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Fluorescence, absorbance, and ion exchange: coupling analysis with water treatment for improved insight on copper complexation with natural organic matter

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ABSTRACT

Groundwater high in dissolved organic carbon (DOC) was spiked with 0–120 μmol/L of copper(II) (Cu) at pH 4, 6, and 8 to observe changes in fluorescence and UV absorbance of natural organic matter (NOM). The groundwater had a fluorescence excitation–emission matrix (EEM) characteristic of humic-like substances. Addition of Cu to the groundwater resulted in fluorescence quenching of EEM peak intensity and increase in UV absorbance at 254 nm (UVA_{254}). Both fluorescence quenching and increase in UVA_{254} suggested the complex formation between Cu and NOM. Importantly, DOC measurements showed that physical loss of NOM did not play a measureable role in contributing to the observed fluorescence quenching and increase in UVA_{254}, which supports the complex formation of Cu–NOM. The conditional stability constant for Cu–NOM was estimated using both fluorescence quenching and UVA_{254} increase at each pH and ranged from 4.26 to 5.77. As a follow up, anion exchange and cation exchange batch experiments were conducted using the groundwater spiked with 120 μmol/L Cu at pH 8 to gain insight on the behavior of Cu–NOM, and attempt to give physical meaning to the fluorescence quenching and UVA_{254} increase. The ion exchange results suggested the possibility of a neutral or partially negatively charged Cu–NOM complex. The significance of this work resides in the idea that fluorescence quenching and UV absorbance of NOM can be used as a tool to evaluate the co-removal of contaminants through water treatment processes.

Keywords: Fluorescence quenching; Natural organic matter; Copper; Ion exchange

1. Introduction

Improved insight on the interactions between natural organic matter (NOM) and metals in water is needed to better understand the transport and toxicity of metals in aqueous systems. In particular, the type, amount, and binding strength of metal–NOM complexes are important factors to consider when studying NOM and metals in water. Fluorescence, and to a lesser extent UV absorbance, has been used to provide both qualitative and quantitative information on metal–NOM interactions [1–12]. For example, copper (Cu), iron, and other metals are known to decrease (or quench) the fluorescence intensity and increase the

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UV absorbance of NOM [9,10], due to complex formation reactions between metal and NOM that changes the electronic structure of NOM. The precise nature of metal–NOM interactions and the most appropriate techniques to investigate these interactions remain active research questions [12,13].

Various analytical methods exist for estimating the conditional stability constants of complexes formed between metals and NOM. A number of these methods are rather complicated, such as cathodic stripping voltammetry and ion selective electrode potentiometry [14,15]. With Ryan and Weber [5,6], fluorescence quenching was being used to estimate conditional stability constants of complexes formed between metals and NOM. Fluorescence quenching and the Ryan–Weber model have since been used extensively by researchers in attempts to estimate complexion capacities and conditional stability constants [1,2,16–20], although not without limitations [12].

While there have been attempts to account or correct for increased scatter caused by precipitation during fluorescence quenching experiments, surprisingly little discussion is made regarding other potential sources of error during the experiment; namely, the possible loss of fluorophores. Filtration is an easy method for removing particulate matter from solution, and therefore, it is an effective way to reduce interference caused by particles formed during the quenching titration. However, the removal of metal–NOM complexes formed can impact the resulting fluorescence intensity of the filtrate by reducing both the concentration of NOM as well as the quencher. Because NOM constitutes the major fraction of fluorophores in natural waters and its concentration is strongly correlated with fluorescence intensity [21,22], physical removal of NOM from samples could lead to exaggerated reductions in fluorescence intensity that could be mistaken for quenching, and thus, complex formation. In addition, comparison between the behaviors of metal–NOM fluorophores with metal–NOM chromophores is sparse in the literature [10,16].

Conditional stability constants estimated from fluorescence quenching and UV absorbance titrations are reported and used as representatives for the association strength of metal–NOM complexes [15–18,23], but little follow-up is made after the estimation of the stability constants that give further insight to the behavior of these complexes. If conditional stability constants are truly representative of binding strength, it is conceivable that they could predict co-transport or co-removal of the quencher–fluorophore (or metal–chromophore) complex within engineered systems. With this in mind, this study proposed that selectively targeting portions of the complex (either the NOM fluorophore/chromophore or the metal quencher) removal from solution can result in the simultaneous removal of both, and shed further light on how these complexes behave in natural and engineered aqueous systems.

The goal of this research was to provide an improved understanding of how fluorescence and UV absorbance can be used to investigate metal–NOM interactions. The specific objectives of the research were to (1) investigate the role of fluorescence quenching, UV absorbance, and physical removal of NOM upon addition of Cu and (2) investigate the strength of interaction between NOM and Cu using an ion-exchange method. The link between these two research objectives is that objective 1 confirms the changes in fluorescence and UV absorbance are true measurements of metal–NOM complex formation, and objective 2 investigates the potential of a physical removal process, such as ion exchange, to remove the metal–NOM complex as a surrogate for co-removal during water treatment.

2. Materials and methods

2.1. Materials

2.1.1. Test waters

All experiments were conducted using groundwater collected from well three at the Cedar Key water treatment plant in Cedar Key, FL, USA. The groundwater was collected in July 2012 and stored at 4°C. Prior to experiments, the groundwater was filtered through 0.45 μm nylon filters (Millipore) to remove any particulates. Table 1 details the water quality of the Cedar Key groundwater (CK). The dissolved organic carbon (DOC) concentration in CK is high due to it being a shallow, carbonate aquifer [24]. In addition to the natural groundwater, synthetic water composed of deionized (DI) water and 1,500 mg/L of sodium chloride was used during the ion-exchange experiments to mimic the ionic strength of the natural groundwater without the presence of NOM. The synthetic water composition is also included in Table 1.

2.1.2. Quencher

Cu(II) was used as the quenching chemical in all experiments. The quenching solution was prepared either by diluting a Cu(II) chloride dihydrate solution (1 M, Fisher Scientific) with DI water or by preparing a solution from Cu(II) chloride dihydrate salt (Fisher Scientific) in DI water. The quenching solution was

Table 1
Water composition measured for CK and synthetic model water

Parameter	Groundwater	Synthetic	Units	Parameter	Groundwater	Synthetic	Units
pH	7.8–8.2	6.1	–	Cl^-	527	nm	mg/L
Conductivity	1,510	1,495	μS/cm	Br^-	0.48	nm	mg/L
Total hardness	431	nm	mg/L $CaCO_3$	SO_4^{2-}	68.9	nm	mg/L
				Na^+	289	nm	mg/L
TOC	6.4	nm	mg/L	K^+	8.5	nm	mg/L
DOC	6.0	nm	mg/L	Mg^{2+}	33.1	nm	mg/L
UV_{254}	0.171	nm	1/cm	Ca^{2+}	120.5	nm	mg/L

Note: Not measured (nm).

prepared fresh before each experiment at a concentration of 0.01 mol/L Cu(II), with varying volumes of solution added to the samples to reach the target concentration. The chloride salt was used because CK had a high background chloride concentration and the anion exchange (AEX) process adds chloride to the solution.

2.1.3. Ion-exchange resins

Amberlite IRA 958 Cl (Dow) was used in the AEX experiments. Amberlite IRA 958 Cl is a strongly basic anion-exchange resin (AER) used to remove organics, with a macroporous structure and acrylic polymer matrix with quaternary ammonium functional groups and chloride used as counterion. Amberlite 200C Na (Dow) was used in the cation-exchange (CEX) experiments. Amberlite 200C Na is a strong acid CEX resin (CER) recommended for demineralization, with a macroporous structure and a polystyrene matrix with sulfonic acid functional groups and sodium used as the counterion. Both AER and CER were used as received from the manufacturer with no regeneration prior to use.

2.2. Experimental methods

2.2.1. Fluorescence quenching and UV absorbance

Fluorescence quenching experiments were conducted at three pH levels and five quencher concentrations. Prior to each experiment, groundwater was filtered through 0.45 μm nylon filters (Millipore) and divided into 125 mL flasks with 50 mL of water in each flask. The samples were spiked with the Cu(II) solution to concentrations of 0, 30, 60, 90, and 120 μmol/L Cu(II), with the 0 (no Cu) sample as a control. Each Cu concentration was tested in duplicate. The pH was measured and adjusted to pH 4 ± 0.1, pH 6 ± 0.1, or kept unaltered at pH 8 using 6 mol/L HCl and 1 mol/L NaOH solutions. The pH was measured continuously during the pH adjustments until it remained within ±0.1 of the target pH for 5 min. During Cu(II) addition, pH adjustment, and subsequent mixing samples were placed in a water bath maintained at 25˚C. The water bath was placed on a 15-place magnetic stir plate and samples were mixed at 400 rpm overnight to ensure complexation reached equilibrium.

After mixing, an aliquot of sample was removed from each flask and measured for fluorescence as excitation–emission matrix (EEM) (hereafter referred to as unfiltered after mixing samples). An aliquot of sample was also filtered after mixing using 0.45 μm nylon filters (Millipore), and EEMs were collected for these samples as well (hereafter referred to as filtered after mixing samples). UV absorbance wavelength scans revealed that a solution containing only Cu(II) in DI water did not appreciably absorb UV light at 254 nm (UVA_{254}), so UVA_{254} of the filtered after mixing samples was also measured. The filtered after mixing samples were also measured for DOC and Cu.

2.2.2. Ion exchange

The ion-exchange experiments were conducted using 250 mL jars containing 250 mL of test water, and experiments were done in triplicate with control samples made containing no ion-exchange resin. Both separate AER and CER experiments were conducted. Resin was rinsed with DI water before use and a small amount of DI water was used to transfer the resin from the graduated cylinder into the sample jars. Resin was measured volumetrically to 0.6 mL of wet resin per 250 mL of water. The pH of water samples following resin addition was measured but left unadjusted (pH 8). Samples were placed in a water bath maintained at 25˚C. The water bath was placed on a 15-place magnetic stir plate and samples mixed for 12 h at 380 rpm. Following mixing, samples were tested for the same measurements as the samples from the quenching experiment: fluorescence EEMs, UVA_{254}, DOC or total organic carbon (TOC), and Cu. Measurements were made on aliquot of sample for unfiltered after mixing as well as filtered after mixing (0.45 μm nylon filters, Millipore).

2.3. Analytical methods

The pH was measured using an Accumet AB-15 + pH meter and calibrated before each use with pH 4, 7, and 10 buffer solutions. Fluorescence was measured on a Hitachi F-2500 spectrophotometer using a 1 cm quartz cuvette. The instrument was allowed to warm up for 1 h prior to use. Fluorescence EEMs were done with an excitation wavelength (Ex) range of 220–450 nm (with 5 nm intervals) and an emission wavelength (Em) range of 300–600 nm (with 2 nm intervals) with 5 nm slit widths for both excitation and emission. Scanning was done at 1,500 nm/min, a PMT voltage of 700 V, and a response time of 0.08 s. Any possible inner filter effect was not corrected for. A DI water EEM was measured before each use. Rayleigh scattering intensities were monitored at Ex/Em = 350/350. UV wavelength scans and UV absorbance measurements were done on a Hitachi U-2900 spectrophotometer using a 1 cm quartz cuvette. The instrument was allowed to warm up for 30 min prior to use. For both fluorescence and UV absorbance, samples were allowed to reach room temperature prior to analysis. TOC (unfiltered) and DOC (filtered) were measured on a Shimadzu TOC-V_{CPH} TOC analyzer with an ASI-V autosampler. Samples were adjusted to pH < 3 using 2 M HCl before analysis to minimize the effects of inorganic carbon on organic carbon measurements. Each separate sample was measured in duplicate. External DOC standards (Ricca Chemical Co.) were also run on the TOC analyzer and were within 11% of their known values. Ionic strength was estimated using specific conductivity measured by an ECTestr 11 + Multi range conductivity meter. Samples for Cu were acidified to 1% acid by volume using trace metal grade nitric acid (Fisher Scientific) prior to analysis. The Cu concentration was measured on a Thermo ICAP 6200 inductively coupled argon plasma atomic emission spectrometer [25]. Cu measurements were verified with quality control checks.

2.4. Data analysis

The CK had a main fluorescence peak at Ex/Em of 260–265/436–450 nm and a secondary peak at Ex/Em of 330–350/430–450 nm, which the literature defines as humic-like peaks [2,26–29] (see Fig. 1). For consistency purposes, the intensity at Ex/Em = 260/440 nm was used to calculate the fluorescence quenching of NOM with Cu(II) addition and the conditional stability constant for Cu(II)–NOM.

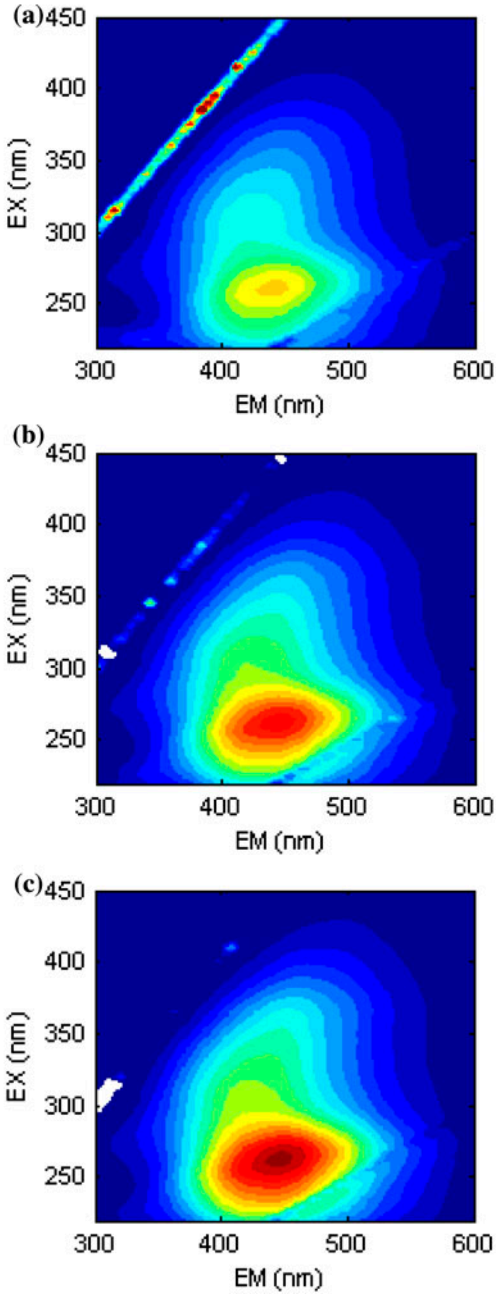

Fig. 1. Fluorescence EEMs of untreated CK at (a) pH 4, (b) pH 6, and (c) pH 8 (unadjusted pH).

The conditional stability constant is an equilibrium constant specific to a certain set of experimental or environmental conditions. In the case here, the conditional stability constant, K', is defined as: $K' = [ML]/([M]\cdot[L])$, where $[ML]$ is the theoretical concentration of the metal–ligand complex (mol/L), $[M]$ is the added quencher concentration (mol/L), and $[L]$ is the theoretical free ligand concentration (mol/L). The conditional stability constant for Cu(II)–NOM was estimated using a non-linear regression analysis based on the model developed by Ryan and Weber (Eq. (1)), and used previously [5,16,18,20], which assumes a one-to-one, metal–ligand complex.

$$\frac{I}{I_{\text{ref}}} = 1 + \frac{\frac{I_{ML}}{I_{\text{ref}}} - 1}{2K'L} \times \left[(1 + K'L + K'M) - \sqrt{(1 + K'L + K'\text{M})^2 - 4K'^2LM}\right] \quad (1)$$

In Eq. (1), I is the measured fluorescence intensity at Ex/Em = 260/440 nm at quencher concentration M (mol/L), and I_{ref} is the fluorescence intensity of the sample at Ex/Em = 260/440 nm with zero quencher concentration.

To reduce the number of parameters to be fit in Eq. (1), I_{ML}/I_{ref}, or the theoretical intensity of the metal-saturated complex normalized to the blank intensity, was assumed constant and was estimated as described by Luster et al. [20] using the equation:

$$\left|\frac{I}{I_{\text{ref}}} - 1\right| = \left|\frac{I_{ML}}{I_{\text{ref}}} - 1\right|\left(1 - e^{-\alpha M}\right) \quad (2)$$

with fitting parameters $I_{\text{ML}}/I_{\text{ref}}$ and α. The estimated $I_{\text{ML}}/I_{\text{ref}}$ from Eq. (2) was inserted into Eq. (1) and the line was fit to the experimental data for K', where K' is the conditional stability constant. The model was also fit to the UVA_{254} data following the same method as the fluorescence quenching data, as done by Bai et al. [16]. For the UV absorbance model, I is the measured absorbance at quencher concentration M, and I_{ref} is the absorbance for the sample at $M = 0$. $I_{\text{ML}}/I_{\text{ref}}$, and α were estimated using Eq. (2) and I_{ML}/I_{ref} was inserted into Eq. (1) for K'.

3. Results and discussion

3.1. Fluorescence quenching and UV absorbance

In this study, the Ryan–Weber model was fit to the fluorescence quenching data, as well as to the UV absorbance data (see Table 2 and Figs. 2 and 3). From the models, conditional stability constants for the Cedar Key NOM with Cu were estimated at each pH level: 4, 6, and 8. Average percent error between methods for the $\log K'$ estimates in this study was 1.5% and average relative standard error was 3.3%. It is possible that estimations could have been improved by using multiple binding sites between NOM and metal, as discussed below.

Literature values of conditional stability constants for humic substances and various organic matter types quenched with Cu are provided for comparison in Table 2. The Cu concentrations are given in Table 2, as well. On the whole, conditional stability constants appear generally consistent for specified pH values. For instance, larger $\log K'$ estimates are seen for higher pH values, smaller estimates for lower, more acidic pH values. Many experiments estimating these stability constants use isolated NOM samples in their experiments [2,16,17,20], contrasted with this study's approach of using a whole natural water sample. The advantage of using isolated NOM is to eliminate the effect of the natural water matrix, whereas natural water has the advantage of the NOM being unaltered. Of the papers studied that report conditional stability constants for Cu–NOM, two were found that also used bulk water in their experiments and used parallel factor analysis (PARAFAC) to deconvolute fluorescence quenching responses of organic matter to Cu addition [19,26]. Of these, Mounier et al. [26] identified a fluorescence peak for their black water samples in the same region observed for the Cedar Key NOM peak. In this work, the one-site model for the pH 6 experiments gives a comparable $\log K'$ estimate ($\log K' = 5.3 \pm 0.01$) to their first binding site under their two-site model at pH 6 ($\log K' = 5.5$) [26]. For multiple site complexation, typically the first presented binding site represents stronger ligands detected at lower metal concentrations, while the secondary and other subsequent sites are considered weaker but more abundant and detectable at higher metal concentrations [17,20,26]. This study used a narrower range of metal concentrations than Mounier et al. [26] but still relevant to natural systems; thus, the quenching observed in the Cedar Key water is most likely representative of Cu complexation by a primary, strong ligand, and therefore may explain the smiliarities in the $\log K'$ estimates. Detailed quality parameters of the surface water used in Mounier et al. [26] was not available, however, for further comparison of experimental conditions and the conditionality of the constants estimated should be kept in mind when attempting to compare constants over different waters, organic matter types, and experimental conditions.

Fluorescence quenching of Cedar Key NOM by addition of Cu with the Ryan–Weber model fit are shown in Fig. 2. As expected, fluorescence intensity at 260/440 nm decreases with increasing Cu concentration, suggesting that complexation occurs between the

Table 2
Comparison of conditional stability constants from this research and the literature

Water or NOM	Quencher (concentration)	log K	Other information	Ref
Fulvic-acid-like component	Cu(II) (0–120 μM)	4.92 ± 0.09	pH 4, fluorescence (260/440)	This study
		4.62	pH 4, UVA_{254}	
		5.30 ± 0.01	pH 6, fluorescence (260/440)	
		5.46	pH 6, UVA_{254}	
		4.77 ± 0.20	pH 8, fluorescence (260/440)	
		–[a]	pH 8, UVA_{254}	
Fulvic-like component	Cu(II) (1.7 nM–0.01 M)	4.4	pH 6, fluorescence (260/450) one-binding site model	[26]
		5.5 and 3.9	pH 6, fluorescence (260/450) two-binding site model	
Humic-like component		4.3	pH 6, fluorescence (332/450) one-binding site model	
		5.8–3.9	pH 6, fluorescence (332/450) two-binding site model	
Surface waters (slough systems) humic-like	Cu(II) (0–70 μM)	4.67–5.21	Natural pH (7.0–7.7); range for multiple components identified using PARAFAC analysis	[19]
Leaf litter extract	Cu(II) (0–40 μM)	7.47 and 5.82	pH 6, two binding sites	[20]
Landfill leachate fulvic acid	Cu(II) (0–300 μM)	4.34 ± 0.03	pH 6, UVA_{254}	[16]
		4.88 ± 0.06	pH 7, UVA_{254}	
Surface water fulvic acid		4.46 ± 0.05	pH 6, UVA_{254}	
Tyrosine		4.77 ± 0.04	pH 6, UVA_{254}	
Algal-derived organic matter	Cu(II) (0–100 μM)	4.85–5.35	pH 7.8; range for multiple components identified using PARAFAC analysis	[17]
Suwannee River fulvic acid isolate		4.97, 5.06		
Pony Lake fulvic acid isolate		5.37, 5.44		
Inogashira soil fulvic acid isolate	Cu(II) (0–240 μM)	3.77–4.00	pH 4.5, values for various excitation wavelengths	[2]
Shinshinotsu peat fulvic acid extract		3.87–4.32		
Fractionated humic acid from soil, 1–10 KDa	Cu(II) (0–125 μM)	4.91–5.86	pH 6, range for multiple identified fluorescent peaks	[23]
Fractionated humic acid from soil, >100 KDa		5.96–6.48	pH 6, range for multiple identified fluorescent peaks	
Suwannee River fulvic acid isolate	Cu(II) (0–100 μM)	4.98–5.62	pH 6, range for multiple identified fluorescent components	[30]

[a] Model was a poor fit; not included in discussion.

Cu and NOM. Quenching is most significant for the pH 6 samples and least significant with increasing Cu concentration for the pH 4 samples. Likewise, log K' for the pH 6 samples is greater than the value for the lower pH samples, which is consistant with other reported trends for samples at varying pH values (see Table 2). NOM tends to have greater charge density at more basic pH levels [31], resulting in more binding or interaction sites available on the NOM molecule. In other words, metal ions are outcompeted by H^+ ions

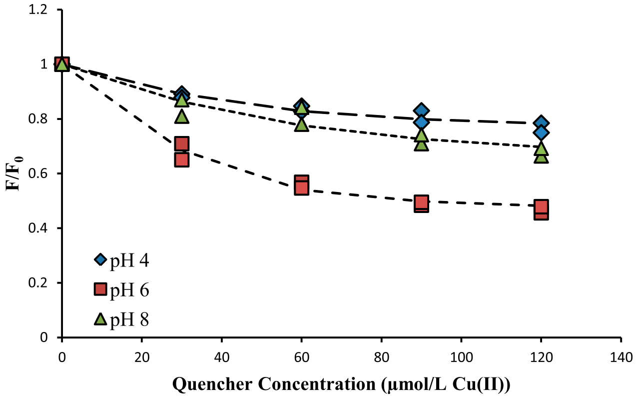

Fig. 2. Fluorescence quenching trends observed for Cedar Key water samples filtered after mixing. Experimental results for replicate samples are shown as symbols. Dashed lines represent results from the Ryan–Weber model (– –pH 4;- - - pH 6; ·· pH 8 (unadjusted pH)).

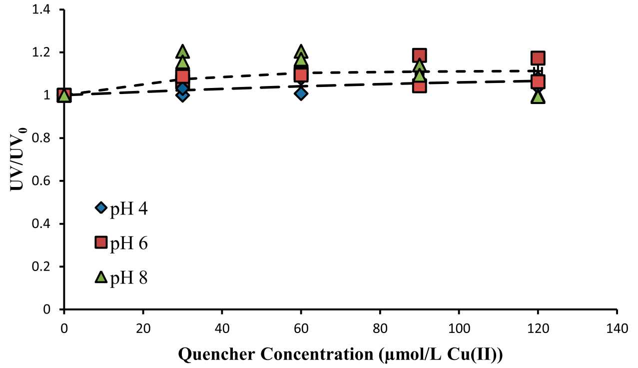

Fig. 3. UVA_{254} absorbance trends observed for Cedar Key water samples filtered after-mixing. Experimental results for replicate samples are shown as symbols. Dashed lines represent results from the Ryan-Weber model (– – – pH 4; - - - pH 6; ·· pH 8 (unadjusted pH) not shown because of poor model fit).

for binding sites at low pH, lower log K' values, and less pronounced quenching trends are expected at high H^+ concentrations (low pH) and were observed from pH 6 to pH 4.

For pH 8, it is possible that some insoluble Cu hydroxide ($Cu(OH)_2$) formed in addition to or instead of a Cu–NOM complex, causing a less pronounced quenching trend when compared with pH 6 despite increased availability of binding sites on the NOM, due to the reduced availability of Cu in solution. Further discussion is provided below. This is supported by chemical equilibrium calculations at 120 μM Cu(II) (Visual MINTEQ, ver. 3.0 [32]) that showed $Cu(OH)_2$ undersaturated at pH 4 and 6 but oversaturated at pH 8.

UV absorbance increased with Cu concentration as shown in Fig. 3 and is consistent with trends observed by Bai et al. [16]. The increase in UV absorbance suggests complex formation between Cu and NOM, similar to the fluorescence quenching results. Estimated conditional stability constants from the UV absorbance trend were used as a comparison with the estimations from the fluorescence quenching trends, except for the unadjusted pH 8 samples for which the UV absorbance model was a poor fit. The two different approaches gave similar conditional stability constants for Cu–NOM as shown in Table 2. Yan et al. showed that the interactions between Suwannee River fulvic acid and Cu were similar to the interactions of salicylic- and polyhydroxyphenolic groups with Cu [10].

To monitor changes in DOC and Cu concentrations in the samples caused by filtration, DOC, and Cu levels were measured on the filtered samples (i.e. filtered after mixing) and compared with unfiltered samples. TOC concentrations in unfiltered samples were assumed equal and constant across all unfiltered samples. Although loss of DOC was observed after filtration (Fig. 4), the loss of DOC was not nearly as marked as the loss of fluorescence intensity (Fig. 2), as evidenced with only approximately 10% DOC loss but up to 50% reduction in fluorescence intensity for the pH 6 titrations. Precipitation formed is likely to have been insoluble Cu–NOM complexes (in addition to potential $Cu(OH)_2$ precipitation at pH 8), but because fluorescence intensity is strongly correlated with DOC concentration [21], it is unlikely that the Cedar Key organic matter was removed from solution via precipitation with the Cu addition to the extent that would

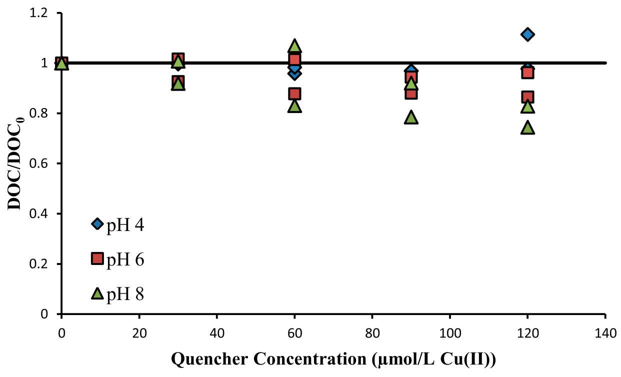

Fig. 4. Changes in DOC for Cedar Key water samples filtered after mixing. Experimental results for duplicate samples are shown as symbols. The solid line represents no change in DOC concentration ($DOC/DOC_0 = 1$) with increasing quencher concentration.

cause the observed decrease in fluorescence. Thus, it can be assumed that the decrease in fluorescence was, in fact, quenching caused by Cu–NOM complexation, rather than exaggerated peak intensity loss caused by physical loss of fluorophores.

For Cu–NOM complexation to occur, Cu must be present in solution to complex with the NOM. The Cu concentrations of the filtered after mixing samples were measured and compared to unfiltered groundwater samples spiked with Cu(II) to monitor for potential Cu loss either through insoluble Cu–NOM complex formation or other loss via precipitation. At pH 8, filtered after mixing samples had up to 1.5 mg/L Cu (approx. 24 μM) remaining in solution vs. the expected 7.6 mg/L (120 μM), likely due to removal of supersaturated $Cu(OH)_2$ as predicted by chemical equilibrium calculations and further explaining the less pronounced quenching trend at pH 8 compared with pH 6. The Cu in filtered after mixing samples for pH 6 and pH 4 samples were also reduced when compared with unfiltered samples; however, the Cu concentrations in samples for pH 6 compared with pH 4 were roughly equal per target concentration. With equal concentrations of Cu, the differing quenching trends are likely caused by competition for binding sites by protons at the lower pH.

The bulk sample main fluorescent peak of the Cedar Key water (Ex/Em = 260/440) is defined by literature as a humic-like peak [2,26,27], and humic-like fluorophores can be assumed to be representative of the whole organic material present [33]. While bulk fluorescence measurements on the Cedar Key water resulted in a single peak that could be assumed as representative of the whole sample, this is not always the case for other samples that contain multiple fluorophores [26,33,34]. Bulk fluorescence measurements do not consider how varying types of organic matter contribute to the overall fluorescence signal. There is a possibility that for some samples, there are multiple fluorophores that interact with both the metal ion added as well as the other fluorophores. For example, Yamashita and Jaffe [19] used PARAFAC to separate the whole water EEM into eight components. From their results, four of the eight components (three terrestrial humic components and a microbially derived component) contributed nearly 80% of the overall fluorescence, while the other four (microbial humic, terrestrial humic, and two protein components) contributed the remaining 23% [19], and so small losses of organic matter of the first four components could result in larger reductions in the overall bulk fluorescence intensity. Additionally, there is the possibility of the presence of non-fluorescent organic matter fractions. If the majority of the organic matter in a sample is non-fluorescent, the quenching of fluorophores can show large reductions in fluorescence intensity without large reductions in overall TOC concentration.

In summary, the use of fluorescence quenching for the estimation of stability constants for aquatic NOM could be improved by understanding the various components that make up organic matter within the water matrix. Because fluorescence quenching only observes changes in NOM fluorophores, conditional stability constants estimated from fluorescence quenching only estimates the parameter for that specific portion of organic matter without giving consideration to the complexation that could occur with the non-fluorescent organic material [26]. The addition of the UV absorbance titrations provides information on NOM chromophores to complement the information on NOM fluorophores. Finally, by employing methods such as PARAFAC to deconvolute the signals given in fluorescence EEMs, additional information can be gained from fluorescence methods [18,19,26,35,36].

3.2. Ion-exchange experiments

The results of the ion-exchange experiments are summarized in Table 3 for the samples not filtered after mixing, and Table 4 for samples filtered after mixing. Columns with the "0" subscript refer to resin-free control samples. Fig. 5 shows fluorescence, UV, DOC, and Cu changes for filtered after mixing samples after ion exchange. Ion-exchange experiments were done at the water's natural pH, approximately 8, to cause minimal changes to the water chemistry.

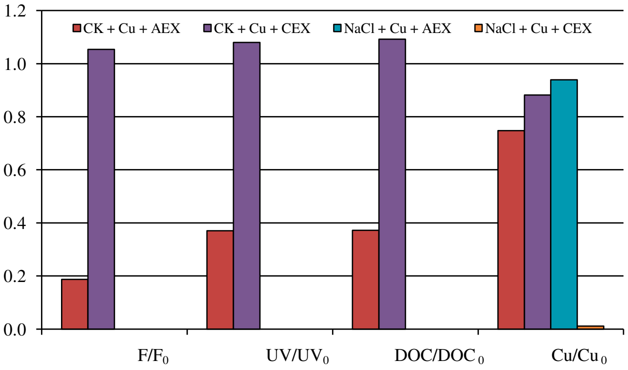

Fig. 5. Ion-exchange results for Cedar Key water samples spiked with Cu(II), filtered after mixing, and normalized to the resin-free controls. Legend: CK, Cu, AEX, CEX, DI water, and NaCl (NaCl).

Because NOM typically has a net negative charge, it is expected to be removed using an AEX process. Indeed, AEX is used in water treatment and has been shown to be an effective method of removing NOM from water [21,37,38]. Removal of Cu (i.e. Cu^{2+}), because of its cationic nature, is not expected with AEX. Marked decreases were seen in the concentration of DOC in both the unfiltered (i.e. not filtered after mixing) and filtered (i.e. filtered after mixing) Cedar Key (CK) samples, while Cu levels in samples spiked with Cu(II) (CK + Cu) were reduced to an average of 70% of their initial concentrations (30% removal) in the filtered samples (Fig. 5). The Cu removal was not seen in the unfiltered samples spiked with Cu(II) and treated with AEX (CK + Cu + AEX). In the synthetic water samples with no NOM, Cu removal was not observed with AEX (7.4 to 7.9 ± 0.1 mg/L). It is noteworthy that there are large differences between the Cu levels detected in the unfiltered and filtered samples, suggesting loss of Cu due to filtration. As in the fluorescence quenching experiment, at pH 8 it is likely that some portion of the Cu in the samples was removed from solution as insoluble $Cu(OH)_2$ and consequently removed from the sample through filtration. UV absorbance results for the no resin samples are consistent with those found in the quenching experiments, and while Cu addition had increased UV absorbance of the CK samples; the removal of NOM through AEX would reduce it again (Fig. 5).

The CEX results are also summarized in Tables 3 and 4. DOC removal through cationic exchange was not expected for reasons mentioned previously and was not observed. Elevated readings for fluorescence, UV, and DOC above the control samples for the unfiltered samples could be due to interference from the CER. UV absorbance is not strongly affected by CEX in the filtered Cedar Key sample, while it increases with Cu as expected. Interestingly, while Cu removal by CEX was observed in the synthetic water samples (those with no NOM, with over 90% Cu removal, see Fig. 5), Cu removal in the Cedar Key samples was not observed to the same extent for either unfiltered or filtered samples. Thus, Cu is removed to great extent with the CEX in samples lacking NOM. However, it should be acknowledged that Cedar Key water does have high calcium concentrations (Table 1), which competes for CEX sites on the resin and can also result in low Cu removal. Because of this potential for interference with the ion-exchange experiments, future experiments should be done that observe the natural water matrix without the NOM, as well as experiments that include only the NOM isolate to better

Table 3
Ion-exchange results for Cedar Key water samples that were not filtered after mixing. Legend for water type: CK, Cu, AEX, CEX, DI water, and NaCl (NaCl)

Water type	F_0	F	UV_0 (cm^{-1})	UV (cm^{-1})	TOC_0 (mg/L)	TOC (mg/L)	Cu_0 (mg/L)	Cu (mg/L)
CK + AEX	5,310	1,165 ± 109	0.186	0.223 ± 0.062	6.4	2.6 ± 0.4	0.01	0.02 ± 0.02
CK + Cu + AEX	2,571	513 ± 12	0.284	0.153 ± 0.02	5.4	2.0 ± 0.1	4.1	4.0 ± 0.2
CK + CEX	5,596	7,850 ± 1,979	0.179	0.396 ± 0.133	6.3	8.8 ± 2.1	0.01	0.02 ± 0.004
CK + Cu + CEX	2,681	4,204 ± 1,264	0.262	0.481 ± 0.106	5.7	8.0 ± 1.4	5.1	5.7 ± 0.4
NaCl + Cu + AEX	–	–	–	–	–	–	7.4	7.9 ± 0.1
NaCl + Cu + CEX	–	–	–	–	–	–	8.3	0.3 ± 0.02

Table 4
Ion-exchange results for Cedar Key water samples filtered after mixing. Legend for water type: CK, Cu, AEX, CEX, DI water, and NaCl (NaCl)

Water type	F_0	F	UV_0 (cm^{-1})	UV (cm^{-1})	DOC_0 (mg/L)	DOC (mg/L)	Cu_0 (mg/L)	Cu (mg/L)
CK + AEX	5,359	842 ± 50	0.186	0.022 ± 0.002	6.0	2.0 ± 0.2	0.010	0.001 ± 0.000
CK + Cu + AEX	2,958	554 ± 18	0.170	0.063 ± 0.010	4.5	1.7 ± 0.03	1.9	1.5 ± 0.3
CK + CEX	5,164	5,336 ± 107	0.170	0.177 ± 0.002	6.2	6.4 ± 0.00	0.01	0.01 ± 0.004
CK + Cu + CEX	3,101	3,268 ± 106	0.187	0.202 ± 0.002	5.2	5.7 ± 0.04	2.1	1.9 ± 0.2
NaCl + Cu + AEX	–	–	–	–	–	–	8.2	7.7 ± 0.2
NaCl + Cu + CEX	–	–	–	–	–	–	8.0	0.09 ± 0.04

understand the interactions and their responses to ion-exchange treatment.

At 1% acid by volume, the samples separated for metals analysis had a pH < 2, and despite this, control samples of Cedar Key spiked with Cu(II) showed lower measurements of Cu than the expected theoretical concentration. This emphasizes the possibility of relatively strong interactions between Cu and NOM, suggesting that Cu is too tightly bound to the NOM to be detected as free Cu during instrument analysis and thus, is too tightly bound to be removed from solution by CEX. It is difficult to say with certainty, however, whether the loss of Cu in the AEX samples is from co-removal with the organic matter through ion exchange, or from precipitate or particulate removal of large-sized complexes through filtration. Data seems to suggest the former.

When coupled with the CEX results, the AEX results also suggest that Cu binds strongly with the NOM and can, to some extent, be removed with NOM via AEX. Because Cu was not removed to the same extent as the NOM in the AEX experiments, the majority of the NOM removed is likely to be the fraction that remains unbound by Cu, followed by preferential removal of NOM fractions that still retain a slight negative charge across all or part of the molecule. Metal-complexed NOM is likely outcompeted for spots on the resin by more densely charged fractions.

Regarding the fluorescence response, standard deviations in absolute intensities between the triplicate samples of most experimental scenarios were 10% or less (in arbitrary units), so reproducible responses for these experimental conditions can be achieved. Only the unfiltered CEX experiments showed high variability in their fluorescence readings, and this can likely be attributed to scattering caused by suspended resin particles present in the water after mixing. With that low variability in mind, if measurable chemistry changes (such as changes in DOC concentration) produce predictable fluorescence changes, then fluorescence has the potential to be a quick, predictive analytical tool for providing insight to changes in the water matrix without directly measuring a specific parameter.

3.3. Implications for water treatment

NOM, while naturally occurring, is a concern in drinking water supplies, causing color, taste, and odor issues. It is also a known precursor for disinfection byproducts (DBPs), making its removal a vital portion of the drinking water treatment process. Source waters themselves are subject to forces that can alter their water qualities, from forces such as land use changes or urbanization, groundwater contamination from salt or wastewater [39], and changes in recharge water quality [40], to more natural causes such as storm events, drought, or seasonal variability. Even climate change impacts the quality of drinking water sources [41].

Fluorescence spectroscopy and fluorescence quenching with their previously mentioned advantages could be a useful tool for water treatment plant operators as a way of quickly alerting plants to changes in their water chemistry. Regular fluorescence EEM readings during treatment plant operation can notify operators on water quality changes caused by changes in NOM concentration, pH changes, or even changes in NOM composition [7,42]. For example, fluorescence has been used to track changes in NOM related to DBP formation [29,43]. Fluorescence tests and quenching results can provide information regarding changes from other aqueous constituents such as metals and, possibly, other unexpected or seasonal contaminants [44]. Fluorescence quenching with treatment methods can further improve understanding of water chemistry by providing practical information beyond the qualitative information from EEM readings. Although quenching and the estimation of stability constants cannot simply be compared over various water and various quenchers, they could be used to track changes in a single water source over time. In the hands of water treatment plants, this information could allow for more effective and more intelligent treatment of water.

As previously mentioned, if the conditional stability constants are indicative of sufficiently strong bonds within the metal complexes, then selectively targeting either portion of the complex (either the organic matter fluorophore or the metal quencher) could result in the simultaneous removal of both. By using ion exchange to selectively target only one contaminant at a time (the negatively charged NOM or the positively charged metal ion) this study hoped that observed co-removal with the ion-exchange process would indicate ion exchange as a potential process for specific removal of one contaminant with the additional benefit of increased co-removal efficiency of another. While it is true that treatment options exist for removing TOC and metals, they are usually separate processes for each contaminant. Additionally, metal removal processes have focused more on wastewater than drinking water treatment [45–47]. And although processes such as membrane separation are able to simultaneously remove organic matter and ionic substances such as metals, it would be more practical for existing conventional plants to be able to predict and achieve co-removal with treatment methods that are already utilized, thereby requiring minimal retrofitting, or do not require extra capital costs (such as the installation of membranes and the consideration of dealing with membrane concentrate).

4. Conclusions

Because of their conditional nature, added consideration should be given when discerning meaning from calculated or experimental conditional stability constants. While precipitation and fluorophore/chromophore loss does not seem to be an issue with regard to fluorescence quenching and UV absorbance results and thus, impacts on stability constant estimations using quenching, factors such as quencher properties, water matrix properties, and NOM type have complicated impacts on fluorescence readings. Simply taking fluorescence quenching readings of bulk NOM could give a shallower understanding of the changes happening in the water matrix than could potentially be gained with currently available analytical methods. The estimation of conditional stability constants, however, could be useful for observing changes over time in locations, where the water chemistry is well known and a good understanding of the water being treated is had.

With regard to water treatment, the ability to predict and rely on co-removal of contaminants depends on deeper understanding of how contaminants interact with each other; in this case, how metal–NOM complexes behave. With further research, coupling fluorescence quenching and UV absorbance with treatment methods can provide reinforcement to each test by giving practical meaning to fluorescence quenching readings, and in turn allow fluorescence readings to be indicative of predictable changes in water chemistry and contaminant removal.

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