Identification of humic acid-like and fulvic acid-like natural organic matter in river water using fluorescence spectroscopy
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ABSTRACT

Identifying the extent of humic acid (HA)-like and fulvic acid (FA)-like natural organic matter (NOM) present in natural water is important to assess disinfection by-product formation and fouling potential during drinking water treatment applications. However, the unique fluorescence properties related to HA-like NOM is masked by the fluorescence signals of the more abundant FA-like NOM. For this reason, it is not possible to accurately characterize HA-like and FA-like NOM components in a single water sample using direct fluorescence EEM analysis. A relatively simple approach is described here that demonstrates the feasibility of using a fluorescence excitation-emission matrix (EEM) approach for identifying HA-like and FA-like NOM fractions in water when used in combination with a series of pH adjustments and filtration steps. It is demonstrated that the fluorescence EEMs of HA-like and FA-like NOM fractions from the river water sample possessed different spectral properties. Fractionation of HA-like and FA-like NOM prior to fluorescence analysis is therefore proposed as a more reasonable approach.

Key words | Natural organic matter (NOM), fluorescence spectroscopy, fulvic acid-like NOM, humic acid-like NOM

INTRODUCTION

Characterization of natural organic matter (NOM) present in natural waters is essential for understanding and controlling some pertinent water-related problems such as membrane fouling, disinfection by-product (DBP) formation and undesirable biological growth experienced in water treatment and distribution systems. For example, individual and combined effects that result due to the interplay between different NOM fractions can lead to different membrane fouling behaviour (Jermann et al., 2007). Also, the potential of DBP formation for different NOM fractions varies considerably (Marhaba et al., 2000). Therefore, knowledge on the major NOM fractions present in water would enable the design and implementation of more efficient treatment processes and control procedures that can mitigate the negative effects of NOM in water treatment and distribution systems.

Major NOM fractions present in natural water are humic substances (HS), protein-like and polysaccharide-like materials, with HS being the most significant component (Thurman, 1985). HS are operationally divided into humic acids (HA), fulvic acids (FA) and humus (Huck, 1999). FA and HA contain both aromatic and aliphatic components with mainly carboxylic, phenolic, alcoholic hydroxyl and keto functional groups (Thurman, 1985). Due to the differences in the NOM matrix effects between different water sources, the characteristics of FA and HA and their make-up by these functional groups are very much source/location dependent and therefore they are generally identified as FA-like and HA-like matter. The aquatic HS are considered to contain largely (FA)-like matter and comparatively smaller amounts of (HA)-like matter. This is because the lower carboxylic content in HA-like matter lowers its solubility and therefore most natural waters contain 5 to 25 times more FA-like matter than HA-like matter (Thurman, 1985). In membrane-based processes, HA-like NOM
is known to affect membrane charge and cause more fouling than FA-like NOM. This due to the higher capacity of HA-like NOM to absorb on to surfaces and inside the pores of the membranes compared to FA-like NOM (Jucker and Clark, 1994). Therefore, increased levels of HA-like matter in natural waters could contribute to high fouling situations than FA-like matter (Kulovaara et al., 1999). In addition, the potential for DBP formation by aquatic HA-like NOM has also been found to be higher than FA-like NOM (Marhaba et al., 2000). Thus, characterization of HS present in natural water to understand its composition in terms of HA- and FA-like components would be beneficial for monitoring and control of NOM related membrane fouling and DBP formation.

This study focused on characterizing HA- and FA-like NOM in water as a method of identifying the presence of HA- and FA-like NOM present in water. For this purpose, a fluorescence excitation-emission matrix (EEM) approach was used as it is able to capture specific fluorescence features that correspond to HS and protein-like materials (Coble et al., 1990; Baker, 2001; Chen et al., 2003; Her et al., 2003; Sierra et al., 2005; Hudson et al., 2008; Henderson et al., 2009). Unlike other available NOM characterization methods (Huber et al., 1992; Her et al., 2003; Gray et al., 2007), fluorescence spectroscopy is able to differentiate the major NOM fractions and is suitable for performing rapid, direct and accurate analysis with high instrumental sensitivity (Coble et al., 1990; Peiris et al., 2008).

The detection of HA-like fluorescence in natural water is more challenging. In most studies the HA-like NOM exhibits a weak fluorescence signal with a peak that appears more or less as a shoulder to the FA-like NOM fluorescence peak (Mobed et al., 1996; Baker, 2001; Sierra et al., 2005; Hudson et al., 2008). Others have observed no significant differences between fluorescence EEMs of aquatic FA-like and HA-like NOM (Alberts and Takács, 2004). These observations suggest that FA- and HA-like fluorescence in most cases overlap, making an accurate identification of HA-like NOM in the presence of FA-like NOM difficult. This difficulty is mainly due to the comparatively weaker fluorescence signals of the less abundant HA-like components which are overshadowed by the stronger fluorescence signals of more abundant FA-like components. Therefore, direct fluorescence EEM measurement of natural waters may not be suitable for identifying the extent of HA-like NOM in the presence FA-like NOM in natural water.

For these reasons, in this study, fluorescence EEMs of the HA- and FA-like NOM were obtained by fractionating the HA- and FA-like components of NOM from river water using pH changes and filtration. The approach presented here would be useful for those who are interested in a simple approach for characterizing HA- and FA-like matter present in natural waters.

**METHODS**

**Sample Preparation**

Grand River water (GRW) (Southwestern Ontario, Canada; collected on July 05, 2009) was filtered through a 0.45 µm GN-6 Metricel® type 47 mm diameter disc filter (Pall Corporation, Ann Arbor, MI; Lot #: 73056) to remove particulate matter. The pH of this filtered GRW was ∼8.2. The pH of the GRW (∼1L) was then adjusted below pH = 1 by adding high purity hydrochloric acid (1 mol/L HCl, 99.999%). At this pH, it is expected that HA-like NOM in the water sample should precipitate (Aiken, 1985; Ghabbour et al., 1994). The pH adjusted water sample was filtered again through a 0.45 µm filter to remove the precipitate. The pH of this filtered water sample was adjusted back to the initial pH level of pH ∼8.2 using high purity NaOH (1 mol/L, 99.998 %). This filtered water sample can be expected to contain the FA-like fraction of the HS present in GRW (Aiken, 1985; Ghabbour et al., 1994). The precipitate collected on the filter membrane was carefully scrapped from the surface of the membrane and dissolved in 50 ml of Milli-Q (Millipore) water (resistivity = 18.2 MΩ cm). This involved three steps: (i) scrapping the membrane surface to remove the precipitated matter and dissolving in 30 ml of Milli-Q water, (ii) pouring an additional 20 ml of Milli-Q water over the membrane surface and into the 30 ml solution with precipitated matter to capture any loosely attached materials on the membrane surface and (iii) mixing the resulting 50 ml solution of precipitated matter using a laboratory vortex mixer. The pH of this dissolved precipitate was adjusted back to pH ∼8.2. During all pH adjustments the addition of HCl acid and NaOH were maintained to a minimum (only a few drops) to minimize the dilution of water samples. The sample preparation method is illustrated in Figure 1. The above procedure was performed in triplicate. As shown, three sets of samples resulted and each sample set included: (i) FA-like NOM solution at pH ∼8.2; (ii) HA-like NOM extract solution at pH < 1 and (iii) HA-like NOM extract solution at pH ∼8.2. These samples were analyzed by fluorescence spectroscopy.
In addition, fluorescence EEM of Aldrich humic acid (AHA) obtained from Sigma Aldrich Inc., (St. Louis, MO; Lot #: 100K0219, Alginic acid, sodium salt) was used to validate the existence of HA-like matter in the HA-like NOM extract of GRW.

**Fluorescence Analysis**

The fluorescence EEMs were recorded using a Varian Cary Eclipse Fluorescence spectrophotometer (Palo Alto, CA) collecting 301 individual emission intensities (Em: 300–600 nm) at sequential 10 nm increments of excitation wavelengths between 250 and 380 nm. Instrument parameters: photomultiplier tube (PMT) voltage = 800 V, scan rate = 600 nm/min and excitation/emission slit width = 10 nm each were maintained during the fluorescence signal acquisition. A more detailed description of the fluorescence analysis procedure and the selection of the spectrophotometer parameter settings used in this study for obtaining reproducible fluorescence signals, especially for low NOM concentrations levels will be found in Peiris et al., (2008; 2009). Corrections for inner filter effects were not applied as inner filtering effects are not expected to be significant at the low concentration levels examined in this study (Hudson et al., 2008). Also, inner filtering effects did not have a significant impact on the fluorescence intensity readings of typical GRW concentration levels (3.9–6.5 mg/L of dissolved organic carbon (DOC) as previously reported (Peiris et al., 2009) and examined in this study. To eliminate water Raman scattering and to reduce other background noise, fluorescence spectra for Milli-Q water, obtained under the same conditions, were subtracted from all spectra. During the course of the fluorescence analyses, the change in Raman scattering peak intensities recorded for Milli-Q water at Ex/Em ~ 348 nm/396 nm was less than 1%, confirming that there were no significant fluctuations in the performance of the spectrophotometer lamp or other hardware. The water samples were maintained at room temperature (~25°C) during the analyses.

**RESULTS AND DISCUSSION**

Typical spectral features and characterization of GRW

The fluorescence EEM of GRW water used in this study shows a peak (α) at the excitation wavelength (Ex) and the emission wavelength (Em) combination: Ex/Em = 520 nm/415 nm (Figure 2), which corresponds to the range reported for FA-like NOM in water (Coble et al., 1990; Sierra et al., 2005). Another secondary peak (β) which also corresponds to HS (Sierra et al., 2005; Peiris et al., 2008) appears to be present in the form of a shoulder to the main peak (α) around Ex/Em = 270 nm/460 nm (Fig. 2). The presence of HS in GRW that is associated with these spectral features was also independently confirmed by the Liquid Chromatography – Organic Carbon Detection (LC-OCD) chromatograph analysis in a previous study (Peiris et al., 2008). The HS in GRW can be expected to comprise predominantly of FA-like matter compared to HA-like matter as reported in other natural waters (Huck, 1999; Sierra et al., 2005). The deviations of the fluorescence EEM contours seen in the region indicated by δ (Ex/Em = 280 nm/330 nm) is due to the presence of protein-like NOM in the water; fluorescence EEM peak around the same region (δ) has been previously observed for protein-like substances (Baker, 2001; Chen et al., 2003; Her et al., 2003). The existence of this protein region (δ) however does not appear to be a significant fluorescence peak due to the very low
concentration levels of the protein-like substances (<0.5 mg C/L as estimated using LC-OCD measurements) present in the GRW. The light scattering regions (first order Raleigh scattering region and second order Raleigh scattering region) observed in the fluorescence EEM are also important areas that provide information related to the particulate/colloidal matter present in water (Peiris et al., 2010b). The DOC content and the turbidity of the GRW used was 4.1 mg/L and 1.6 NTU, respectively.

Fluorescence EEMs of FA-like and HA-like NOM in GRW

The fluorescence EEM of FA-like NOM (Figure 3a) demonstrates similar fluorescence spectral characteristics to that of GRW. This indicates that GRW contained predominantly FA-like NOM as expected in river water. However, Figure 3a also demonstrates a shift (about 10 nm) in the peak (α) towards the higher excitation wavelength (red shift) as illustrated by peak (α'). A red shift is generally related to the
increase of carbonyl, hydroxyl, alkoxyl, amino, and carboxyl groups in the structures of fluorophores (Chen et al., 2002; Uyguner and Bekbolet, 2005). Since FA-like NOM contains higher levels of carbonyl content than HA-like NOM (Thurman, 1985), it is reasonable to conclude that this red shift is attributed to the separation of FA-like NOM from GRW. The fluorescence EEM of HA-like NOM extract at pH < 1 (Figure 3b), on the other hand, does not seem to contain fluorescence spectra related to HS-like matter that is seen in Figure 2. This can be explained by the poor solubility of HA at pH < 1 (Aiken, 1985) resulting in little or weak fluorescence.

When the pH of the HA-like NOM extract was increased to the normal pH level of GRW (pH ~ 8.2), a new fluorescence peak (α′) appeared at Ex/Em = 296 nm/400 nm (Figure 3c). This peak fell within the general fluorescence region related to the HS-like NOM in GRW as demonstrated in Figure 2 (i.e. between peak (α) and (β)). It also has a closer resemblance to the fluorescence EEM of Aldrich humic acid (AHA) obtained under the same conditions (Figure 4) with respect to the excitation wavelength at which the EEM peak is situated. The fluorescence EEM peak of HA-like NOM extract of GRW is situated at Ex = 296 nm and that of AHA at around Ex = 295 nm (Figure 3c and Figure 4). The difference in the EEM peak position of AHA and HA-like NOM in GRW on the emission scale is expected as similar differences were also observed in other studies in which AHA and HA extracts of river water were used (Parlanti et al., 2000; Sierra et al., 2005). The existence of peak (α′) at pH ~ 8.2 but not at pH < 1 can be related to the HA-like NOM being completely re-dissolved at higher pH levels and hence its fluorescence properties.

In addition, the presence of a clear protein-like fluorescence EEM peak in the HA-like NOM fraction indicates that some of the protein-like NOM present in GRW was also present in this HA-like NOM extract (Figure 2, Figure 3b and 3c). The spectral properties related to the protein-like NOM is also present in the fluorescence EEM of the FA-like NOM fraction of GRW (δ-region in Figure 3a). Therefore, it appears that protein-like NOM in GRW is soluble at lower pH conditions that were used during the fractionation of HS. Nevertheless, since the fluorescence EEM peak related to protein like NOM, is situated reasonably outside the fluorescence EEM region of HS, the presence of protein-like NOM in both HA-like and FA-like fractions did not impede the detection of HA-like and FA-like fluorescence signals. Low levels of protein content present in GRW also appears to have contributed positively to the detection of HA- and FA-like fractions. With higher protein levels in water it is possible to have higher fluorescence intensities around (δ) region that could interfere with detection of HA- and FA-like NOM. However, protein concentration levels capable of such interferences, is unlikely to be present in natural river water.

**Significance of this approach and potential for future research**

This study demonstrates that fluorescence signals related to HA-like NOM extracts of GRW are located within the general fluorescence region related to HS-like NOM. Therefore, if un-fractionated natural water were analysed using fluorescence EEM, one would not expect to be able to clearly identify the existence of HA-like NOM as the unique fluorescence properties related to HA-like NOM is overshadowed by the fluorescence signals of the more abundant FA-like NOM. This has been the case in many fluorescence-based analyses as described in the introduction. Since the predominant component of HS present in natural water is FA-like matter, fluorescence spectral properties of natural water is largely associated with fluorescence properties of the more abundant FA-like NOM. Due to these reasons, one cannot make an accurate characterization of the HA-like NOM present in natural water samples by direct fluorescence analysis. In this context, fractionation of natural water samples prior to fluorescence analysis, as proposed in this study, could provide more information on the content of HA-like and FA-like NOM in natural water. The approach presented here is relatively simple to perform compared to conventional NOM isolation protocols that involve complicated and time consuming acidification, XAD fractionation and concentration steps (Leeher et al., 2000; Croué 2004). Hence, it could be useful for those with an
interest in the effect of HA-like and FA-like NOM in water treatment processes and distribution systems.

The NOM fractionation procedure presented here was performed in triplicate and all fluorescence EEM measurements of the fractionated samples in triplicate showed similar fluorescence peak intensities for $\alpha$, $\alpha'$, $\beta$ and $\delta$ regions (<5% difference in all cases, results not shown). This indicates that this procedure which involves, acidification, filtration, precipitation, and physical removal of precipitated material (i.e. manual scrapping) steps can be performed to obtain repeatable results. Similar observations were also made with different GRW water samples collected at different time periods than the one that was used in this study (results not shown). During the proposed NOM fractionation procedure, absorption of precipitated matter onto the filter surface is possible to a certain extent, but this is not believed significant compared to the amount of recovered matter by manual scrapping. The co-precipitation of FA-like NOM with HA-like NOM can also be considered insignificant due to the absence of FA-like NOM related fluorescence regions in the fluorescence EEM of the dissolved precipitated matter extract at pH < 1 (Figure 3b).

CONCLUSIONS

In this study, we have performed fluorescence EEM-based characterization of HA-like and FA-like NOM in GRW by fractionation using a combination of pH adjustments and filtration steps. The HA-like NOM fraction of GRW water exhibited a fluorescence EEM peak that lies within the general fluorescence region related to HS-like NOM. This highlights the challenges of performing direct fluorescence-based analysis when interested in detecting HA-like NOM in natural water as the unique fluorescence properties related to HA-like NOM are overshadowed by the fluorescence signals of the more abundant FA-like NOM. A simple pH-based separation of HA- and FA-like NOM fractions is proposed followed by fluorescence EEM analysis that could be helpful for obtaining more accurate qualitative information on the HA- and FA-like NOM content in natural water.

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