Spectroscopic Characterization of Changes of DOM

Deprotonation-protonation Properties in Water

Treatment Processes

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Abstract

The deprotonation-protonation properties of dissolved organic matter (DOM) in drinking water produced at critical treatment points were quantified using absorbance spectra in combination with DOM fractionation data. Analysis of differential spectra of DOM present in inlet, settled and filtered waters from two large treatment plants and their fractions were obtained. The data demonstrated the presence of six Gaussian bands largely associated with carboxylic and phenolic DOM functionalities. Properties of the protonation-active groups of DOM in raw and treated waters were further examined based on data of potentiometric titrations at pH from 2.5 to 10. Interpretation of the differential log-transformed absorbance at wavelength 350 nm ($\text{Dln}A_{350}$) based on the NICA-Donnan model showed that the normalized concentrations of low- and high-affinity protonation-active groups in residual DOMs increases as a result of water treatment. This was consistent with the results of DOM fractionation. This study demonstrates that changes of the composition and reactivity of DOM found in drinking water treatment sequences can be quantified based on the examination of their optical properties.

Keywords: absorbance, dissolved organic matter (DOM), NICA-Donnan model, protonation, fractionation, drinking water treatment

1. Introduction

Dissolved organic matter (DOM) is ubiquitous in aquatic systems. It affects color, taste and odor and generation of disinfection by-products (DBPs) in drinking water (Leenheer and Croué, 2003; Richardson et al., 2007; Ding and Zhang, 2009; Richardson and Ternes, 2011). DOM also facilitates the transport of many heavy metals and hydrophobic pollutants and increases coagulant demand (Croue et al., 2000; Liu et al., 2007; Yan et al., 2008; Park, 2009; Yan et al., 2013a).

DOM fractionation by XAD8, XAD4 and other resins (Thurman and Malcolm, 1981; Croue et al., 2000; Muller et al., 2000; Schafer et al., 2002; Croue et al., 2003) and structure-sensitive methods such as nuclear magnetic resonance (NMR), pyrolysis GC/MS, Carbon K-edge Near Edge X-Ray Absorption Fine Structure (NEXAFS) spectroscopy, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), and optical (absorbance and fluorescence) spectroscopy have been used to probe the chemistry of DOM (Korshin et al., 1998; Wong et al., 2002; Abbt-Braun et al., 2004; Cook, 2004; Del Vecchio and Blough, 2004; McDonald et al., 2004; Christl and Kretzschmar, 2007; Helms et al., 2008; Minor and Stephens, 2008; Schäfer et al., 2009; Berwick et al., 2010; Janot et al., 2010; Matilainen et al., 2011; Zhang et al., 2012). These efforts have conclusively shown that DOM fractions with high molecular weight and hydrophobicity are preferentially removed in water treatment processes

(Vilg-Ritter et al., 1999; Leenheer and Crou, 2003; Zhang et al., 2005; Yan et al., 2007).

Despite the extent of these studies, quantitative data correlating water treatment conditions and resultant changes on the intrinsic chemistry of DOM, for instance its proton- and metal-binding capacity and applicable stability constants are limited while such data are highly important for achieving a detailed understanding of DOM interactions with coagulants (Yan et al., 2008; Yan et al., 2013a), absorbents (Janot et al., 2011; Janot et al., 2012) and metal cations (e.g., Cu²⁺, Cd²⁺, Fe³⁺ and Al³⁺) (Bai et al., 2008; Marang et al., 2008; Marang et al., 2009; Xiao et al., 2013; Yan et al., 2013a; Yan et al., 2013b; Yan et al., 2013c).

Earlier research has demonstrated that spectrophotometric titrations allow ascertaining interactions of DOM with protons and metal cations (Dryer et al., 2008; Janot et al., 2010; Yan et al., 2013b). In this paper, we expanded this approach and adopted it to characterize DOM originating from critical points in drinking water treatment plants, notably after coagulation and filtration. The protonation properties of DOM present in the inlet (or influent) and in treated water were further quantified via deconvolution that helped discern contributions of discrete bands present. Changes of absorbance-derived parameters determined for DOM in the influent, treated water and selected DOM fractions were interpreted based on the NICA-Donnan Model (Milne et al.,

2001; Ritchie and Perdue, 2003; Dryer et al., 2008; Janot et al., 2010) that helped ascertain properties of low- and high-affinity protonation-active groups in DOM.

2. Material and Methods

2.1. Samples. Grab samples of inlet, settled and filtered waters after coagulation from two water treatment plants denoted Plant A and B, and located in the Atlantic coast of the United States were collected in 5-gallon cubitainers by the treatment plant personnel and immediately shipped to the research laboratory at the University of Washington. The inlet water for Plant A and B is the same. DOC concentrations in the examined waters were relatively low (Table S1), and prior to fractionation experiments these samples were RO-preconcentrated as described in Supporting Information Section. All chemicals were AR-grade unless otherwise specified. All solutions were prepared using Milli-Q water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$, Millipore Corp., MA, USA).

2.2. Spectrophotometric titration. Spectrophotometric titration of all five RO isolated DOM samples was carried out as described in previous literature (Dryer et al., 2008; Yan et al., 2013b). During these titrations, the RO-preconcentrated DOM samples were diluted with Milli-Q water to reach a 5.0 mg L^{-1} DOC concentration.

2.3. DOM Fractionation. DOM fractionation for five RO-preconcentrated water samples was repeated twice using XAD8 and XAD4 resin fractionation scheme (Croue et al., 2000). The DOC in the RO-preconcentrated samples was adjusted to 15 ± 1 mg L⁻¹, and the sample volume was 520 mL. The first two steps in the fractionation procedure included the separation of the hydrophobic basic (HPOB) and hydrophobic acidic (HPOA) fractions of DOM. The HPOB fraction was retained by XAD8 (or DAX8) resin at pH 12 and eluted by 0.1 M perchloric acid, while the HPOA fraction was retained by XAD8 at pH 12 and is eluted by 0.1 M sodium hydroxide.

The remainder of DOM that was retained by XAD8 and not eluted by either basic or acidic eluents was assigned to the fraction of hydrophobic neutrals (HPON). Following the separation of hydrophobic (HPO) fractions, similar operations were carried out with XAD4 resin, which retained the transphilic fractions (TPH) of DOM. These included the fractions of transphilic bases (TPHB) retained by XAD4 at pH 12 and eluted by perchloric acid, transphilic acids (TPHA) adsorbed at pH 2 and eluted by sodium hydroxide. The remainder of DOM retained by XAD4 was assigned to the fraction of transphilic neutrals (TPHN). The DOM that was not retained by either XAD8 or XAD4 was classified as hydrophilic DOM fraction (HPI). The effluent of XAD8 or XAD4 resin at pH 2 and pH12 were collected also to deduce the normalized differential absorbance spectra of HPON and TPHN fraction.

2.4. Analytical methods. DOC concentrations were determined with a Shimadzu TOC-Vcsh carbon analyzer. Absorbance spectra were recorded using a Perkin-Elmer Lambda 18 UV/Vis spectrophotometer with a 5 cm cell. All absorbance spectra were measured at pH 2.0, except in the spectrophotometric titration experiment where pH was varied as needed.

3. Results and Discussion

3.1. DOMs fractionated by RA in water treatment processes

DOM fractionation showed that the examined samples had similar compositions (Table 1). The most notable difference in their fractional compositions was a relatively larger contribution of the HPOA fraction in inlet water DOM. The percentages of HPON fractions were relatively high, and the contributions of all hydrophobic (HPO) fractions were on the average close to 60%.

The contributions of transphilic (TPH) and HPI fractions were comparable for all five examined samples, with the percentages of the HPI fraction (typically close to 10%) being low. This also possibly indicates that a fraction of hydrophilic organic molecules associated with the HPI fraction in the original samples may have been lost during RO isolation. The average recovery of DOC during RO operations was close to 90% (Table S1).

Table 1

3.2. Changes of spectroscopic properties of DOMs in water treatment processes

The absorbances of DOM decreased gradually after settling and filtering with DOC removal. To compare the changes of spectroscopic properties of DOMs caused by settling and filtering, the absorbance of DOMs in inlet, settled and filtered water from the examined treatment plants were normalized by their DOC concentrations (Figure S2). The DOC-normalized absorbance of DOMs decreased also in settled and filtered water. In all cases, the absorbance intensity decreased exponentially with the observation wavelength, as observed in prior studies (Twardowski et al., 2004; Yan et al., 2013b; Yan et al., 2013c). To ascertain changes of DOM absorbance caused by the treatment of the inlet water in more detail, the differential DOC-normalized spectra of treated waters were calculated using the spectrum of the inlet water as a reference (Eq 1):

$$\Delta A_{\lambda} = \frac{A_{\lambda,i}}{DOC} - \frac{A_{\lambda,ref}}{DOC} \quad (1)$$

where $A_{\lambda,i}$ and $A_{\lambda,ref}$ are absorbances of the treated and inlet water, respectively (Dryer et al., 2008). Results of these calculations (Figure 1) demonstrate that the intensity of the differential spectra of both settled waters decrease with the wavelength while the differential spectra for filtered waters have a peak with maximum located near to wavelength 270 nm. The degree of

change of absorbance was more obvious for Plant A than Plant B, which was consistent with the efficiency of DOM removal and indicates that the DOC with higher absorbance is more removable (Table S1).

Figure 1

The differential DOC-normalized spectra were processed to determine the presence of constituting bands (Yan et al., 2013b). Results of this processing (Figure 2) demonstrate that the fitting of the experimental spectra with six Gaussian distribution band components resulted in a very close fit ($R^2 > 0.95$). The constituting bands operationally referred to as A0 (210 nm), A1 (236 nm), A2 (270 nm), A3 (313 nm), A4 (374 nm) and A5 (523 nm). The positions of bands A1, A2, A3 and A4 are consistent with the results in our prior studies (Yan et al., 2013b). Figure 2 indicates the difference between the differential DOC-normalized spectra calculated for settled and filtered water was primarily associated with the high contribution of Band A2 while that of Band A1 is low.

Figure 2

To examine differences in the properties of DOM chromophores present in different fractions, the differential spectra were normalized by their intensity at 220 nm (Yan et al, 2012). The normalized differential absorbance spectra of each fraction calculated using the data for the sample before fractionation were calculated using Eq 2.

$$R\Delta A_{\lambda} = \frac{A_{\lambda,i}}{A_{220,i}} - \frac{A_{\lambda,ref}}{A_{220,ref}} \quad (2)$$

In this expression, $R\Delta A_{\lambda}$ is the normalized differential absorbance at wavelength λ . $A_{\lambda,i}$ and $A_{\lambda,ref}$ are absorbance spectra at a particular wavelength measured for selected fraction and DOM before fractionation, respectively.

Figure 3

The normalized differential spectra could be fitted the same Gaussian components which were found in the differential spectra of DOM in previous study ($R^2 > 0.95$) (Figure 4) but the contributions of these bands were different (Yan et al., 2013b). HPOB fraction had the highest intensity of Band A1 while the HPOA fraction had significantly higher contributions of bands A1 and A2, but relatively lower contribution of band A4; TPHA had higher contribution of Band A1 and A2, especially for A2, and lowest contribution of Band A3; TPHB was of negative value for all the bands except A1 and the contribution of A3 was very significant; TPHN had high contributions of Band A1 and Band A2, and relatively lower contributions of A3 and A4. HPI had significant negative value of all the bands.

Based on the comparison of the results shown in Figure 1 and 2, it could be also concluded that settling and filtering appears to remove efficiently the

fractions such as HPOB, HPOA, HPON, and TPHA, with the filtration step associated with further removal of the HPOA and TPHA fractions.

Figure 4.

3.3. Effects of pH variation on DOM absorbance spectra.

To ascertain changes of DOM acid-base property induced by water treatment, the absorbances of all DOM samples were measured at pH values ranging from 2.5 to 10.0. The corresponding zero-order and pH-differential spectra calculated using Eq 1 as described in prior studies (Dryer et al. 2008; Yan et al. 2013b) from the reference pH value of 2.5 are shown in Figure S4 and Figure 5, respectively.

The intensities of differential absorbances differ significantly between the five samples but major trends discerned from the figures were similar. All pH-differential spectra had peaks located at approximately 245, 280 and 370 nm and for all five DOM samples, the spectra could be deconvoluted using the six Gaussian components ($R^2 > 0.995$) as shown in Figure 6.

Figure 5.

Figure 6.

Prior research has shown that in the system of Gaussian components used to deconvolute the differential spectra of DOM, bands A4 and A5 are most

characteristic for humic substances (Yan et al., 2013b) because they appear to reflect contributions of the inter-chromophore interactions in humic species (Del Vecchio and Blough, 2004; Ma et al., 2010). The relatively low intensity of Band A4 necessitates that the log-transformed and differential spectra be calculated using the log-transformed data using Eq 3:

$$D \ln A_{\lambda} = \ln A_{\lambda,i} - \ln A_{\lambda,ref} \quad (3)$$

Results of data processing using the above equation are shown in Figure 7. As discussed in previous literature (Yan et al., 2013 a, 2013c), the intensity of the differential log-transformed absorbance at wavelength 350 nm is reflective of changes of Band A4. Accordingly, DlnA₃₅₀ values for all five isolated DOMs in the entire ranges of pH used in the experiments were calculated and presented in Figure 8.

Figure 7.

Figure 8.

3.4. Modeling proton binding onto DOMs using NICA-Donnan Model

Major trends in the behavior of DlnA₃₅₀ vs. pH for were similar for the five examined waters. To quantify the modification of DOM in water treatment processes, the DlnA₃₅₀ vs. pH datasets were interpreted based on the NICA-Donnan theory as described in previous studies (Milne et al., 2001; Ritchie and Perdue, 2003; Dryer et al., 2008; Janot et al., 2010):

$$\begin{aligned}
237 \quad D \ln A_{pH} = & \left[\frac{D \ln A_{LAS}}{1 + \left(\tilde{K}_{LAS} [H^+] \right)^{m_{LAS}}} + \frac{D \ln A_{HAS}}{1 + \left(\tilde{K}_{HAS} [H^+] \right)^{m_{HAS}}} \right] - \\
& - \left[\frac{D \ln A_{LAS}}{1 + \left(\tilde{K}_{LAS} [H^+]_{ref} \right)^{m_{LAS}}} + \frac{D \ln A_{HAS}}{1 + \left(\tilde{K}_{HAS} [H^+]_{ref} \right)^{m_{HAS}}} \right] \quad (4)
\end{aligned}$$

238 In the above formula, $D \ln A_{LAS}$ and $D \ln A_{HAS}$ correspond to the maximum
 239 change of absorbance associated with the deprotonation of the low-affinity
 240 protonation-active groups (LAS, mostly carboxylic) and high-affinity
 241 protonation-active groups (HAS, mostly phenolic), respectively, where
 242 $D \ln A_{LAS}$ and $D \ln A_{HAS}$ refer to DlnA values at wavelength 350 nm. It needs to
 243 be noted that $D \ln A_{LAS}$ and $D \ln A_{HAS}$ are parameters independent of DOC
 244 concentration, and they reflect the amount of protonation-active groups in per
 245 mg L⁻¹ DOM. $\tilde{K}_{LAS}$ and $\tilde{K}_{HAS}$ are the median values of the protons affinity
 246 distributions for these groups, m_{LAS} and m_{HAS} define the width of these
 247 distributions and are measures of the heterogeneity of DOM (Milne et al., 2001;
 248 Dryer et al., 2008; Yan et al., 2014).

249 The behavior of $D \ln A_{350}$ values for all five DOMs vs. pH and their fitting are
 250 shown in Figure 8 and Table 2. The data show that excellent agreement can
 251 be reached between the $D \ln A_{350}$ data for all samples and the predictions made
 252 based on the revised NICA-Donnan Model ($R^2 > 0.99$).

253 **Table 2**

254 The $\tilde{K}_{LAS}$ and $\tilde{K}_{HAS}$ values for all five DOMs determined in this study are
255 similar, and are in the range of those reported in the literature (Milne et al.,
256 2001; Ritchie and Perdue, 2003). $\tilde{K}_{LAS}$ values increase slightly after
257 settlement and filtration in Plant A water plantwhile $\tilde{K}_{HAS}$ values for waters of
258 both plants decreased after treatment and it is lower in DOM after settled
259 waters than that in after filtered water.

260 The $DlnA_{LAS}$ and $DlnA_{HAS}$ values increased in the treated waters, especially for
261 Plant A. This demonstrates that the normalized amounts of the LAS and HAS
262 groups in DOM remaining in water after settling and filtering steps is more
263 abundant than in Inlet water, and these groups are more sensitive to variations
264 of pH. This is consistent with hydrophobic DOM being more available to be
265 removed in water treatment. The ratio of $DlnA_{LAS}/DlnA_{HAS}$ also increased in
266 treated waters indicating that DOM with high-affinity groups (mostly
267 phenolic-like groups) are preferentially removed during water treatment.

268 The relative changes of the concentrations of LAS and HAS groups in DOM
269 determined based on NICA-Donnan modeling was compared with the results
270 of DOM fractionation. Results of this comparison are shown in Figure 9 and S5.
271 These indicate that the relative contributions of the LAS and HAS groups
272 increase linearly with the percentage of HPI fraction (R^2 0.91, excluding the
273 data for filtered water from Plant B which appear to be an outlier) in Figure
274 S5(a)). The concentrations of HAS decreased with the total percentages of

HPOB and TPHB fractions (Figure S5(b), R^2 0.92, excluding the data for the settled water produced by Plant B)). The contribution of HAS in the total protonation active groups increased with the total percentages of HPOA, TPHA and HI fractions (Figure 9, $R^2=0.95$).

Figure 9

4. Conclusions

This study used absorbance spectroscopy to quantify changes of DOM deprotonation-protonation properties in drinking water produced by two treatment plants.

(1) The data demonstrate that properties of the chromophores in DOM present in inlet, settled and filtered waters and their fractions can be quantified via numeric deconvolution of the relevant differential spectra. This approach indicated the presence of six well-resolved Gaussian bands A0 (210 nm), A1 (236 nm), A2 (270 nm), A3 (313 nm), A4 (374 nm) and A5 (523 nm) that comprise the spectra in all examined cases.

(2) The emergence of these bands is associated with the carboxylic and phenolic functionalities. DOM fractions with high contributions of band A1 and A2, e.g. the HPOB, HPOA, HPON, and TPHA fractions are more easily removed by settling and filtering. Filtration is more efficient than

settle in removing DOM with high contribution of band A2 on verse and the band A1 is low, e.g. the HPOA and TPHA fractions.

(3) The differential absorbance of DOM presented at each water treatment stage caused by deprotonation could reflect the amount of protonation-active chromophores in DOM. When combined with the NICA-Donnan model, the constants describing DOM/proton equilibria could be calculated based on the observed changes of the selected parameter $D\ln A_{350}$ vs pH.

(4) The normalized content of low- and high-affinity protonation-active groups (LAS and HAS) in DOM remaining in the treated water after settling and filtering steps was found to be higher than that in Inlet water. The observed changes of the LAS and HAS were generally consistent with the polarity characteristics of DOMs fractionated by XAD8/XAD4 RA.

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Captions of Table and Figure

Table 1 Contributions of hydrophobic, transphilic and hydrophilic fractions in the concentrations of dissolved organic carbon in RO isolates of influent (inlet), Plant A settled (AS) and filtered (AF), and Plant B settled (BS) and filtered (BF) water.

Table 2 NICA modeling parameters for protonation-active groups in DOMs determined by DInA350 in spectrophotometric titrations. DOMs in RO isolates of influent (inlet), Plant A settled (AS) and filtered (AF), and Plant B settled (BS) and filtered (BF) water.

Figure 1 DOC-normalized differential absorbances of DOM in RO isolates of Plant A settled (AS) and filtered (AF), and Plant B settled (BS) and filtered (BF) waters vs. the absorbance spectrum of the influent (inlet water).

Figure 2 Gaussian band fitting of the DOC-normalized differential absorbance of DOM in RO isolates of Plant A settled (AS, a) and filtered (AF, b), and Plant B settled (BS, c) and filtered water (BF, d). Absorbance spectrum of the influent (inlet water) was used as the reference.

Figure 3. Normalized differential absorbance of hydrophobic, transphilic and hydrophilic fractions in DOM in RO isolates of influent (inlet) water. Normalized by absorbance at wavelength 220 nm, reference to DOM before fractionation. The normalized differential absorbance spectra of HPON and TPHN fraction

were deduced by the normalized differential absorbance spectra of the effluent of XAD8 or XAD4 resin at pH 2 and pH12, respectively.

Figure 4. Gaussian band fitting of the normalized differential absorbance by intensity at 220 nm and DOC concentration of DOM fractions in water treatment processes referenced to the inlet waters.

Figure 5. pH-differential absorbance spectra of normalized differential absorbance of DOMs in RO isolates of DOM in the influent (inlet, a), Plant A settled (AS, b) and filtered (AF, c), and Plant B settled (BS, d) and filtered (BF, e) water. Reference pH value 2.5.

Figure 6. Gaussian band fitting of the differential DOM spectra in the influent (inlet, a), Plant A settled (AS, b) and filtered (AF, c), and Plant B settled (BS, d) and filtered (BF, e) water at pH about 7.0 referred to pH 2.5.

Figure 7. Log-transformed differential absorbance spectra of normalized differential absorbance of DOM in the influent (inlet, a), Plant A settled (AS, b) and filtered (AF, c), and Plant B settled (BS, d) and filtered (BF, e) water. Reference pH value 2.5.

Figure 8. Comparison of effects of pH on the differential log-transformed absorbance at wavelength 350 nm and their NICA-based fitting for DOMs in RO isolates of influent (inlet), Plant A settled (AS) and filtered (AF) and Plant B settled (BS) and filtered (BF) water.

Figure 9. Correlation between the percentage of HAS in total protonation-active groups vs. HPOA, THPA and HPI. DOMs in RO isolates of influent (inlet), Plant A settled (AS) and filtered (AF), and Plant B settled (BS) and filtered (BF) water.

493 **Table 1 Contributions of hydrophobic, transphilic and hydrophilic fractions in**
 494 **the concentrations of dissolved organic carbon in RO isolates of influent (inlet),**
 495 **Plant A settled (AS) and filtered (AF), and Plant B settled (BS) and filtered (BF)**
 496 **water.**

DOM fraction	Abbreviation	Inlet	AS	AF	BS	BF
Hydrophobic bases	HPOB	10%	9%	8%	8%	9%
Hydrophobic acids	HPOA	24%	15%	14%	17%	14%
Hydrophobic neutrals	HPON	39%	48%	48%	46%	44%
Transphilic bases	TPHB	3%	3%	2%	1%	2%
Transphilic acids	TPHA	3%	3%	4%	3%	4%
Transphilic neutrals	TPHN	12%	13%	13%	15%	14%
Hydrophilic fraction	HPI	9%	10%	11%	10%	13%

497 Table 2 NICA modeling parameters for protonation-active groups in DOMs
 498 determined by DlnA350 in spectrophotometric titrations. DOMs in RO isolates
 499 of influent (inlet), Plant A settled (AS) and filtered (AF), and Plant B settled (BS)
 500 and filtered (BF) water.

	Inlet	AS	AF	BS	BF
$\tilde{K}_{LAS}$	2.68	2.80	2.88	2.64	2.64
$\tilde{K}_{HAS}$	6.58	6.07	6.22	6.10	6.30
DlnA _{LAS}	0.214	0.371	0.472	0.303	0.334
DlnA _{HAS}	0.238	0.272	0.327	0.241	0.278
m_{LAS}	0.99	0.71	0.73	0.75	0.85
m_{HAS}	0.67	0.68	0.73	0.84	0.64

Figure 1 DOC-normalized differential absorbances of DOM in RO isolates of Plant A settled (AS) and filtered (AF), and Plant B settled (BS) and filtered (BF) waters vs. the absorbance spectrum of influent (inlet water)

Figure 2 Gaussian band fitting of the DOC-normalized differential absorbance of DOM in RO isolates of Plant A settled (AS, a) and filtered (AF, b), and Plant B settled (BS, c) and filtered water (BF, d). Absorbance spectrum of the influent (inlet)

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512 **Figure 3. Normalized differential absorbance of hydrophobic, transphilic and**
 513 **hydrophilic fractions in DOM in RO isolates of influent (inlet) water. Normalized**
 514 **by absorbance at wavelength 220 nm, reference to DOM before fractionation.**
 515 **The normalized differential absorbance spectra of HPON and TPHN fraction**
 516 **were deduced by the normalized differential absorbance spectra of the effluent**
 517 **of XAD8 or XAD4 resin at pH 2 and pH12, respectively.**

522 **Figure 4. Gaussian band fitting of the normalized differential absorbance by**
523 **intensity at 220 nm and DOC concentration of DOM fractions in water**
524 **treatment processes referenced to the inlet waters.**

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553 **Figure 9. Correlation between the percentage of HAS in total**
 554 **protonation-active groups vs. HPOA, TPHA and HPI. DOMs in RO isolates of**
 555 **influent (inlet), Plant A settled (AS) and filtered (AF), and Plant B settled (BS)**
 556 **and filtered (BF) water.**

Graphical Abstract