Characterization of dissolved organic matter using high-performance liquid chromatography (HPLC)–size exclusion chromatography (SEC) with a multiple wavelength absorbance detector

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ABSTRACT

High-performance liquid chromatography–size exclusion chromatography (HPLC–SEC) coupled with a multiple wavelength absorbance detector (200–445 nm) was used in this study to investigate the apparent molecular weight (AMW) distributions of dissolved organic matter (DOM). Standard DOM, namely humic acid, fulvic acid and hydrophilic acid, from the Suwannee River were tested to ascertain the performance and sensitivity of the method. In addition to four compounds groups: humic substances (Peak 1, AMW 16 kD), fulvic acids (Peak 2, AMW 11 kD), low AMW acids (Peak 3, AMW 5 kD), and low AMW neutral and amphiphilic molecules, proteins and their amino acid building blocks (Peak 4, AMW 3 kD), an new group that appears to include low AMW, 6–10 kD, humic substances was found based on investigating the spectra at various elution times. The spectroscopic parameter $S_{>365}$ (slope at wavelengths >365 nm) was determined to be a good predictor of the AMW of the DOM. The detector wavelength played an important role in evaluating the AMW distribution. For some fractions, such as the humic and low AMW non-aromatic substances, the error in measurement was $\pm 30\%$ as determined by two-dimensional chromatograms detected at an artificially selected wavelength. HPLC–SEC with multiple wavelength absorbance detection was found to be a useful technique for DOM characterization. It characterized the AMW distributions of DOM more accurately and provided additional, potentially important information concerning the properties of DOM with varying AMWs.

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1. Introduction

Dissolved organic matter (DOM) is ubiquitous in natural waters and is derived from plant or terrestrial (allochthonous) and algal (autochthonous) sources. It is composed of relatively high apparent molecular weight (AMW) humic and fulvic acids, as well as low AMW proteins, organic acids, carbohydrates and other compounds (Leenheer and Croue, 2003). The molecular properties of DOM, notably its molecular weight, strongly affect its reactivity in both natural and engineered aquatic systems (Amy et al., 1987; Her et al., 2002a; Leenheer and Croue, 2003; Yan et al., 2006, 2007; Korshin et al., 2009). High-performance liquid chromatography–size exclusion chromatography (HPLC–SEC) is one of the most widely used techniques to characterize DOM due to its important advantages, such as little need for DOM pre-concentration, small

injection volume, and ease and speed of analysis (Chin et al., 1994; Her, 2003; Korshin et al., 2009). In principle, the separation of molecules in a SEC column is based on size, and compounds with lower AMW are eluted later than those with higher AMW. However, the interpretation of HPLC–SEC data is greatly affected by the type of post-column detection used (Her, 2003; Korshin et al., 2009), typically UV absorbance, fluorescence, or multi-angle light scattering (MALS) (Baker, 2001; Wong et al., 2002; Her, 2003; Allpike et al., 2005; Akagi et al., 2007; Liu et al., 2010a). Post-column absorbance detection is most common due to its sensitivity and relatively low cost (O'Loughlin and Chin, 2001; Her et al., 2002b, 2008; Hur and Kim, 2009). DOM chromophores produce overlapping bands, so their absorbance spectra are largely featureless and cannot be deconvoluted to determine the contributions of discrete functional groups (Korshin et al., 1997, 2009; Her et al., 2008). In most cases, such measurements have been limited to using a single wavelength (e.g., 254 nm).

However, a number of phenomenological parameters (e.g., $SUVA_{254}$, ratios of DOM absorbances at fixed wavelengths, and slopes of DOM absorbance vs. wavelength) have been correlated

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with important DOM properties (e.g., aromaticity and apparent molecular weight) that have been estimated using structure-sensitive methods (e.g., ^{13}C NMR).

These phenomenological parameters have rarely been used to interpret HPLC–SEC data. For instance, Her et al. (2008) used the ratio of absorbance of varying-MW fractions measured at 210 nm and 254 nm to estimate the relative contributions of fulvic acids and microbial biopolymers. Korshin et al. (2009) used the ratio of the absorbance slopes of wavelengths 254–272 nm vs. 220–230 nm (ISA) to evaluate the DOM removal and DBP formation potential.

In this paper, we examined the performance of alternative absorbance-based indexes (e.g., absorption ratios and slopes). Our conclusions were based on the analysis of absorbance spectra, from 200 nm to 445 nm, of HPLC–SEC fractions with varying AMWs. This approach was applied to the Suwannee River humic acid, fulvic acid and hydrophilic acid standards to determine the performance and sensitivity of the method.

2. Methods and materials

2.1. Samples

Suwannee River humic acid (SRHA) (1R101H) and Suwannee River fulvic acid (SRFA) (1S101F) standards were obtained from the International Humic Substances Society (IHSS). Suwannee River hydrophilic acid (SRHPiA) was isolated and provided for this study by Dr. J.A. Leenheer. SRHPiA was obtained by passing an acidified (pH 2) sample of Suwannee River organic matter, eluted from column containing Amberlite XAD-8 resin, through a column containing Amberlite XAD-4 resin. This sample was then eluted from the XAD-4 resin with sodium hydroxide. SRHA, SRFA and SRHPiA solutions were prepared at a 5.0 mg L^{-1} final TOC concentration in Milli-Q water. Dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC-Vcsh carbon analyzer. The ionic strength of each was maintained at 0.04 M by adding the requisite amount of NaClO_4 .

2.2. HPLC–SEC analyses

The HPLC–SEC analysis used a DIONEX Ultimate 3000 HPLC system. Isocratic flow of 0.01 M NH_4HCO_3 was delivered through an Agilent PL aquagel-OH 30 column (300×7.5 , $8 \mu\text{m}$) at 0.5 mL min^{-1} . Elution profiles were monitored with an Ultimate 3000 diode array detector, which acquired absorbance spectra from 200 nm to 445 nm at a resolution of 1.0 nm.

A highly linear calibration curve for the apparent molecular weight (AMW) was generated using polyethylene glycol (PEG) standards (Agilent EasiVial PEG) (see Fig. 1S in Supplementary information (SI)), but it suggested that the AMW of the DOM was much larger than that reported by other researchers (Chin et al., 1994). Therefore, although the HPLC–SEC profiles are useful for comparisons among samples in a particular experiment, the absolute AMW values should not be interpreted literally (Cai and Benjamin, 2011).

The absorbance spectra of DOM samples were recorded in a 5 cm quartz cell on a Perkin-Elmer Lambda 18 UV/vis spectrophotometer using wavelengths from 200 nm to 600 nm.

2.3. AMW evaluation

AMW distributions can be represented by number-averaged AMW (M_n), weight-averaged MW (M_w), and polydispersity (p , M_w/M_n). M_n and M_w values were determined using the following Eqs. (1) and (2):

$$M_w = \frac{\sum_{i=1}^n (b_i \text{MW}_i)}{\sum_{i=1}^n b_i} \quad (1)$$

$$M_n = \frac{\sum_{i=1}^n b_i}{\sum_{i=1}^n (b_i/\text{MW}_i)} \quad (2)$$

$$p = \frac{M_w}{M_n} \quad (3)$$

where b_i and MW_i are the height of the chromatogram and the MW of an organic matter sample corresponding to retention time i , respectively (Chin et al., 1994).

3. Theoretical aspects of the interpretation of absorbance spectra

The energies of various types of molecular orbits differ significantly. It was reported that most detectable UV absorbance by organic compounds is based on transitions of n or π electrons to the π^* excited state. The energies required for these processes bring the absorption peaks into an experimentally convenient spectral region (200–700 nm) (Her et al., 2002b). The absorbance spectra of pure, model aromatic compounds can often be represented by three bands in the UV region from $\pi \rightarrow \pi^*$ transitions: the electron-transfer (ET) band centered at 254 nm, the benzenoid (Bz) band centered at 203 nm, and the local excitation (LE) band centered at 180 nm. The ratio of the intensities of the electron transfer and benzenoid bands ($A_{\text{ET}}/A_{\text{Bz}}$, or A_{254}/A_{203}) is quite sensitive to substitutions on the rings, increasing dramatically when the rings are substituted with activating functional groups (e.g., hydroxyl, ester, and carboxyl) (Korshin et al., 1997; Li et al., 2006). The main DOM functional groups associated with non-aromatic groups display absorption maxima only at shorter wavelengths, and, in the case of the non-conjugated form, the absorption maxima wavelengths were 206 nm (for carboxylic acid and esters) and 210 nm (for amides) in water for the $n \rightarrow \pi^*$ transition. When carboxylic acid or esters are conjugated, the peak maxima absorption occurs by $\pi \rightarrow \pi^*$ transitions at an experimentally detectable spectral region (longer than 200 nm), and due to a substituent effects, the peak maxima absorption shifts to longer wavelengths from the base absorption of 187 nm in water. The absorption band increase is dependent upon the substituent type and its position on the unsaturated carbonyl chromospheres (Her et al., 2008). The overall spectrum of a DOM sample represents a superposition of these bands. However, by refining spectral parameters, some useful information could be obtained from the overall spectrum of a DOM sample. A review and relevant values of SRHA, SRFA and SRHPiA are provided in Table 1.

The conventional absorbance spectra of SRHA, SRFA and SRHPiA are shown in Fig. 1a. Except for a shoulder located in the 250–270 nm range of wavelengths, these spectra did not exhibit any prominent peaks. In all cases, the absorbance gradually decreased at increased wavelengths. While the TOC concentrations of these three samples were equal, the absorbance intensities were different. Due to its higher aromaticity, SRHA absorbance values were twice as high as those of SRFA and SRHPiA. When the spectra were normalized by their absorbance measured at an a priori selected reference wavelength (e.g., 220 nm), additional properties of these spectra became more evident, as shown in Fig. 1b. At wavelengths > 250 nm, the absorbance of SRHA was higher than that of SRFA and SRHPiA. At the same time, the normalized spectrum of SRHPiA was slightly higher than that of SRFA, and close to that of SRHA, in the 250–270 nm range.

Several indexes were derived based on the ratios of the absorbance of SRHA, SRFA and SRHPiA at varying wavelengths and are presented in Table 1. It was also recognized that the

Table 1

Review of spectroscopic parameters measured for SRHA, SRFA and SRHPiA.

Spectral parameter	SRHA	SRFA	SRHPiA	References
$S_{200-205}$	0.0014	0.0018	0.0038	Helm et al., 2008; Twardowski et al., 2004; Carder et al., 1989 ^a
$S_{206-240}$	0.0061	0.0062	0.0061	
$S_{241-272}$	0.0031	0.0044	0.0032	
$S_{273-350}$	0.0046	0.0060	0.0067	
$S_{>365}$	0.0062	0.0081	0.0081	
$S_{200-205}/S_{206-240}$	0.2342	0.2824	0.6123	Korshin et al., 2009 ^b
$S_{240-272}/S_{206-240}$	0.5100	0.7109	0.5162	
$S_{273-350}/S_{206-240}$	0.7483	0.9567	1.0935	
$S_{>365}/S_{206-240}$	1.0084	1.2928	1.3129	Helm et al., 2008; Twardowski et al., 2004
A_{203}/A_{254}	1.8331	1.9036	1.8548	
A_{210}/A_{254}	1.7289	1.8254	1.7454	Korshin et al., 1997; Li et al., 2006 ^c
A_{280}/A_{350}	2.1294	2.6578	2.9891	Her et al., 2008 ^d
A_{250}/A_{365}	3.2768	4.7869	5.1549	Korshin et al., 1997, 2009 ^e
				Helm et al., 2008; De Haan and De Boer, 1987; Peuravouri and Pihlaja, 1997 ^f

^a $S((A_1 - A_2)/(\lambda_1 - \lambda_2))$, was used to semi-quantitatively describe the property of a DOM sample. $S_{275-295}$ (slope of 275–295 nm) and $S_{275-295}/S_{350-450}$ (ratio of slopes of 275–295 nm and 350–450 nm) were inversely related to the AMW fractionated by ultrafiltration or gel filtration fraction.

^b ASI and $S_{220-230}/S_{254-272}$ (ratio of slopes of 220–230 nm and 254–272 nm) were used to evaluate DOM removal and DBP formation potential.

^c A_{254}/A_{203} was used to determine A_{ET}/A_{Bz} (absorption spectral ratios for electron-transfer (ET) and benzenoid (Bz) bands) to evaluate the degree of activation of the PHA moiety in DOM.

^d A_{210}/A_{254} was used to estimate relative contributions of fulvic acids and microbial biopolymers.

^e A_{280}/A_{350} , like A_{203}/A_{253} , was used to evaluate the degree of activation of the PHA moiety in DOM.

^f A_{250}/A_{365} (called E2:E3) or A_{465}/A_{665} (called E4:E6) were used to track changes in the relative size of DOM molecules.

characterization of these spectra can be done by calculation of their spectral slopes. For instance, it was demonstrated that the absorbance of DOM changes near exponentially relative to the observation wavelength (Twardowski et al., 2004):

$$a_g(\lambda) = a_g(\lambda_r)e^{-S_e(\lambda - \lambda_r)} \quad (4)$$

where a_g is the Napierian absorption coefficient (m^{-1}), λ and λ_r are selected and the reference wavelength, respectively, and S_e (nm^{-1}) is the spectral slope parameter that describes the relative steepness of the spectrum. Like the absorption ratios, the S_e parameter is independent of the DOM concentration and is often used as a proxy for changes in the composition of DOM (Twardowski et al., 2004). Measurements of spectral slopes provided further insight into the selected characteristics of the DOM over the absorption values alone.

Log-transformed absorption spectra of SRHA, SRFA and SRHPiA are shown in Fig. 1c. All three log-transformed spectra show the presence of five ranges of wavelengths with different slopes. These ranges corresponded to 200–205 nm, 206–240 nm, 240–272 nm, 273–350 nm and > 350 nm. The slopes of SRHA in all these intervals were the lowest relative to the three examined samples except in the 206–240 nm wavelength range, where the slopes were almost equal for the three samples.

The absorbance spectrum of SRHPiA had the highest slope values except in the 241–272 nm ($S_{241-272}$) wavelength range. The ratio of slopes was calculated and $S_{206-240}$ was used as a reference. The slopes and slope ratios are provided in Table 1.

4. Results and discussion

4.1. HPLC–SEC chromatograms

AMW distribution profiles of SRHA, SRFA and SRHPiA characterized by HPLC–SEC with multiple wavelength detection are displayed in Fig. 2. The chromatograms for these samples were measured at several representative wavelengths and are shown in Fig. 2S in Supplemental information (SI).

Compared with the two-dimensional chromatograms plotted against absorbance at a single or pair of wavelengths, the AMW data were plotted against the entire response range (200–445 nm) of the UV detector and gave a comprehensive perspective

on the relative absorbance intensity of the various AMW compounds.

The chromatograms of SRHA and SRFA exhibit four peaks with maxima located at AMW 16 kD, 11 kD, 5 kD, and 3 kD and denoted P1, P2, P3, and P4, respectively. The HPLC–SEC profiles for SRHPiA showed only three peaks that corresponded to P1, P2, and P4.

The relative intensities of each peak in the three samples differed significantly, consistent with the well-known characteristics of these materials. SRHA and SRFA were derived primarily via biogeochemical reactions associated with the decomposition of woody plant materials that have a high lignin content. As a result, SRHA and SRFA had structures with larger AMW values and higher overall aromatic content (37% and 24% for SRHA and SRFA, respectively) (Her et al., 2002b). Accordingly, SRHA and SRFA had relatively higher intensities at P1 and P2, with SRHA having a higher relative intensity at P1. In contrast, SRHPiA was mostly composed of a low AMW hydrophilic fraction, and the contribution of the low AMW P4 was high.

The absorbance intensities at various elution times decreased when the wavelength increased, and the spectral slopes were inconsistent. The results of the AMW distribution evaluation were significantly influenced by the wavelength utilized in Eqs. (1) and (2), as discussed later.

4.2. Characterizing spectra in three-dimensional HPLC–SEC chromatograms

To gain more insight into the nature of the DOM molecules with varying AMW values, the behavior of several spectral parameters as a function of elution time were examined. The selected results of these analyses are presented in Figs. 3 and 4 for absorption ratios and slopes, respectively.

The trends in the behavior of A_{203}/A_{254} and A_{210}/A_{254} vs. AMW (elution time) were similar, therefore, only the results for A_{210}/A_{254} are shown in Fig. 3a. In general, the value of A_{210}/A_{254} increased when AMW decreased. This was consistent with the expectation that DOM with lower AMWs were likely to have lower concentrations of activated aromatic rings but were enriched with non-aromatic groups (Korshin et al., 1997; Li et al., 2006; Her et al., 2008). There are three obvious peaks observed for SRHA in Fig. 3a. These peaks corresponded to elution times P2, P3 and P4 in Fig. 2. There were only two peaks found for SRFA at P3 and P4; the peak

Fig. 1. Absorbance spectra of SRHA, SRFA and SRHPiA scanned by UV spectrophotometer. (a) Absorbance spectra; (b) absorbance spectra normalized to the reference absorbance at 220 nm and (c) natural log-transformed absorbance spectra.

corresponding to P2 was not obvious because SRFA had a higher A_{210}/A_{254} value. There were only two peaks found for SRHPiA at P2 and P4. The values of A_{210}/A_{254} showed similar values at two AMW ranges, 14–20 kD and 6–10 kD.

The A_{280}/A_{350} values for the three samples exhibited similar trends when AMW decreased. These ratios decreased gradually at AMWs larger than 6 kD, except for a peak at the AMWs of 11–14 kD. This may indicate that DOM with an AMW of 11–14 kD had specific groups with non-aromatic functional character. The A_{280}/A_{350} values increased at an AMW of 6 kD and decreased gradually as the AMW decreased.

The behavior of the spectral slopes calculated for wavelength ranges 206–240 nm and 273–350 nm is shown in Fig. 4. These data showed similar trends to those observed for the A_{210}/A_{254} and A_{280}/A_{350} values.

The results shown in Figs. 2–4 and data of prior research (Huber and Frimmel, 1994; O'Loughlin and Chin, 2001; Her et al., 2002a,b;

Fig. 2. HPLC-SEC chromatograms for (a) SRHA, (b) SRFA and (c) SRHPiA.

Korshin et al., 2009; Liu et al., 2010b; Huber et al., 2011) revealed that DOM can be divided into five distinct component fractions based on AMW. The first fraction (F1) had an AMW larger than 14 kD (observed at elution times from 14.2 min to 14.7 min), corresponded to P1 and was likely comprised of humic substances (Rosenberger et al., 2006) that had relatively high aromaticity and contributions from functional groups with conjugated bonds. This fraction had a relatively low A_{210}/A_{254} and $S_{206-240}$ values and relatively high A_{280}/A_{350} and $S_{273-350}$ values. The second fraction (F2) had an AMW of 10–14 kD (observed at elution times from 14.7 min to 15.2 min). It corresponded to P2 and was comprised of fulvic acids, characterized by highly aromatic components and high conjugated no-aromatic activating functional groups. It had

Fig. 3. Comparison of the absorption ratios of SRHA, SRFA and SRHPiA.

relatively higher A_{210}/A_{254} , A_{280}/A_{350} , $S_{206-240}$, and $S_{273-350}$ values. The third component (F3) had an AMW of 6–10 kD (observed at elution times from 15.2 min to 15.8 min). It corresponded to the tailing part of the second peak and appeared to include humic substances with relatively low AMWs and operationally defined building blocks (sub-units of humic substances), characterized by high aromatic components and low non-aromatic functional groups. It had relatively lower A_{210}/A_{254} , A_{280}/A_{350} , $S_{206-240}$, and $S_{273-350}$ values. The DOM with larger MWs contained more aliphatic carbons. The aliphatic C chains had a lower content of C=C, carbon double bonds, and have higher UV absorptivity at shorter wavelengths. Conversely, the fractions with smaller MWs, such as aromatic structures, had a greater content of C=C and have a higher UV absorptivity at longer wavelengths (Liu et al., 2010b). The fourth fraction (F4) had an AMW of 4–6 kD (observed at elution times of 15.8 min to 16.2 min) and corresponded to P3 that has been generally assigned to low MW acids (O'Loughlin and Chin, 2001; Liu et al., 2010b). This fraction had relatively high A_{210}/A_{254} , A_{280}/A_{350} , $S_{206-240}$, and $S_{273-350}$ values. The fifth fraction (F5) had an AMW lower than 4 kD (observed at elution times of 16.2–17.0 min) and corresponded to P4 that has been generally assigned to low MW neutrals and amphiphilics, proteins and their amino acid building blocks. This fraction had the highest A_{210}/A_{254} , $S_{206-240}$ values and relatively low A_{280}/A_{350} , $S_{273-350}$ values.

4.3. MW evaluation by UV spectra

In previous studies, several parameters, such as A_{365}/A_{250} , A_{465}/A_{665} , spectral slope in the 275–295 nm range and the ratio of slopes

Fig. 4. Comparison of the slopes of absorbance spectra of SRHA, SRFA and SRHPiA.

of 275–295 nm and 350–450 nm ranges, were used to evaluate the relative sizes of DOM molecules found in fractions obtained from ultrafiltration or Gel filtration chromatography. In this paper, several spectroscopic parameters, including those outlined above, were used to evaluate the AMW base of the HPLC–SEC data at various elution times.

The A_{365}/A_{254} values for SRHA, SRFA and SRHPiA are shown in Fig. 5a. In accord with the results of previous studies (Twardowski et al., 2004) the A_{365}/A_{254} values were observed to generally increase with elution time. However, a notable peak located at elution times 15.2–15.8 min (with an AMW of 6–10 kD, as calculated by a standard AMW calibration curve) was observed for SRHA. It corresponded to F3, the fraction with low AMW humic substances and was characterized by highly aromatic components with high relative UV absorbance values at longer wavelengths. The MW of this DOM fraction was underestimated if A_{365}/A_{254} was used to evaluate the AMW.

The A_{365}/A_{254} values and the spectral slopes calculated for wavelengths > 365 nm ($S_{>365}$) represented a more accurate parameter with which to estimate the AMW of DOM molecules, as shown in Fig. 5b. The $S_{>365}$ values tended to decrease gradually and showed a more robust linear correlation vs. the elution time. However, the $S_{>365}$ values had higher scatter because the absorption intensities measured at longer wavelengths were closer to the detection limit of the detector.

4.4. Error in MW distribution estimation

The observation wavelength was evaluated to determine its effect on the estimate of AMW distributions in HPLC–SEC measurements. The HPLC–SEC spectra at various elution times were

Fig. 5. MW estimation indexes of (a) A_{356}/A_{254} and (b) $S_{>365}$.

normalized by the absorbance at a priori selected reference wavelength (e.g., 220 nm). The data were processed further by subtracting the normalized absorbance spectrum of the DOM by absorbance intensity at the same reference wavelength. This calculation can be expressed in the following equation:

$$RI_{w,t} = \frac{I_{w,t}}{I_{220,t}} - \frac{t_w}{t_{220}} \quad (5)$$

where $RI_{w,t}$ is the error at wavelength w and elution time t ($i = 200$ – 445 nm; $w = 14.2$ – 17.0 min). $I_{w,t}$ is the intensity measured by HPLC–SEC at wavelength w and elution time t ($w = 200$ – 445 nm; $t = 14.2$ – 17.0 min). $I_{220,t}$ is the intensity measured by HPLC–SEC at wavelength 220 nm and elution time t ($t = 14.2$ – 17.0 min). i_w is the intensity measured by UV/vis spectrophotometer at wavelength w ($w = 200$ – 445 nm). i_{220} is the intensity measured by UV/vis spectrophotometer at wavelength 220 nm.

The relative errors in MW distribution estimation for SRHA, SRFA and SRHPiA are shown in Fig. 6a–c, respectively. As shown in Fig. 6a for SRHA, there were two areas at wavelengths longer than 220 nm that have negative values beyond 10%. These areas corresponded to DOM with an AMW larger than 10 kD and smaller than 6 kD. Additionally, there were several peak areas found at wavelengths lower than 220 nm, but these areas were overlooked in this study due to interference from inorganic ions at low wavelengths (e.g., NO_3^- , SO_4^{2-} , and PO_4^{3-}) (Her et al., 2008; Korshin et al., 2009). SRFA and SRHPiA also showed the same phenomena as SRHA, though the areas and depths of the washes were different. The areas and depths of washes at AMWs larger than 10 kD were smaller for SRFA and SRHPiA. Furthermore, the areas and depths

Fig. 6. Normalized HPLC–SEC chromatogram subtracted by the normalized absorbance spectra of (a) SRHA, (b) SRFA and (c) SRHPiA.

of the washes at AMWs smaller than 6 kD are bigger for SRFA and especially SRHPiA.

There was an error in evaluating M_n and M_w based on Eqs. (1) and (2). The error changed with the wavelength utilized and the characteristics of the DOM. In general, the error was within $\pm 10\%$. For some special fractions, such as humic substance or low WM non-aromatic matters, the error was greater than 30% at certain wavelengths.

The HPLC–SEC coupled with a multiple wavelength absorbance detector was a useful technique to characterize DOM. It not only characterized the MW distribution of the DOM more accurately, but it also provided some interesting information on DOM characteristics at various MWs. However, further work is needed to understand the UV spectra.

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Appendix A. Supplementary material

HPLC–SEC calibration curve and the chromatograms for these samples measured at several representative wavelengths and are shown in Figs. 1S and 2S in the Supplemental Information (SI), respectively. Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.chemosphere.2012.01.029](https://doi.org/10.1016/j.chemosphere.2012.01.029).

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