Acquiring reproducible fluorescence spectra of dissolved organic matter at very low concentrations

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ABSTRACT

A method that would allow for fast and reliable measurements of dissolved organic matter (DOM), both at low and high concentration levels would be a valuable tool for online monitoring of DOM. This could have applications in a variety of areas including membrane treatment systems for drinking water applications which is of interest to our group. In this study, the feasibility of using fluorescence spectroscopy for monitoring DOM at very low concentration levels was demonstrated with an emphasis on optimizing the instrument parameters necessary to obtain reproducible fluorescence signals. Signals were acquired using a cuvette or a fibre optic probe assembly, the latter which may have applications for on-line or in-line monitoring. The instrument parameters such as photomultiplier tube (PMT) voltage, scanning rate and slit width were studied in detail to find the optimum parameter settings required. The results showed that larger excitation and emission slit widths were preferred, over larger PMT voltage or lower scanning rates, to obtain reproducible and rapid measurements when measuring very low concentration levels of DOM. However, this approach should be implemented with caution to avoid any reduction of the signal resolution.

Key words | dissolved organic matter (DOM), drinking water treatment, fluorescence spectroscopy, humic substances, measurement noise

INTRODUCTION

Interest in dissolved organic matter (DOM) is increasing in the area of water treatment, particularly as it relates to membrane fouling, monitoring and control of disinfected by-products and issues arising due to interactions with other environmental contaminants (Mobed et al. 1996; Marhaba & Kochar 2000). Most methods available for characterizing DOM rely on traditional analysis techniques such as light absorptivity, dissolved organic carbon (DOC), aromaticity and fractionation using XAD resins although characterization is difficult due to the complex structural aspects of DOM (Her et al. 2003). Some studies have been performed on molecular weight (MW) fractions of DOM using nuclear magnetic resonance (13C NMR), pyrolysis-GC/MS, IR and thermogravimetric methods (Wilson et al. 1999). Aside from the fact that these methods are time consuming, expensive, complex and require operator expertise (Her et al. 2003) they are also not amenable to on-line or in-line applications for monitoring DOM.

In this context, fluorescence spectroscopy is becoming an increasingly popular method for characterizing DOM due to the requirement for minimal sample pre-treatment and preparation, ability to interpret DOM fluorescence properties, high instrumental sensitivity and the non-destructive nature of the technique (Peiris et al. 2008). Chen et al. (2003) identified various fluorescence spectroscopic techniques, out of which, the emission-excitation matrix (EEM) analysis method was identified as providing an overall view of all features of DOM existing within a selected spectral range (Sierra et al. 2005). This mode of fluorescence data acquisition has been used satisfactorily in
water analysis (Her et al. 2005; Sierra et al. 2005) and, with these advantages, is promising as an on-line or in-line monitoring approach (Peiris et al. 2008).

The DOM content in drinking water is generally modest with the most significant component being humic substances (HS) and some minor levels of polysaccharides and protein related substances. HS may represent up to 90% of the DOM with the concentration varying from 0.5 to 30 mg-C/l in streams and rivers (Thurman 1985). Especially where membrane treatment methods are being considered, the DOM content at the permeate side can be very low resulting in challenges associated with the characterization of the DOM content in the permeate. In such cases, most of the analytical techniques described above require that the DOM content be concentrated by means of extraction or other physical pre-treatment methods prior to analysis. In this paper, we have performed a comprehensive study on the instrument parameters that are necessary in order to obtain reproducible fluorescence signals for DOM at very low concentration levels. The main emphasis was to obtain reproducible spectra at low concentration levels for application in areas such as characterizing the DOM matter at the permeate side for a membrane-based water treatment process. Even though fluorescence spectrophotometer literature, including instrument manuals, provides generally useful information on how the instrument parameters may be changed to obtain reproducible fluorescence readings, manipulation of these instrument parameters, especially at very low concentration levels, has been rarely demonstrated. This may explain in part the reason for limited direct applications of fluorescence spectroscopy in the characterization of very low DOM concentration levels such as the levels found in nanofiltration (NF) permeates. In this context by using the optimum instrument parameters settings determine in this study, we have managed to characterize DOM in the NF permeate of drinking water (Peiris et al. 2008). These findings also have value in developing a fluorescence-based sensor for an on-line approach for monitoring DOM content in drinking water treatment applications. For these reasons, this paper will serve as a useful reference for those interested in developing fluorescence spectroscopy as a tool to monitor low concentration DOM, for example in post water treatment strategies.

**METHODS**

**Water samples and preparation**

Water samples were obtained on March 6th, 2007 from the Grand River in Kitchener, Ontario, Canada. Grand River Water (GRW) samples were filtered using a 0.45 μm Nylon Acrodisc type filter to remove any particulate material present. The samples were then diluted to different dilution levels using Milli-Q (Millipore) water as the dilution medium. Different dilution levels spanning from no dilution to dilutions up to 10% of the original strength of the GRW. The value “10% dilution level” was selected to match the dissolved organic carbon (DOC) content of the permeate, when GRW was filtered through a nanofiltration (NF) system currently in use. During our preliminary studies, permeate with ~0.7 mg-DOC/L was obtained from GRW with an average 6.7 mg-DOC/L. The pH of the GWR was ~8.2 so the pH of all diluted samples was adjusted to ~8.2 using high purity 0.1 M HCl acid (99.999%) or 0.1 M NaOH (99.998%).

**Fluorescence analysis**

Fluorescence excitation emission matrices (EEMs) of each sample were acquired using a Varian Cary Eclipse Fluorescence Spectrofluorometer (Palo Alto, CA) at ~25°C. A Peltier multicell holder, which can accommodate cuvettes, and a Fluorescence Remote Read Fibre Optic Probe coupled to an Eclipse Fibre Optic Coupler with a 20 mm fluorescence probe tip, were used for signal acquisition. UV-grade polymethylmethacrylate cuvettes with four optical windows were used for cuvette-based analysis. The excitation and emission ranges used were 280–380 nm and 350–600 nm, respectively which fall in most reported ranges for HS (Chen et al. 2003; Her et al. 2003; Sierra et al. 2005). Fluorescence emission spectra were obtained at different photomultiplier tube (PMT) voltages (V), scanning rates and emission and excitation slit widths. To eliminate Raman scattering due to water and to reduce other background noise, all spectra were subtracted from the spectra for Milli-Q water obtained under the same conditions. Data processing was performed using Matlab 7.3.0 software (The Mathworks Inc., Natick, MA). Analysis of the reproducibility of the spectra was based on the measurement...
noise (or % error with respect to signal intensity) which was calculated using error at a 95% confidence level of the peak intensity measurement for all dilution levels.

RESULTS AND DISCUSSION

DOM in surface and ground water consists of a variety of organic compounds with various fluorophores that are responsible for producing unique spectral fingerprints (Sierra et al. 2005). HS and protein-related substances are the primary sources of fluorescence in DOM with HS being the dominant fluorescent component. However, with the low concentration levels of HS found in drinking water treatment applications, the weak fluorescence signals are compromised due to large measurement noise. This issue becomes more significant for on-line measurements acquired using fibre optic probes as signal strength in this approach is further attenuated in the fibre optic system. As a result, off-line measurements are often obtained using cuvettes which allow for higher signal strength. To optimize signal acquisition we systematically examined the effect of three primary variables on signal strength and measurement noise, something which has not been previously reported in the literature. These variables included PMT voltage, scanning rate and slit width. Raman scattering peak intensities of Milli-Q water, recorded with the different instrument parameter combinations examined, are also reported to facilitate replication of the methods described in this study.

Figure 1  | Fluorescence EEMs of GRW obtained with (a) cuvettes, (b) fibre optic probe. Fluorescence EEMs of GRW diluted up to 10% of the original strength obtained with (c) cuvettes and (d) fibre optic probe. All measurements were performed at scanning rate of 600 nm/min, PMT voltage = 800 V and excitation and emission slit widths of 5 nm each.

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The fluorescence EEMs of GRW (undiluted) and GRW diluted up to 10% of the original strength are shown in Figure 1a–d respectively. These EEMs were obtained at a scanning rate = 600 nm/min, PMT voltage = 800 V and excitation and emission slit widths = 5 nm each with cuvettes (Figure 1a and c) and fibre optic probe (Figure 1b and d). The EEM peaks are located at excitation wavelength (Ex) of approximately 330 nm and emission wavelength (Em) between 415–435 nm, in the range reported for HS (Chen et al. 2003; Her et al. 2003; Sierra et al. 2005). In spite of the general spectral similarities in the EEMs obtained with cuvettes and the fibre optic probe in terms of peak positions, there is however significant differences in terms of peak intensities and the signal quality as seen in Figure 1a–d.

In this study, the weak fluorescence properties of very low concentrations of HS demanded instrument settings that are different from the settings needed for undiluted water samples. Our preliminary studies with PMT voltage in the medium range (600 V), excitation and emission slit widths at 5–10 nm each did not give reproducible spectra, especially for highly diluted samples acquired either with cuvettes or the fibre optic probe. When the fibre optic probe was used to acquire signals from highly diluted samples the problem was more significant; virtually no signal other than noise was observed in some instances (results not shown). This can be explained due to the loss of energy that occurs in the fibre optic assembly when in operation (Lewzey 2007, personal communication). Due to this reason the results presented here were acquired with PMT voltages of 800 V (Figure 1) and higher. From the EEM spectra obtained with the given instrument settings in Figure 1, it is clear that the fibre optic probe produced noisier spectra. At low concentrations both signal acquisition methods were hampered by significant levels of measurement noise (Figure 1c and d; Table 1). When the signal was acquired using the fibre optic probe, % error ranged from 11.6–34.8%, increasing with increasing dilution up to 10% GRW. Error was reduced when the signal was acquired using cuvettes with a range from 3.6–28.2%, increasing with increasing dilutions up to 10% GRW.

### PMT Voltage

Since the fluorescence EEM spectra of GRW, diluted up to 10% of its original strength, was not clear at PMT voltage = 800 V, the PMT voltage was further increased up to 1,000 V in order to obtain a much higher signal strength. The scanning rate, and excitation and emission slit widths were unchanged. This resulted in generally higher EEM peak intensities for highly diluted samples, compared to the spectra acquired at 800 V (Table 2). EEM spectra obtained with the fibre optic probe however remained fuzzy with no clearly visible peak as in Figure 1d (results not shown).

Even though higher PMT voltages contributed to higher signal strengths in all samples measured, the error associated with the measurements increased significantly (Table 2). This observation is consistent with the theoretical background of fluorescence spectroscopy and other observations made elsewhere (Casado-Terrones et al. 2007).

### Table 1 | Measurement error observed with the fibre optic probe vs. cuvettes for different dilutions of GRW at PMT = 800 V, scanning rate of 600 nm/min and excitation and emission slit widths of 5 nm each

<table>
<thead>
<tr>
<th>% GRW</th>
<th>Probe (PMT = 800 V) Peak intensity (a.u) at Ex/Em: 330/415 nm</th>
<th>Error at 95% confidence</th>
<th>% Error</th>
<th>Cuvettes (PMT = 800 V) Peak intensity (a.u) at Ex/Em: 330/430 nm</th>
<th>Error at 95% confidence</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.65</td>
<td>0.23</td>
<td>34.85</td>
<td>11.15</td>
<td>3.14</td>
<td>28.18</td>
</tr>
<tr>
<td>20</td>
<td>1.22</td>
<td>0.25</td>
<td>20.85</td>
<td>16.81</td>
<td>3.35</td>
<td>19.92</td>
</tr>
<tr>
<td>40</td>
<td>2.74</td>
<td>0.44</td>
<td>16.13</td>
<td>31.84</td>
<td>3.87</td>
<td>12.16</td>
</tr>
<tr>
<td>60</td>
<td>3.75</td>
<td>0.55</td>
<td>14.73</td>
<td>49.99</td>
<td>3.95</td>
<td>7.90</td>
</tr>
<tr>
<td>100</td>
<td>6.40</td>
<td>0.74</td>
<td>11.63</td>
<td>79.03</td>
<td>2.84</td>
<td>3.59</td>
</tr>
<tr>
<td></td>
<td>Raman scattering peak intensity at Ex/Em ~ 348 nm/396 nm (a.u)</td>
<td>2.17</td>
<td>±0.23</td>
<td>13.57</td>
<td>±0.65</td>
<td></td>
</tr>
</tbody>
</table>

Raman scattering peak intensity at Ex/Em ~ 348 nm/396 nm (a.u)
Due to these reasons higher PMT voltages (>800 V) were deemed to be unfavourable for obtaining reproducible spectra of water samples containing very low DOM concentrations.

**Scanning rate**

Another method of reducing signal noise is to reduce the speed at which spectra are acquired. With a high signal averaging time or by keeping smaller intervals between successive data points, the noise associated with measurement can be minimized. This improvement however comes with the cost of increased scanning time. When the scanning rate was reduced from 600 nm/min to 120 nm/min, the measurement noise for low concentration levels was reduced significantly with the fibre optic probe (Tables 1 and 3). The same observation was made when cuvettes were used (results not shown). At a scanning rate of 120 nm/min, it takes almost 24 minutes to acquire a complete fluorescence EEM, with emission scans performed from 350 nm to 600 nm for excitation levels from 280 nm to 380 nm in 10 nm increments. However, at a scanning rate of 600 nm/min, it would only take less than 5 minutes to acquire the same EEM. Long scanning times diminish the likelihood of the fluorescence spectroscopy being used as an on-line monitoring tool. Therefore slower scanning rates were not considered as a viable approach and no further studies were pursued with this parameter.

**Emission and excitation slit widths**

Measurement noise can also be reduced by increasing the excitation energy using larger excitation and emission slit widths. Increasing the slit widths is however constrained to a limit given by the formula (Westerhoff et al. 2001; Lewzey 2007, personal communication):

\[ 2 \times (\text{Excitation slit width} + \text{Emission slit width}) \leq \text{Stokes Shift} \] (1)

For GRW the Stokes shift is approximately 90 nm (Figure 2). Therefore the measurement noise levels at different slit width combinations were studied in detail adhering to the above constraint (Equation (1)). In general, as the excitation and/or emission slit widths were increased...
the measurement noise decreased in all dilution levels studied. Since the main objective was to reduce measurement noise at low concentration levels only the results relevant to GRW diluted up to 10% of its original strength are presented here (Table 4).

From these results (Table 4) it is evident that as the excitation and emission slit width is increased, the measurement noise decreases. A similar observation was made, with same slit width combinations, when undiluted GRW was analyzed by both cuvette and fibre optic approach (results not shown). However, slit widths may not be increased without checking the following constraints (Equations (2) and (3)) in order to preserve the resolution of the signal (Creighton 1997):

\[
\text{Ex\_slit\_width} \times 10 \leq \text{BW\_ex}
\]

\[
\text{Ex\_slit\_width} \times 10 \leq \text{BW\_em}
\]

Where BW\_ex and BW\_em are the band width (BW) of the excitation and emission signals, respectively. The BW of both excitation and emission signals of GRW is approximately 120 nm each (Figure 2). According to the above criteria, the maximum slit width levels that can be

![Figure 3](https://iwaponline.com/wst/article-pdf/60/6/1385/447591/1385.pdf)

Table 4 | Signal noise at different slit width combinations, obtained with the fibre optic probe and cuvettes on GRW (diluted up to 10%)—calculated at the EEM Peak at Ex: 330 nm; Em: 415 nm

<table>
<thead>
<tr>
<th>Emission slit width (nm)</th>
<th>Excitation slit width (nm)</th>
<th>% error at different slit width combinations</th>
<th>Fibre optic probe</th>
<th>Cuvettes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>34.9</td>
<td>8.7</td>
<td>6.0</td>
<td>28.2</td>
</tr>
<tr>
<td>10</td>
<td>8.2</td>
<td>4.5</td>
<td>4.1</td>
<td>13.1</td>
</tr>
<tr>
<td>20</td>
<td>5.1</td>
<td>4.3</td>
<td>3.8</td>
<td>8.5</td>
</tr>
</tbody>
</table>
Raman scattering peak intensity at Ex/Em \(\sim\) 348 nm /396 nm (a.u) 12.05 \(\pm\) 0.63 \(=\) 87.21 \(\pm\) 0.83

![Figure 3](https://iwaponline.com/wst/article-pdf/60/6/1385/447591/1385.pdf)

From these results (Table 4) it is evident that as the excitation and emission slit width is increased, the measurement noise decreases. A similar observation was made, with same slit width combinations, when undiluted GRW was analyzed by both cuvette and fibre optic approach (results not shown). However, slit widths may not be increased without checking the following constraints (Equations (2) and (3)) in order to preserve the resolution of the signal (Creighton 1997):

\[
\text{Ex\_slit\_width} \times 10 \leq \text{BW\_ex}
\]

\[
\text{Ex\_slit\_width} \times 10 \leq \text{BW\_em}
\]

Where BW\_ex and BW\_em are the band width (BW) of the excitation and emission signals, respectively. The BW of both excitation and emission signals of GRW is approximately 120 nm each (Figure 2). According to the above criteria, the maximum slit width levels that can be
used without compromising the resolution of the signal are excitation and emission slit widths of 10 nm each. This phenomenon can be further confirmed by examining the spectra of different slit width combinations examined in this study (Figure 3). Figure 3 illustrates how the signal resolution diminishes as the slit widths are increased beyond 10 nm. The spectral information available in the emission spectra (Figure 3; circled regions), in the form of a Raman peak, obtained with emission and excitation slit widths of 10 nm or below, appeared to be absent in the emission spectra obtained with emission and excitation slit widths greater than 10 nm.

Using the optimum instrument parameter settings: PMT = 800 V, scanning rate of 600 nm/min and excitation and emission slit widths of 10 nm each, that were identified in this study, we have obtained reproducible fluorescence EEMs of the residual DOM present in NF permeates that contained DOC about 0.7 mg/L (Peiris et al. 2008). In addition, this parameter combination also allowed us to capture fluorescence EEMs of very low concentration levels of DOM that are present in other water sources such as drinking water and rain water with reasonable reproducibility (i.e. signal noise is <5–10% of the peak EEM intensities measured; results not shown). The manipulation of the instrument parameters demonstrated in this study could therefore serve as a useful guideline for those interested in using fluorescence spectroscopy to analyze very low concentrations levels of DOM, without having to pre-concentrate samples using tedious, expensive and time consuming experimental procedures that could contribute to additional experimental error. When implementing these methods, however, instrument biases in different fluorescence spectrophotometers may need to be accounted for using the Raman scattering peak intensities reported in Tables 1–3.

CONCLUSIONS

When acquiring fluorescence signals for very low concentrations of DOM in water, special attention needs to be given to capture reliable signals with good signal resolution. The instrument parameters that are commonly used to produce quality spectra from strongly fluorescing substances proved to be less suitable in dealing with highly diluted HS present in very low DOM concentrations levels. Low PMT voltages (600 V) failed to produce signals with significant strength when the fibre optic probe was used. Therefore, the PMT voltage was increased up to 800 V to obtain improved signal strength. However, the measurement noise of samples at low concentrations increased significantly when increasing the PMT voltage, and therefore increasing the PMT voltage above 800 V was seen as unfavourable. Nevertheless, even with PMT = 800 V, very poor reproducibility of the fluorescence spectra of highly diluted samples was produced using cuvettes and the fibre optic probe measurements with the later being most affected. Slower scanning rates showed potential for producing good reproducibility. Slower scan rates, below 600 nm/min, were however not seen as a viable option, due to longer scanning times reducing the likelihood of the fluorescence spectroscopy being used as an on-line monitoring tool. As the excitation and/or emission slit widths were increased the measurement noise decreased at all dilution levels studied. This approach was seen as the best method to reduce the measurement noise since it generated the most improvement out of the three approaches studied. Nevertheless, this approach should be practiced with care to avoid diminishing signal resolution. In conclusion, excitation and emission slit widths up to 10 nm are recommended to preserve the spectral information and to obtain reproducible signals with minimum signal noise. The approach demonstrated here shown was successfully applied in the analysis of very low DOM concentrations levels and could be useful for researchers interested in using fluorescence spectroscopy to analyze similar concentrations levels.

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REFERENCES


