Fluorescence spectroscopy for assessing trihalomethane precursors removal by MIEX resin

P. Jutaporn, W. Laolertworakul, M. D. Armstrong and O. Coronell

ABSTRACT

This study investigated the applicability of fluorescence excitation–emission matrix spectroscopy (EEMS) to assess total trihalomethane formation potentials (TTHMFPs) and the ability of magnetic ion exchange (MIEX®) resin to reduce TTHMFP. We treated a surface water and secondary wastewater effluent with MIEX mimicking full-scale operation by repeatedly exposing the same resin batch to additional feed water, with batches ranging from 500 to 5,000 resin bed volumes. Results showed that MIEX was more effective at removing or reducing ultraviolet absorbance (UVA254), dissolved organic carbon (DOC), and TTHMFP in surface water than in secondary effluent. The greater UVA254, DOC and TTHMFP removal for surface waters was explained by the stronger affinity of MIEX for terrestrial dissolved organic matter (DOM) compared to microbial DOM. Fluorescence EEMS results showed that the ratio between terrestrial and microbial fluorescent signals of DOM was significantly greater in surface water than in secondary effluent. Fluorescence surrogate parameters were strongly correlated with TTHMFP, namely, fluorescence intensity of humic-like peak C ($R^2 = 0.98$, $p < 0.01$), protein-like peak T ($R^2 = 0.96$, $p < 0.01$), and fulvic-like peak A ($R^2 = 0.87$, $p < 0.01$). Correlations between fluorescence surrogate parameters and TTHMFP were substantially stronger than correlations between DOC and TTHMFP. Overall, the results indicate that fluorescent parameters extracted from EEMS data can be used as quick surrogate parameters to monitor TTHMFP for a diverse group of raw and MIEX-treated waters.

Key words | dissolved organic matter (DOM) characterization, excitation–emission matrices (EEMs), fluorescence, MIEX®, trihalomethane formation potentials

INTRODUCTION

Disinfection is an essential step to provide a clean and safe water supply. While chemical disinfectants (e.g., free chlorine, monochloramine, and chlorine dioxide) are effective at protecting human health from pathogens, their reactions with dissolved organic matter (DOM) can form harmful disinfection by-products (DBPs). Despite the availability of other chemical disinfectants, free chlorine disinfection remains the most widely used disinfectant in the USA (US EPA 2016). Free chlorine is of particular concern because its reaction with DOM forms trihalomethanes (THMs) preferentially over other DBPs and in higher mass concentration (Singer 1999).

Since the discovery of associations between DBPs and adverse health effects, DBPs have become a focus of study in water treatment. The National Cancer Institute declared chloroform to be carcinogenic in laboratory animals (US EPA 2016) and long term exposure to THMs has been associated with higher risk of bladder cancer (US EPA 2016). In 2001, the US Environmental Protection Agency (EPA) promulgated the Stage 1 Disinfectants and Disinfection Byproducts Rule, which set the maximum contaminant level to 80 μg/L for total trihalomethanes (TTHM) (US EPA 2001).

As a DBP precursor, the structure of DOM species plays an important role in DBP formation because aqueous chlorine species tend to react with electron-rich sites (e.g., aromatic rings and amino nitrogen) in organic structures (Harrington...
et al. 1996). For example, THM formation potential (THMFP) was found to be strongly correlated with indicators of DOM aromaticity (i.e., UV254 (Pifer & Fairey 2014) and specific UV absorbance (SUVA) (Singer 1999)), and phenolic carbon content of hydroporphic natural organic matter (Nom) (Harrington et al. 1996). The importance of DOM structure on DBP formation is important because the characteristics of organic matter vary among water types. Specifically, compared to organic matter in natural water, effluent organic matter (EfOM) in treated wastewater is less aromatic and more hydrophilic, and is enriched with nitrogen, refractory substrates, and soluble microbial products (Chen et al. 2005). Most EfOM contains high levels of DBP precursors that form a wider range of DBPs than do natural waters when treated with chlorine for pathogen control (Krasner et al. 2008).

The identification of DOM as a DBP precursor has had a major influence on the selection, design, and operation of water and wastewater treatment strategies to control DBP formation. Magnetic ion exchange (MIEX®) resin was developed by Ixom Watercare Inc. (Australia) specifically to remove DOM in water treatment plants. MIEX is a strong base anion-exchange resin with magnetic properties and a small resin bead size. The magnetic properties make the resin beads act as weak individual magnets, which enhances particle agglomeration and facilitates rapid particle settling. The small resin beads provide high surface areas that enhance DOM removal kinetics and regeneration capacity (Singer & Bilyk 2002).

The effectiveness of MIEX resin to remove bulk dissolved organic carbon (DOC), including THM precursors, in surface water is well established (Singer & Bilyk 2002; Boyer & Singer 2003). For example, THMFP was reduced by more than 60% in MIEX batch experiments using surface water from nine different water utilities across the USA (Singer & Bilyk 2002). By contrast, there are fewer studies that reported the ability of MIEX resin to remove DOC or THM precursors from synthetic wastewater (Nguyen et al. 2011) and biologically treated secondary effluent (Zhang et al. 2006). Notably, Gan et al. (2013) reported that 8–12 mL/L MIEX resin reduced 45–58% of DOC and 39–62% of THMFP from effluent impacted waters. Further, few studies have focused on the quantifiable characteristics of DOM to explain differences in MIEX resin performance in treating effluent and natural water.

DOM has a broad range of concentrations and compositions; its chemistry is complex and highly variable in water (Cory et al. 2010). Because organic molecules primarily contain humic substances, which are aromatic chromophores that absorb both visible and UV light, spectrophotometric analysis can be used to characterize DOM. When excited by UV and blue light, a sub-fraction of DOM emits energy as fluorescent light (Stedmon et al. 2003; Stedmon & Bro 2008). 3D-fluorescence excitation–emission matrix (EEM) spectroscopy, a series of emission scans in a range of excitation wavelengths, characterizes DOM with minimal sample pretreatment (Coble 1996). Fluorescence excitation–emission matrix spectroscopy (EEMS) is both qualitative and quantitative, providing information regarding the type, structure, and abundance of functional groups. Fluorescent organic matter is generally classified into two distinct groups: terrestrially derived (humic-like substances) and microbially derived (protein-like substances) (McKnight et al. 2003; Leenheer & Croué 2005; Stedmon et al. 2003). Quantitative fluorescence parameters (e.g., fluorescence index (fi), peak intensity ratios, and maximum fluorescence intensity in different regions) can be extracted from EEM spectra. For example, fi is the ratio of emission intensity (450 nm/500 nm) at 370 nm excitation (McKnight et al. 2004) and can be used to distinguish sources of aquatic DOM. A previous study (McKnight et al. 2003) reported that terrestrially derived fulvic acids had a low fi (~1.4) and microbially derived DOM had a high fi (~1.9). Typically, fi was low for DOM with high aromaticity and high for DOM with low aromaticity.

EEMS has been used to track DOM in both drinking water and wastewater treatment plants (Chen et al. 2003). Strong correlations between THMFP and humic-like fluorophores in samples from drinking water treatment plants were reported previously (Pifer & Fairey 2014), suggesting the application of fluorescence parameters to assess THMFP. Using EEMS analysis, Jutaporn et al. (2016) demonstrated that MIEX resin was more effective at removing terrestrially derived DOM than microbially derived DOM, and Arias-Paic et al. (2016) reported that MIEX treatment produced water with predominantly microbial character due to its preferential removal of terrestrially derived DOM. However, the correlations of raw water fluorescence parameters with THMFP and the ability of MIEX resin to reduce THMFP remain under-explored, especially with wastewater effluent and wastewater-effluent-impacted water. Moreover, most of the results in the published literature on MIEX performance were obtained using batch experiments with single use of MIEX resin; batch tests do not mimic MIEX conditions in full-scale applications and might overestimate the removal efficiency of MIEX (Kitis et al. 2007) Only a limited number of studies use experimental protocols that mimic full-scale operation.

Accordingly, the objective of this study was to investigate the applicability of fluorescence EEMS analysis to
assess: (1) the THMFP of surface water and wastewater effluent and (2) the ability of MIEX resin to remove THM precursors from surface water and wastewater effluent. We examined fluorescence spectra of DOM from surface water and wastewater effluent, before and after a MIEX treatment protocol that replicated full-scale, continuous plant operation.

METHODS

Water samples

We collected a surface water and secondary effluent from Orange County, North Carolina, USA. Surface water samples were collected on 10 October 2013 from a drinking water treatment plant intake that used University Lake as source water. Secondary effluent before UV disinfection was collected on 24 January 2014 from Mason Farm Wastewater Treatment Plant (WWTP), a conventional secondary activated sludge process with nitrification. Water samples (50 L) were collected once from each water source and used for all experiments in this work. Upon arrival at the laboratory, water samples were filtered with 1.2 μm glass fiber filters (Whatman GF/C) to remove suspended materials. All samples were stored at 4 °C in the dark and allowed to reach room temperature (20 ± 2 °C) prior to each experiment.

MIEX treatment

MIEX resin (Ixon Watercare Inc., Australia) was stored wet in a 5% NaCl solution, then rinsed with laboratory-grade deionized water prior to use. The resin was allowed to settle in a graduated cylinder for 15 min before measuring the settled resin volume. The resin contact experiments were performed using a six-paddle stirrer jar test (Phipps and Bird Inc., VA, USA) as described by Singer & Bilyk (2002). The experiment followed the multiple-loading test protocol as advised by the manufacturer. The purpose of the MIEX multiple-loading procedure was to replicate the performance of a full-scale continuously operated process using the same resin multiple times without regeneration. The initial resin dose was 2 mL per litre of water (500 bed volume (BV)).

The procedure required mixing the resin with 2 L of raw water for 15 minutes at 100 rpm, followed by 1 minute of settling. We doubled the settling period recommended by the manufacturer from 30 seconds to 1 minute; we also used a magnet to aid settling and decanting. Treated water was decanted into collection vessels. Another 2 L of raw water was added to the resin in the jars and mixing was repeated. The bed volume was calculated as the ratio between the volume of treated water to MIEX resin (Kitis et al. 2007). We used the same quantity of resin for each experiment, meaning BV values are defined by the volume of treated water; larger BV values indicate consecutive resin uses. The resin was put in contact with six consecutive volumes of raw water (i.e., repeated six times using the same resin without regeneration), spanning 500–5,000 BV. One sample treated with fresh resin (500 BV) and five composite samples from 1,000–5,000 BV treatments were collected for each water source. MIEX-treated samples were vacuum filtered with 1.2 μm glass fiber filters (Whatman GF/C) after MIEX treatment, as resin fines could have interfered with sample analysis. Each treatment condition was performed in triplicate and the average value was reported.

DOM measurement and characterization

DOC (mg-C/L) and total dissolved nitrogen (TDN, mg-N/L) were measured using a TOC-V organic carbon analyzer (Shimadzu, Atlanta, GA, USA). UVA254 measurements were made using a 1-cm quartz cell (Starna, Australia) and a U-2000 spectrophotometer (Hitachi Instruments Inc., Danbury, CT, USA). SUVA values were calculated as UVA254 normalized by DOC. As mentioned above, the effluent water was sampled from Mason Farm WWTP, which has good nitrification capability; thus, we expected that the majority of TDN in the effluent was composed of nitrate from the nitrification process (Gan et al. 2013).

EEM spectra of the water samples were acquired using a 1-cm quartz cell in a Fluorolog-321 spectro-fluorometer with a xenon arc lamp as the excitation source and a synapse CCD detector (Horiba JobinYvon, Edison, NJ, USA). Excitation wavelengths of 240–450 nm in 5-nm intervals and emission wavelengths of 320–550 nm in 1-nm intervals were used. The blanks were collected from deionized laboratory-grade water daily on the same day as the sample measurement. Inner-filter correction (McKnight et al. 2001) was performed with UV absorption spectra data acquired from a diode array UV-Vis spectrophotometer (Hewlett Packard, Palo Alto, CA, USA) and a 1–5 cm quartz cell for an absorption range of 200–600 nm. All EEMs in this study were collected in ratio mode (signal from the sample normalized to the signal from the reference detector: S/R), which corrects the non-uniform output of the lamp over the excitation range (Cory et al. 2010). Sample EEMs were corrected for instrumental bias using the excitation and emission correction factor, then corrected for inner filtering...
effects, and the blank subtracted. The fluorescence character of organic matter in the two water sources could be represented by three main peaks: Peak A (terrestrial fulvic-like, ex 250 nm/em 450 nm), Peak C (terrestrial humic-like, ex 350 nm/em 450 nm), and Peak T (microbial protein-like, ex 275 nm/em 340 nm) (Coble 1996). The fluorescent intensities were normalized by the area under the water-Raman peak (ex 350 nm/em 368–450 nm), reporting data in Raman units (RU) (Stedmon et al. 2003). All samples were analyzed at least in duplicate and average values were reported. EEM correction and data processing were performed using Matlab (MATLAB R2016a, MathWorks) and DOM-Fluor Toolbox (Stedmon & Bro 2008). Statistical analyses (i.e., analysis of variance (ANOVA), paired t-test, and regression analysis) were performed using Stata IC 13.1 (StataCorp LP, College Station, TX, USA).

MIEX treatment resulted in minor pH increases of 0.07–0.12 units for surface water and 0.10–0.32 units for secondary effluent. Previous studies reported that the effect of pH on the fluorescence intensity of humic and fulvic-like substances was minor in the pH range of 6–8 (Patel-Sorrentino et al. 2002) and that no significant pH effect was observed for tryptophan-like fluorescence (Spencer et al. 2007). Thus, EEM spectra measurement in this study was performed at the native pH of the samples.

We chlorinated water samples and evaluated THMFP under uniform formation conditions (UFC) (Summers et al. 1996). The UFC correspond to the chlorination conditions that yield 1 mg/L free chlorine residual at pH 8 after 24-hour incubation at 20 °C in the dark. Chlorine residuals were measured by a DPD (N,N-diethyl-p-phenylenediamine) colorimetric method (Ha et al. 2004) using a Hach DR890 portable colorimeter. The samples with chlorine residuals of 1 ± 0.2 mg/L as Cl2 were selected for THM4 analysis (chloroform (Cl3CH), bromodichloromethane (BrCl2CH), dibromochloromethane (Br2CICH), and bromoform (Br3CH)). THM4 were extracted with methyl tert-butyl ether and analyzed on a Hewlett-Packard 5890 gas chromatograph with electron capture detector. We followed procedures published by the US EPA (EPA Method 551.1; US EPA 1995) with minor modifications. The following temperature program was used: oven held at 35 °C for 22 min, increased at 10 °C/min to 145 °C and held for 2 min, increased at 20 °C/min to 225 °C and held for 10 min, then increased at 20 °C/min to 260 °C and held for 5 min (Lyon et al. 2014). The injection volume was 2 μL (splitless), and after 0.5 minutes, the sample was split at a 1:1 ratio. The injection temperature was set at 117 °C and the detector temperature was 290 °C. All samples were analyzed in replicate.

### RESULTS AND DISCUSSION

#### Raw water characteristics

Water quality parameters of the raw water samples are summarized in Table 1. Both water sources had pH values close to neutral (6.85 and 6.95). The surface water had slightly lower DOC (6.55 mg-C/L) than the secondary effluent (7.17 mg-C/L). The secondary effluent had higher conductivity and TDN concentration, and lower UVA254 and SUVA values than the surface water. With similar DOC concentrations, high conductivity in the effluent indicated higher concentration of inorganic materials, which can also influence DBP formation (Krasner et al. 2008). The low SUVA of the secondary effluent sample is indicative of the non-aromatic and hydrophilic characters of EfOM, consistent with Krasner et al. 2008. Additionally, surface water had a lower FI value compared to effluent of (1.47 and 1.99, respectively), indicating that DOM in surface water was terrestrially derived, while EfOM was from microbial origin (McKnight et al. 2001). Therefore, these two source waters provide two distinctly different types of waters with which we can evaluate the applicability of EEMS to assess total THMFP (TTHMFP) and the ability of MIEX resin to reduce TTHMFP. Other important aspects of THM formation such as the effect of nitrate, nitrite, and bromide on THM formation have been well established in previous studies (Boyer & Singer 2005).

#### Removal of DOC and UVA254 and reduction of SUVA

Figure 1 illustrates changes in DOC, UVA254, and SUVA in raw water (0 BV) and samples treated with 500–5,000 BV

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Water quality of raw waters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>University Lake</td>
</tr>
<tr>
<td>Source</td>
<td>Surface water</td>
</tr>
<tr>
<td>Turbidity</td>
<td>3.34 NTUs</td>
</tr>
<tr>
<td>pH</td>
<td>6.85</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>6.55 ± 0.03 mg-C/L</td>
</tr>
<tr>
<td>Total dissolved nitrogen</td>
<td>0.43 ± 0.02 mg-N/L</td>
</tr>
<tr>
<td>UV254 absorbance</td>
<td>0.150 ± 0.01 cm⁻¹</td>
</tr>
<tr>
<td>Specific UV absorbance</td>
<td>2.29 ± 0.04 L/mg.m</td>
</tr>
<tr>
<td>Conductivity</td>
<td>116 ± 5.4 μS/cm</td>
</tr>
<tr>
<td>Fluorescence index</td>
<td>1.47 ± 0.01</td>
</tr>
</tbody>
</table>
MIEX treatment removed a substantial amount of UVA254 (37–53%) and DOC (28–44%) from the surface water samples, but less (13–33% for UVA254 and 8.5–19% for DOC) from the secondary effluent. This result agrees well with previous studies (Gan et al. 2013) that reported greater removal of DOC from samples with high SUVA, as the raw water SUVA of secondary effluent was 1.59 L/mg.m (with corresponding lower removals) and that of the surface water was 2.29 L/mg.m (with corresponding higher removals). MIEX treatment preferentially removed UV-absorbing material as indicated by higher removal of UVA254 (13–53%, Figure 1(a)) than of DOC (8.5–44%, Figure 1(b)), which resulted in lower SUVA values for the MIEX-treated water (Figure 1(c)). SUVA was reduced by MIEX treatment by 11–28% for surface water and 4.6–18% for secondary effluent. Since high SUVA waters are generally enriched in hydrophobic DOM (Leenheer & Croué 2005), this result is consistent with previous reports that MIEX preferentially removed hydrophobic over hydrophilic DOM (Boyer & Singer 2005).

For this study, the 500–5,000 BV range, equivalent to a resin dose range of 0.2–2 mL/L in a batch operation, covered a typical commercial resin dose of 600 BV, but is relatively low compared to previous batch studies. For example, Gan et al. (2013) used 8–12 mL/L MIEX for DBP control and reported 44–82% removal of DOC. With the continuous BV treatment, the low DOC removal reported in this study was as expected.

For both surface water and secondary effluent, increase in BV (i.e., the number of consecutive resin uses) resulted in a decline in removal efficiency of DOM, consistent with a study by Arias-Paic et al. (2016) that showed lower BV treatment removed more DOC. Figure 1(b) shows that in this study, for surface water, the 1,000 BV treatment achieved the lowest DOC concentration of 3.91 mg-C/L (40% removal) and the 3,000 BV loading achieved ∼4.6 mg-C/L (∼31% removal), while larger loadings (∼3,000 BV) had marginal differences in DOC removal. A similar trend was observed in secondary effluent, as fresh MIEX resin achieved the greatest removal efficiency at 500 BV (19% removal), and a constant DOC concentration of ∼6.0 mg/L (∼11% removal) was achieved at larger BVs. In other words, no detectable DOC removal by the resin occurred after a loading of 3,000 BV for surface water and 1,000 BV for secondary effluent. The same trend of resin saturation (i.e., inability to remove additional DOM) was observed for UVA254 reductions after 500–1,000 BV (Figure 1(a)). Overall, our results showed that MIEX treatment achieved greater UVA254 and DOC removal efficiency in surface water compared to secondary effluent. This greater removal can be explained by the difference in SUVA of these two water sources. DOC removal by MIEX resin was better for high SUVA values of surface water than low SUVA values of secondary effluent, similar to previous studies (Boyer et al. 2008; Singer et al. 2009). Surface water with high SUVA values has more hydrophobic DOM, whereas secondary effluents with low SUVA values tend to be dominated by hydrophilic acids and neutral DOM. Low DOC removal in waters with low SUVA values occurs because hydrophilic acids contain more carboxylic acid functional groups per milligram of carbon than hydrophobic acids, thereby occupying more sites on the resin (Singer et al. 2009) and resulting in low removal.
THMFP formation and its reduction by MIEX

Total THM (TTHM) is the sum of the four chloro- and bromo-substituted THM species (Cl\textsubscript{3}CH, BrCl\textsubscript{2}CH, Br\textsubscript{2}ClCH, and Br\textsubscript{3}CH). Illustrative effects of MIEX treatment on the formation potential of THM speciation in surface water and secondary effluent is shown in Figure 2(a) and 2(b), respectively. The dominant THM species formed during chlorination of surface water was Cl\textsubscript{3}CH, while secondary effluent had higher concentrations of BrCl\textsubscript{2}CH and Br\textsubscript{2}ClCH. Considering the similarity in DOC concentration (6.55 and 7.17 mg-C/L in surface water and secondary effluent, respectively), TTHMFP was significantly higher in secondary effluent (611 μg/L) than in surface water (256 μg/L). This result suggested the different characteristics of DOM present in surface water and secondary effluent played a major role in formation potentials. NOM in the surface water was rich in humic materials from terrestrial origin, while EfOM was comprised of both refractory substrates and soluble microbial products (SMPs) (Chen et al. 2003); thus, an aggregate DOC measurement was not an effective predictor of THMFP for water sources containing different DOM matrices (Krasner et al. 2008).

TTHMFP reduction by MIEX treatment is shown in Figure 2(c). MIEX treatment achieved greater TTHMFP reduction in surface water than in secondary effluent, which is consistent with the DOC and UVA\textsubscript{254} results shown in Figure 1. For surface water, 500 BV loading achieved 49% TTHMFP reduction, while increasing the loading from 500 to 1,000 BV did not cause a significant performance change; however, the reduction efficiency decreased substantially to 35% at a loading of 5,000 BV. For secondary effluent, 21% TTHMFP was achieved at a loading of 500 BV, but TTHMFP reduction efficiency dropped to 11% at 5,000 BV. Therefore, differences in DOM composition and source not only affected the speciation of THMs, but also influenced the DOM removal efficiency of MIEX treatments and consequently affected the reduction of TTHMFP.

Fluorescence excitation–emission matrices characterization

Surface water and secondary effluent samples before and after MIEX treatment were characterized using fluorescence excitation–emission matrices spectroscopy (EEMS). Representative EEM spectra obtained for raw and treated water samples are shown in Figure 3. Effects of Rayleigh and Raman scatter were removed for a better representative plot using DOMFluor Toolbox (Stedmon & Bro 2008). Locations of common re-occurring fluorescence components are designated as letter A, C, and T. For the visible region peaks, peak A (ex 250 nm/em 450 nm) in this study had similar positions to those reported for the terrestrial fulvic acids, and peak C (ex 350 nm/em 450 nm) resembled humic-like fluorophore. The position of peak T (ex 275 nm/em 340 nm), which can be seen distinctively in secondary effluent samples, resembled microbially derived protein-like fluorophore (Coble 1996). Therefore, we defined peak A as fulvic-like, peak C as humic-like, and peak T as protein-like DOM.

In all EEM spectra, the highest intensity was observed at peak A and moderate intensity was seen at peak C. Peak T, which is related to SMPs (Chen et al. 2003), was
observed more apparently in secondary effluent than in surface water. Comparing the EEM spectra of raw surface water (Figure 3(a)) to 500 BV-treated surface water (Figure 3(b)), loss in fluorescence intensity was observed visually, suggesting effectiveness in fluorescent DOM removal by MIEX treatment. Note that the color scales of Figure 3(a) and 3(b) are different from each other: maximum intensity was 3.0 RU for raw surface water and 1.4 RU for 500 BV-treated water. In the case of secondary effluent (Figure 3(c) and 3(d)), a qualitative difference in the EEM spectra was not observed, suggesting that loss in fluorescence intensity as a result of MIEX treatment was small and that MIEX was not as effective at removing fluorescent DOM from secondary effluent compared to surface water.

To analyze the EEM spectra quantitatively, we extracted fluorescence parameters including maximum fluorescence intensity at peaks A, C and T, as well as peak intensity ratios, FI, and fluorescence signal reduction caused by MIEX treatment. Maximum fluorescence intensity at peaks A, C, and T of untreated surface water and secondary effluent is presented in Figure 4(a). Both water samples had highest intensity at peak A (1.65 and 2.52 RU for surface water and secondary effluent, respectively), followed by moderate intensity at peak C (0.48 and 1.33 RU for surface water and secondary effluent, respectively) and the lowest intensity at peak T (0.26 and 0.77 RU for surface water and secondary effluent, respectively). The quantitative peak intensities confirmed the greater abundance of humic substances compared to protein-like DOM as observed qualitatively from the EEM spectra shown in Figure 3. This result is consistent with literature findings for most natural waters (Stedmon et al. 2006) and secondary effluent (Henderson et al. 2014). The fluorescence spectra observed for the surface water samples were similar to samples collected from the same treatment plant in 2013 for a separate study (Jutaporn et al. 2016).

Figure 4(b) depicts a fluorescence signal ratio of fulvic-like to protein-like DOM peaks (A/T) and fulvic-like to humic-like DOM peaks (A/C) in surface water and secondary effluent. Peak intensity ratio was previously used as a parameter to compare the removals of the related DOM fractions across different treatment processes (Baghoth et al. 2011). In this study, surface water had an A/T ratio of 6.4, which was significantly ($p < 0.01$) greater than the A/T ratio of 3.0 of secondary effluent. This result suggests...
that the two water sources were different in DOM composition and that the surface water was more humic-like, while the secondary effluent had greater abundance of microbial protein-like DOM. After MIEX treatment, the A/T ratio of the surface water decreased substantially from 6.4 to 3.9 at a loading of 1,000 BV, but increased to 4.7 as the BV loading increased from 1,000 BV to 5,000 BV, suggesting the MIEX treatment changed the DOM composition of the surface water. The surface water became less humic after MIEX treatment, as demonstrated by the decrease in A/T ratio compared to the raw surface water, but then humic content increased with continuous resin use. In contrast, the secondary effluent A/T ratios remained constant at ~2.9 across the range of BV treatments (changes were statistically insignificant). Thus, changes in the composition of DOM in the secondary effluent upon MIEX treatment were less apparent. Raw and treated waters had relatively unchanged A/C ratios at ~3.4 for surface water and at ~1.8 for secondary effluent, indicating no significant change in the ratio of fulvic-like to humic-like DOM by MIEX treatment.

FI values were extracted from EEM spectra of all samples and are shown in Figure 4(c). FI is the ratio of emission intensity (450 nm/500 nm) at 370 nm excitation (McKnight et al. 2001), and can serve as an index to distinguish sources of aquatic DOM. A previous study (McKnight et al. 2001) reported low FI values (~1.4) for terrestrially derived fulvic acids and high FI values (~1.9) for DOM from microbial origin. Typically, FI decreased as aromaticity of DOM increased.

In this study, raw surface water had a low FI value of 1.47 compared to a high FI value of 1.99 of raw secondary effluent (Table 1 and Figure 4(c)), indicating that DOM in surface water was terrestrial and humic, while secondary effluent was enriched with microbial DOM. This result agrees well with the nature of the samples in this study. Surface water was sampled from a lake in North Carolina, in which the DOM sources are dominated by terrestrial plants and soil organic matter. Secondary treated effluent was sampled from an activated sludge wastewater treatment system, where microbial activities are abundant.

With MIEX treatment, the FI value of treated surface water increased, resulting in a maximum FI value of 1.54 at a loading of 2,000 BV. This phenomenon aligns with our observation that MIEX resins remove terrestrially derived organic matter preferentially, resulting in a treated water with predominantly microbial DOM. For raw and treated secondary effluent, FI values remained unchanged at the range of 1.98–1.99, confirming results from the peak ratio analysis that the A/T and A/C ratios generally stayed the same in experiments with secondary effluent. Taken together, the peak ratio and FI results imply that MIEX treatment had a strong affinity for humic-like DOM in surface water, but did not cause significant change in the DOM composition in secondary effluent. It is also noteworthy that the standard deviations of FI values from replicate experiments were narrower than those of peak ratios or the intensity at different peaks themselves. Therefore, the precision in the FI measurement would allow its use as a reliable parameter to analyze or predict DOM sources from EEM spectra.

To investigate quantitatively the apparent preferential affinity of the MIEX treatments for humic-like DOM,
fluorescence signals were extracted from the EEM spectra of selected MIEX loadings. The reductions in fluorescence signals are depicted in Figure 5. MIEX treatment achieved greater fluorescence signal reduction with surface water (Figure 5(a)) compared to secondary effluent (Figure 5(b)). One-way ANOVA was performed to ascertain whether there were statistically significant differences in the levels of removal for humic-like and protein-like DOM. For surface water, preferential removal of humic-like materials by MIEX can be seen distinctively, as intensity reduction at peaks A and C was significantly greater ($p < 0.01$) than that at peak T. There was no significant difference ($p = 1.0$) between the fluorescence signal reduction at fulvic-like peak A and humic-like peak C in surface water by MIEX treatment for any loading tested. These results agree well with literature findings for most surface waters and DOM isolates (Arias-Paic et al. 2016; Jutaporn et al. 2016). In contrast to the surface water and in accordance with the qualitative analysis, preferential fluorescence reduction at peaks A and C was not observed in MIEX treatments with secondary effluent. There was no significant difference ($p = 0.12$) between the reduction of intensity at peak A, C, and T by MIEX treatment. This statistical analysis confirmed that MIEX treatment had preferential removal of humic substances only in surface water.

### Correlations between THMFP and surrogate parameters

To investigate relationships between THMFP and water quality parameters, all 42 samples with and without MIEX treatment were included in the linear regression analysis and the results are shown in a correlation matrix in Table 2. Results from triplicate experiments are represented by average values and standard deviation in

<table>
<thead>
<tr>
<th>R²</th>
<th>Peak A</th>
<th>Peak C</th>
<th>Peak T</th>
<th>FI</th>
<th>UVA$_{254}$</th>
<th>DOC</th>
<th>SUVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl$_3$CH-FP</td>
<td>0.10</td>
<td>0.002</td>
<td>0.01</td>
<td>0.12</td>
<td>0.94*</td>
<td>0.24</td>
<td>0.50</td>
</tr>
<tr>
<td>BrCl$_2$CH-FP</td>
<td>0.71*</td>
<td>0.92*</td>
<td>0.97*</td>
<td>0.99*</td>
<td>0.00</td>
<td>0.53</td>
<td>0.61</td>
</tr>
<tr>
<td>Br$_2$ClCH-FP</td>
<td>0.71*</td>
<td>0.93*</td>
<td>0.97*</td>
<td>0.98*</td>
<td>0.00</td>
<td>0.53</td>
<td>0.61</td>
</tr>
<tr>
<td>Br$_3$CH-FP</td>
<td>0.76*</td>
<td>0.96*</td>
<td>0.99*</td>
<td>0.95*</td>
<td>0.00</td>
<td>0.58</td>
<td>0.54</td>
</tr>
<tr>
<td>TTHMFP</td>
<td>0.87*</td>
<td>0.98*</td>
<td>0.96*</td>
<td>0.90*</td>
<td>0.03</td>
<td>0.72*</td>
<td>0.44</td>
</tr>
<tr>
<td>Peak A</td>
<td>1.00</td>
<td>0.90*</td>
<td>0.82*</td>
<td>0.60</td>
<td>0.74*</td>
<td>0.94*</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak C</td>
<td>1.00</td>
<td>0.98*</td>
<td>0.85*</td>
<td>0.01</td>
<td>0.74*</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Peak T</td>
<td>1.00</td>
<td>0.92*</td>
<td>0.01</td>
<td>0.64</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FI</td>
<td>1.00</td>
<td>0.02</td>
<td>0.42</td>
<td>0.71*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UVA$_{254}$</td>
<td>1.00</td>
<td></td>
<td>0.43</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>1.00</td>
<td></td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUVA</td>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.01 level (two-tailed).
The correlation coefficient ($R^2$) marked by an asterisk indicates a correlation that is significant at 0.01 confidence level ($p < 0.01$). There were significant correlations observed between formation potentials and UVA$_{254}$, DOC concentration, fluorescence intensity at peaks A, C, and T, and FI.

The correlations of formation potentials were substantially higher with fluorescence parameters ($R^2 = 0.87$–0.98) than with DOC, UVA$_{254}$, or with SUVA. The formation potential of chloroform (Cl$_3$CH-FP), the predominant THM species in the surface water, correlated well only with UVA$_{254}$ ($R^2 = 0.94$, Figure 6(a)). In contrast to earlier findings that UVA$_{254}$ was the most accurate TTHMFP precursor surrogate parameter among natural water sources (river, lake, and groundwater) (Pifer & Fairey 2014), TTHMFP in this study did not correlate with UVA$_{254}$ ($R^2 = 0.05$, $p > 0.01$). A contributing factor to the disparity between our study and Pifer & Fairey (2014) is that the two water sources used in this study, surface water and secondary effluent, contained substantially different DOM matrices as evidenced by the fluorescence parameters and THM formation discussed above. Based on FI values, the DOM in the surface water was from terrestrial sources (FI $\sim 1.4$), while the DOM in the secondary effluent (FI $\sim 1.9$) was enriched from microbial sources (McKnight et al. 2001). These two water sources also had different predominant THM species: Cl$_3$CH for surface water and BrCl$_2$CH for secondary effluent. Even though Cl$_3$CH correlated well with UVA$_{254}$ ($R^2 = 0.94$, Figure 6(a)), BrCl$_2$CH did not; therefore, a significant correlation between TTHMFP and UVA$_{254}$ was not found. Accordingly, our results suggested that UVA$_{254}$ is not a good surrogate parameter for TTHMFP for all waters. Furthermore, individual formation potentials of THM species did not correlate with DOC or SUVA (Table 2).

Figure 6 presents some selected regressions from Table 2. The linear fits in Figure 6 correspond to fits to the data from the two water sources combined. Results suggest that UVA$_{254}$ is a good indicator of Cl$_3$CH-FP ($R^2 = 0.94$), consistent with previous studies (Pifer & Fairey 2014; Yang et al. 2018). The correlation between TTHMFP and DOC was significant ($R^2 = 0.72$, Figure 6(b)), but was weaker than the correlations between TTHMFP and fluorescence parameters ($R^2 = 0.87$–0.98, Figure 6(c)). The correlation between TTHMFP and DOC in this study using surface water and effluent water was lower than a previous study using only water from drinking water treatment plants.

![Figure 6](https://iwaponline.com/wst/article-pdf/79/5/820/562117/wst079050820.pdf)

**Figure 6** | Regressions describing the relationships between (a) UVA$_{254}$ and formation potential of chloroform (Cl$_3$CH-FP), (b) DOC and formation potential of TTHM (TTHMFP), (c) fluorescence intensity at peaks A, C, and T and TTHMFP, and (d) DOC and fluorescence intensity at peaks A, C, and T. The regression lines correspond to fits to the data from both water sources combined.
The surface water and effluent data series in Figure 6(b) were also analyzed separately and the correlation between TTHMFP and DOC was \( R^2 = 0.98 \) for surface water and \( R^2 = 0.97 \) for effluent. The difference in correlations of the individual water sample types compared to the aggregate data suggests that DOC can be a good indicator of TTHMFP for individual types of water sources, but not for a group of water sources with different DOM characteristics.

Of the fluorescence parameters, the intensity of humic-like peak C was most strongly correlated to TTHMFP (\( R^2 = 0.98 \), Figure 6(c)), suggesting this parameter was the strongest fluorescence-based predictor for TTHMFP. Peak T (protein-like) and peak A (fulvic-like) also correlated with TTHMFP with slightly lower \( R^2 \) values of 0.96 and 0.87, respectively. These strong correlations between TTHMFP and fluorescence parameters support a previous finding that fluorescence spectroscopy is a better DOM characterization tool than UV absorbance because it is more sensitive to organic matter than are UV measurements (Leenheer & Croué 2005). A previous study also reported that TTHMFP correlated strongly with humic-like DOM (Yang et al. 2015). The results of this study confirm that fluorescence is a useful tool in tracking DBP formation during chlorination of both surface water and secondary treated effluent and that humic-like peak C was the most accurate TTHMFP precursor surrogate parameter.

To better understand differences in DOM composition based on fluorescence surrogates, linear correlations between DOC concentration and fluorescence intensities at peaks A, C, and T were identified (Figure 6(d)). The two terrestrial peaks were good predictors of DOC, with peak A the strongest (\( R^2 = 0.94, p < 0.01 \)) and peak C moderate (\( R^2 = 0.74, p < 0.01 \)). The correlation between DOC concentration and peak T was the weakest (\( R^2 = 0.64, p = 0.02 \)). These correlations suggest that the humic fraction of DOM makes up the majority of DOC, which is consistent with a previous study (Leenheer & Croué 2005) that found humic substances (humic and fulvic acid) typically comprise ~50% of the total DOM composition. While terrestrially and bacterially derived DOM may have contributed to the DOC in surface water, EfOM is comprised of SMPs and refractory materials (Chen et al. 2005), so EfOM is derived from different sources than DOM in surface water (Krasner et al. 2008). DOC is an aggregate measurement of carbon content and not all fractions of DOC contribute to THM formation equally, which explains the lower correlation coefficients of TTHMFP with DOC. Fluorescence characterization has an advantage over an aggregate DOC measurement because it is more sensitive and selective to DOM fractions and the individual parameters can be used to monitor DOM and TTHMFP for a diverse group of waters.

Correlations in Table 2 should be evaluated together with plots (Figures 2 and 6) of each data set. For example, the correlation between BrCl₂CH-FP and peak A was influenced by lower reduction of BrCl₂CH-FP (5–37%) compared to those of Cl₃CH-FP (37–50%) (Figure 2(b)). When regression analyses were conducted using both water sources combined as a data set, the \( R^2 \) of 0.71 (Table 2) was calculated from the line drawn between two groups of cluster samplings (Figure 7(a)). Even though this correlation was significant (\( p < 0.01 \)), peak A only moderately predicted BrCl₂CH-FP for the combined data set. A similar trend was noticed with the other Br-THM species and fluorescence-peak correlations. When we conducted separate correlation analyses for the surface water and effluent water, we discovered the significance of the correlation from the combined data set was driven by the strong
correlation between peak A and BrCl2CH-FP for effluent samples ($R^2 = 0.98$). Peak A and BrCl2CH-FP were only moderately correlated for surface water ($R^2 = 0.75$); therefore, peak A may not be an appropriate predictor for Br-THM precursors in all water sources. We performed a similar analysis for the combined data set to calculate a correlation coefficient for TTHMFP and FI values ($R^2 = 0.90$, $p < 0.01$) (Figure 7(b)). Despite the promising correlation, the FI values varied marginally with MIEX treatment (1.47–1.54 for surface water and 1.97–1.99 for effluent), making FI values of each water source clustered together. Thus FI values are poor TTHMFP predictors for two water sources combined, even though they are reliable parameters to analyze or predict DOM sources. A more diverse set of water samples containing a variety of DOM sources is needed to investigate whether FI values could be used to assess TTHMFP.

CONCLUSIONS

We investigated the applicability of fluorescence EEMS to assess TTHMFP and the ability of MIEX resin to reduce TTHMFP. We drew the following conclusions from our study:

- MIEX resin removed DBP precursors more effectively from surface water than from secondary effluent, but also changed the final DOM composition of the treated surface water. We successfully treated surface water with up to 3,000 BV of MIEX resin, which suggests that MIEX resin can be regenerated less frequently than existing practices. Water treatment plants can use this information to help them assess whether MIEX resins are an affordable and appropriate treatment technology for their source water.

- Although UVA254 has been used successfully in the past as a surrogate measurement for TTHMFP, our data show it was not a good surrogate parameter for TTHMFP of surface water and secondary effluent combined; therefore, UVA254 is not a good surrogate parameter for all water types of different DOM origins.

- Fluorescence parameters, including fluorescence intensity of humic-like peak C, fulvic-like peak A, and protein-like peak T, can potentially be used as surrogate parameters to assess the TTHMFP of different raw and MIEX-treated source waters.

Our results demonstrate that fluorescence EEMS can be used as a tool to assess the TTHMFP of raw water sources that contain DOM from different origins and to assess the efficacy of MIEX to reduce TTHMFP.

ACKNOWLEDGEMENT

This work was partially supported by Ixom Watercare Inc. The Royal Thai Office of the Civil Service Commission supported Panitan Jutaporn through a Royal Thai Government Scholarship. M. D. Armstrong acknowledges funding from the US EPA-UNC-CH Cooperative Training Agreement CR-83591401-0. The authors thank Dr Rose Cory and Katherine Harrold for their assistance in fluorescence EEMS analysis, Dr Philip Singer and Ariel Atkinson for their suggestions in TTHMFP analysis, and Dr Miguel Arias for helpful discussions about MIEX experiments. We also thank our collaborators at the Orange Water and Sewer Authority (Carrboro, NC) who facilitated and helped with water samples collection.

REFERENCES


Gan, X., Karanfil, T., Kaplan Bekaroglu, S. S. & Shan, J. 2015 The control of N-DBP and C-DBP precursors with MIEX®. Water


