Two analytical approaches quantifying the electron donating capacities of dissolved organic matter to monitor its oxidation during chlorination and ozonation

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Abstract

Electron-donating activated aromatic moieties, including phenols, in dissolved organic matter (DOM) partially control its reactivity with the chemical oxidants ozone and chlorine. This comparative study introduces two sensitive analytical systems to directly and selectively quantify the electron-donating capacity (EDC) of DOM, which corresponds to the number of electrons transferred from activated aromatic moieties, including phenols, to the added chemical oxidant 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) radical cation (i.e., ABTS•+). The first system separates DOM by size exclusion chromatography (SEC) followed by a post-column reaction with ABTS•+ and a spectrophotometric quantification of reduction of ABTS•+ by DOM. The second system employs flow-injection analysis (FIA) coupled to electrochemical detection to quantify ABTS•+ reduction by DOM. Both systems have very low limits of quantification, allowing determination of EDC values of dilute DOM samples with <1 mg carbon per liter. When applied to ozonated and chlorinated model DOM isolates and real water samples, the two analytical systems showed that EDC values of the treated DOM decrease with increasing specific oxidant doses. The EDC decreases detected by the two systems were in overall good agreement except for one sample containing DOM with a very low EDC. The combination of EDC with UV-absorbance measurements gives further insights into the chemical reaction pathways of DOM with chemical oxidants such as ozone or chlorine. We propose the use of EDC in water treatment facilities as a readily measurable parameter to determine the content of electron-donating aromatic moieties in DOM and thereby its reactivity with added chemical oxidants.
Keywords

Electron donating capacity
Dissolved organic matter
Ozonation
Chlorination
Size exclusion chromatography
Flow-injection analysis

Abbreviations

$A_{254}$: Absorbance at 254 nm
ABTS: 2,2'‐azino‐bis(3‐ethylbenzothiazoline‐6‐sulphonate)
ABTS$^{•+}$: One‐electron oxidation product of ABTS
DOC: Dissolved organic carbon
DOM: Dissolved organic matter
EDC: Electron donating capacity
EDC$_0$: Electron donating capacity of unaltered DOM
FIA: Flow‐injection analysis
MEO: Mediated electrochemical oxidation
SEC: Size exclusion chromatography
SUVA$_{254}$: Specific UV absorbance at 254 nm
1. Introduction

Dissolved organic matter (DOM) is a complex mixture of low- and high-molecular weight organic molecules originating from various biological precursor materials (Stenson et al. 2003). Because of the diversity of its source materials and extents of biogeochemical processing, DOM covers a wide range of physicochemical properties. In water treatment, DOM interferes with coagulation, membrane filtration, adsorption, and chemical oxidation processes (Amy 2008, Kennedy and Summers 2015, Köhler et al. 2016, Velten et al. 2011, von Sonntag and von Gunten 2012, Yuan and Zydney 1999). In the latter case, DOM is a significant sink for chemical oxidants such as chlorine and ozone and may thus lower the efficiency for disinfection and micropollutant abatement (Deborde and von Gunten 2008, Lee and von Gunten 2016, von Gunten 2003, von Sonntag and von Gunten 2012). While the oxidant dose may be increased to compensate for oxidant consumption by DOM, this may also increase the formation of undesired disinfection by-products (DBPs) from the reaction of the oxidant with DOM. In the case of chlorine, these by-products are potentially carcinogenic (Hammes et al. 2006, Krasner et al. 2013, Lavonen et al. 2013, Richardson 2003, Richardson 2011, Richardson et al. 2007, Zhang and Minear 2006). Reaction of DOM with ozone produces assimilable organic carbon, which in turn leads to biological growth in the treated water (Collins and Vaughan 1995, Escobar and Randall 2001, Hu et al. 1999, Van der Kooij et al. 1989). High ozone exposures also increase bromate formation in bromide-containing waters (von Gunten 2003). Therefore, the oxidant dose has to be optimized for an effective disinfection and abatement of micropollutants while minimizing the DBP formation. For this purpose, the concentration and the reactivity of the DOM plays a crucial role.
A common approach in water treatment for online determination of DOC concentration is the measurement of the UV-visible light absorption of DOM (Her et al. 2002, Huber et al. 2011, Korshin et al. 1997, Leenheer and Croue 2003) or the specific UV-absorbance at 254 nm (SUVA\textsubscript{254}), which is obtained by normalizing the measured absorbance at 254 nm by the DOC concentration. SUVA\textsubscript{254} is commonly used as a proxy for DOM aromaticity, the fraction of aromatic to total carbon. The SUVA\textsubscript{254} values of numerous organic matter isolates were found to correlate with aromaticity determined by \textsuperscript{13}C-NMR (Traina et al. 1990, Weishaar et al. 2003, Westerhoff et al. 1999). Several studies showed that the concentrations of chlorinated DBPs formed by reaction of chlorine with DOM were positively correlated with SUVA\textsubscript{254} values and that the chlorination of DOM resulted in a decrease in the UV absorbance (Korshin et al. 1997, Reckhow et al. 1990). However, the correlations between SUVA\textsubscript{254} and DBP formation vary among differing DOM samples and SUVA\textsubscript{254} has only limited value as a predictor for the formation of DBPs during chlorination of DOM from varying sources (Fram 1999, Weishaar et al. 2003). These limitations were ascribed to SUVA\textsubscript{254} values providing only a measure for DOM aromaticity but not for the entire fraction of DBP-producing moieties (trihalomethanes, Weishaar et al. 2003). For ozone consumption kinetics during reactions with DOM (natural waters and isolates) no satisfying correlations could be found with SUVA\textsubscript{254} (Elovitz et al. 2000). Nevertheless, UV absorbance changes during ozonation have been successfully applied as a surrogate parameter for the abatement of micropollutants during enhanced wastewater ozonation (Bahr et al. 2007, Wert et al. 2009). This approach is based on the competition for ozone consumption between chromophoric DOM reactive sites and micropollutants.
We recently introduced mediated electrochemical oxidation (MEO) to quantify electron-donating activated aromatic moieties (mostly phenols) in DOM (Aeschbacher et al. 2009, Aeschbacher et al. 2011). This method relies on the electrochemical quantification of the number of electrons transferred from DOM to the radical cation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) (i.e., ABTS$^{•+}$). ABTS$^{•+}$ was used as chemical oxidant because it has a sufficiently high standard reduction potential ($E_{\text{H}^0}(\text{ABTS}^{•+}/\text{ABTS})= 0.7 \ \text{V}$) (Scott et al. 1993) to oxidize phenols and is readily reduced in a pH-independent, reversible one-electron transfer. The number of electrons transferred from DOM to ABTS$^{•+}$ per mass of DOM is referred to as the electron donating capacity (EDC). While the EDC is operationally defined (i.e., EDC increases with increasing pH, $E_{\text{H}}$ and reaction time, (Aeschbacher et al. 2012)), the EDC values of a diverse set of model DOM isolates were found to linearly correlate with their titrated phenol contents (Aeschbacher et al. 2012). We quantified changes in the EDC of DOM upon oxidation with chlorine dioxide (ClO$_2$), chlorine (HOCl) and ozone (O$_3$) (Wenk et al. 2013). We demonstrated that chlorination resulted in pronounced decreases in EDC values but comparatively small decreases in SUVA$_{254}$ values, suggesting that chlorine primarily reacted by electrophilic aromatic substitution leading to chlorophenols, which still absorb UV light, but cannot be oxidized by ABTS$^{•+}$. Ozonation of the same DOM resulted in more comparable relative EDC and absorbance losses, suggesting that ozone reacted by electrophilic addition to aromatic structures, followed by ring cleavage (Ramseier and von Gunten 2009). These findings demonstrated that MEO allows quantifying changes in DOM redox state during chemical oxidation and—in combination with SUVA$_{254}$ measurements—provided insights into possible oxidation pathways of DOM. This is further supported by a recent study in which the decreases in both EDC
and SUVA$_{254}$ during DOM ozonation were applied as proxies for micropollutant abatement and bromate formation (Chon et al. 2015). Furthermore, in a chemical oxidation study of low molecular weight model phenols, EDC was shown to correlate with chlorine demand, suggesting that monitoring EDC during chemical water treatment may help identifying optimal oxidant doses (de Vera et al. 2017).

While these studies demonstrated the usefulness of EDC for monitoring the oxidation state of DOM during chemical oxidation and determining DOM oxidant demand, the original MEO method (Aeschbacher et al. 2010, Aeschbacher et al. 2012) required advancements on two levels for a more widespread application during oxidative water treatment: an automated sample analysis and an improved sensitivity to quantify EDC under realistic conditions. To this end, two analytical methods have recently been introduced. First, an LC-based system in which DOM is first passed through a SEC column followed by post-column oxidation of DOM by preformed ABTS$^{•+}$. The extent of reduction of ABTS$^{•+}$ was quantified based on the loss of absorbance of ABTS$^{•+}$ at 405 nm (Chon et al. 2015). Second, a flow-injection analysis (FIA) system in which DOM is reacted with electrochemically-generated ABTS$^{•+}$, followed by chronoamperometric –instead of a spectrophotometric– detection in an electrochemical flow-through detector to quantify the extent of ABTS$^{•+}$ reduction by DOM (Walpen et al. 2016). While both methods were shown to reliably determine EDC in water samples with low DOM concentrations, a systematic investigation of the capabilities of the methods to monitor chemical DOM oxidation was missing.

The objective of this study was to provide a systematic assessment of the performances of the two analytical methods, subsequently abbreviated as SEC-EDC and FIA-EDC, to quantify decreases in the EDC of diverse DOM samples upon chemical oxidation. To this end, we treated water samples containing DOM with
different doses of ozone or chlorine and quantified the resulting losses in EDC using the two methods. To span a wide range of DOM characteristics, the treated samples included solutions prepared from three model DOM isolates and three real water samples collected in Switzerland and in Sweden. Based on the results, we evaluate the applicability of the two MEO methods in water treatment facilities to monitor DOM oxidation during chlorination and ozonation.

2. Material and methods

2.1 Chemicals

The chemicals used in the study are listed in the Supplementary Material in Appendix A.

2.2 DOM samples

2.2.1 Model DOM isolates

Three model DOM isolates were obtained from the International Humic Substances Society (St. Paul, Minnesota, USA) and used as received: Pony Lake Fulvic Acid (PLFA, 1R109F), Nordic Lake Aquatic Natural Organic Matter (NNOM, 1R108N) and Suwannee River II Standard Fulvic Acid (SRFA, 2S101F). We selected these three isolates because they cover a range of physicochemical properties and have different origins. Detailed information on the isolates, including selected chemical characteristics (aromaticity and elemental composition), are provided in Table S1.

Stock solutions of the DOM isolates were prepared by dissolving the material in unbuffered, ultrapure water (approximately 50 mgC·L⁻¹). The exact concentration of each stock solution was determined by diluting aliquots 10- and 50-fold in phosphate buffer (5 mM phosphate buffer, pH 7; 0.1 M NaCl), measuring the
absorption coefficient at 254 nm ($a_{254}$) using a spectrophotometer (Cary 100, Varian, USA) and calculating the DOC concentration from predetermined SUVA$_{254}$ values (Table S2).

2.2.2 Real water samples

Three real water samples were included in this study to investigate chemical oxidation of DOM that have not been pre-extracted. The first sample was collected from the wastewater effluent (WWE after biological treatment (Bourgin et al. 2018)) of the wastewater treatment plant Neugut in Dübendorf, Switzerland. The other two samples were collected from the surfaces of Lake Sjököp (SLW) and Lake Vomb (VLW) in Sweden. We selected Lake Sjököp because it has high DOC concentrations, presumably due to organic matter input from a peatland surrounding the lake. In contrast, Lake Vomb is a drinking water source with low DOC concentrations. The exact sampling locations and selected physicochemical properties of the samples (pH, DOC, and SUVA$_{254}$) are provided in Table S3. The water samples were filtered through pre-rinsed cellulose nitrate membranes (0.45-µm pore size; Sigma Aldrich, Switzerland) on the day they were collected to remove particulate organic matter and microorganisms. The samples were stored at 4 °C in the dark until use (within 10 days of sampling). The absorbance spectrum of the filtered real water samples did not change between sampling and analysis, suggesting minimal if any microbial degradation of the DOM during sample storage.

2.3 Chemical oxidation of DOM

We used a commercially available hypochlorite solution (6-14%) to prepare chlorine stock solutions (10 mM HOCl) in unbuffered, ultrapure water. The exact chlorine concentrations of the stocks were determined spectrophotometrically either by absorbance measurements at 290 nm ($ε_{290} = 350$ M$^{-1}$·cm$^{-1}$ (Soulard et al. 1981)) or...
by reaction with \(N,N\)-diethyl-p-phenylenediamine (DPD) followed by quantification of the oxidation product of DPD by absorbance measurement at \(\lambda = 515\) nm (American Public Health Association, 2012).

Ozone stock solutions of approximately 1.5 mM ozone were obtained with an ozone generator (Innovatec CMG 3-3 ozone generator (Rheinbach, Germany)) from pure oxygen. The generated ozone gas was sparged through unbuffered, ultrapure water cooled on ice. The exact ozone concentration in the resulting stock solutions was determined spectrophotometrically at 260 nm (\(\varepsilon_{260} = 3200\) M\(^{-1}\)·cm\(^{-1}\) (von Sonntag and von Gunten 2012)) or by reaction with indigo (Bader and Hoigné 1981, von Sonntag and von Gunten 2012).

Prior to the chemical oxidation with chlorine or ozone, the model DOM isolate stock solutions were diluted to 2.5 mg\(\text{C} \cdot \text{L}^{-1}\) in phosphate buffer (final phosphate concentration: 10 mM; pH 7). The real water samples were diluted to 2.5 mg\(\text{C} \cdot \text{L}^{-1}\) with ultrapure water and subsequently adjusted to pH 7 using HCl (0.2 M; a final concentration of 24 and 45 \(\mu\)M of HCl was required for VLW and WWE, respectively). We oxidized the DOM solutions by adding a defined volume of the oxidant stock solutions to obtain the desired specific oxidant dose from 0 to 0.2 mol\(\text{HOCl} \cdot \text{molC}^{-1}\) for chlorine and from 0 to 0.5 mol\(\text{ozone} \cdot \text{molC}^{-1}\) for ozone. We stored the ozonated samples at room temperature for 24 h and stored the chlorinated samples in the dark for 48 h at room temperature, to ensure complete reaction of the respective oxidant prior to sample analyses. Complete reaction was confirmed by control measurements of the residuals of the respective oxidant. All experiments were carried out in the presence of \(t\)-BuOH (100 mM). In ozonation experiments, \(t\)-BuOH served to quench hydroxyl radicals, which are formed during ozone decay (von Sonntag and
von Gunten 2012). To match the solution matrix of the ozonation experiments, chlorination experiments were also run in the presence of 100 mM t-BuOH.

2.4 Quantification of EDC of DOM samples

Figure 1 shows a scheme of the two analytical system setups to quantify EDC, SEC-EDC and FIA-EDC. The components and the sample analysis principles of the two systems are described in the following.

2.4.1. SEC-EDC system

The SEC-EDC system was briefly described in a previous publication (Chon et al. 2015). In this system, DOM samples (volume: 2.5 mL) were analyzed using a high performance liquid chromatography system (Ultimate 3000, Thermo Fisher Scientific) connected to a SEC column (8 mm x 300 mm, Toyopearl HW50S, Tosoh Bioscience, Japan) packed with macroporous hydroxylated methacrylic polymer beads with a mean bead diameter of 35 µm. The eluent consisted of 50 mM borate at pH 7.8 with a flow rate of 0.2 mL min⁻¹. The SEC column had an exclusion limit of 80'000 Da (Tosoh Bioscience, 2018). Following SEC, the solution passed through a UV detector (PDA 3000, Thermo Fisher Scientific). The SEC column was used to separate DOM from dissolved ions in the injected solution that may affect the reaction of DOM with ABTS⁺⁺ in the post-SEC reaction. The solution was then passed through a mixing tee, in which the ABTS⁺⁺ reactant solution was mixed in at a flow rate of 50 µL min⁻¹. The ABTS⁺⁺ reactant solution was delivered pneumatically (PC10, Dionex, USA) from a reservoir to the mixing tee and was prepared by adding sodium hypochlorite sub-stoichiometrically to ABTS (Chon et al. 2015, Pinkernell 2000). The combined SEC-effluent and the reactant solution had a pH of 7.8 and were passed through a knitted polytetrafluoroethylene (PTFE) reaction coil (Dionex, USA; internal volume of 750 µL, corresponding to a delay time of 3 min), in which ABTS⁺⁺ was
reduced by electron transfer from electron-donating moieties of the DOM. The extent of ABTS$^{+}$ reduction was subsequently quantified spectrophotometrically in a flow-through cell (path length: 1 cm, UltiMate 3100 VWD UV-detector, Thermo Fisher Scientific) in which the absorbance at 405 nm was continuously recorded. ABTS$^{+}$ has a strong absorbance at 405 nm, whereas the reduced species, ABTS, shows negligible absorbance at this wavelength (Pinkernell et al. 1997).

The EDC was calculated from the absorbance loss peaks at 405 nm as follows (eq. 1):

$$EDC = - \frac{1}{m_C} \cdot \left( q_v \cdot \frac{\int (A_{405}(t) - A_{405, baseline}) dt}{\varepsilon_{405} l} \right) \quad \text{Eq. 1}$$

where $m_C$ is the mass of carbon injected, $q_v$ is total volumetric flow rate passing through the detector ($2.5 \cdot 10^{-3}$ L·min$^{-1}$), $\varepsilon_{405}$ is the molar absorption coefficient of ABTS$^{+}$ at 405 nm ($\varepsilon_{405} = 31'600$ M$^{-1}$·cm$^{-1}$) (Pinkernell et al. 1997), $l$ is the optical path length of the flow cell (1 cm), $A_{405}$ is the absorbance measured at 405 nm, and $A_{405, baseline}$ is the baseline absorbance measured at 405 nm (i.e., the absorbance in the absence of a sample). The term in parenthesis on the right side of equation 1 converts the ABTS$^{+}$ absorbance loss peak to moles of electrons donated by the injected DOM sample to ABTS$^{+}$. This conversion from a concentration dependent reading (i.e., absorbance) to an absolute amount of substance converted (i.e., ABTS$^{+}$ reduced) is well established for spectrophotometric detections on flow injection analysis systems and is considered to also apply to the SEC-EDC system studied herein.

(Figure 1)

2.4.2 FIA-EDC system

We used a previously described FIA system (Walpen et al. 2016) to quantify EDC values of DOM samples (Figure 1). Briefly, DOM samples were sequentially
loaded through a 11-port/10-position selector valve into a sample loop (volume 100 µL) of a 10-port/2-position injector valve, from which they were injected into a buffered carrier stream (pH 7, 100 mM phosphate; \( q_{\text{carrier}} = 75 \, \mu\text{L} \cdot \text{min}^{-1} \)). The carrier stream was delivered to a mixing tee where it was continuously mixed with the reagent stream (\( q_{\text{reagent}} = 15 \, \mu\text{L} \cdot \text{min}^{-1} \)). The reagent stream contained electrochemically produced ABTS\(^{•+}\) (450 µM) and was weakly buffered to pH 4 (1 mM acetate, 100 mM KCl) at which ABTS\(^{•+}\) was stable (note that the stability of ABTS\(^{•+}\) decreases with increasing pH (Walpen et al. 2016)). The combined carrier and reagent solutions were then delivered through a knitted PTFE reaction coil (Biotech, Sweden) with an internal volume of 2 mL, corresponding to a reaction time of 22 min. The extent of reduction of ABTS\(^{•+}\) by DOM was quantified amperometrically in an electrochemical flow cell with a glassy carbon working electrode (ALS, Japan). The working electrode in the detector was polarized to the \( E_H \) measured in the solution in the absence of injected samples (i.e., the open-circuit potential defined by the ABTS\(^{•+}/\text{ABTS} \) couple in solution; \( E_H = +0.71 \pm 0.01 \) V), resulting in background currents <10 nA. Electron transfer from DOM to ABTS\(^{•+}\) lowered the ratio of ABTS\(^{•+}\) to ABTS and hence the \( E_H \) of the solution relative to the \( E_H \) applied to the working electrode. The \( E_H \) offset led to the oxidation of ABTS and hence oxidative current peaks.

To calibrate the current response of the electrochemical detector, we injected solutions containing the redox standard ascorbate (EDC\(_{\text{ascorbate}} = 2.00 \, \text{mol}_e \cdot \text{mol}_{\text{ascorbate}}^{-1} \) (Walpen et al. 2018)) at concentrations of 10, 5, 1 and 0 µM. The calibration curve of oxidative current peak area versus number of electrons donated by ascorbate to ABTS\(^{•+}\) was used to convert the measured oxidative current responses of DOM samples to EDC values according to the following equation:
EDC = −\( \frac{1}{m_C} \cdot \frac{E_{DC_{ascorbate}}}{a_{ascorbate}} \cdot \int (I(t) - I_{baseline}) \, dt \)  

\text{Eq. 2}

where \( m_C \) is the mass of carbon injected, \( a_{ascorbate} \) (C·mol\(_{ascorbate}^{-1}\)) is the slope of the linear calibration curve of the area of the oxidative current peaks from the injections of ascorbate standards (see above), \( I \) is the oxidative current in response to an injected sample, and \( I_{baseline} \) is the oxidative current baseline (i.e., the oxidative current in the absence of an injected sample).

The ABTS\(^*\) reagent solution was prepared by direct electrochemical oxidation of an ABTS solution (total ABTS concentration of 750 µM, pH 4 (1 mM acetate)) in an electrochemical cell with a glassy carbon cylinder as working electrode polarized to a reduction potential of \( E_{H_2} = 0.82 \) V until the concentration of ABTS\(^*\) reached 450 µM (Walpen et al. 2016).

All electrochemical analyses were controlled with an electrochemical analyzer (630D, CH Instruments, Austin, TX, USA). All reduction potentials (\( E_{H_2} \)) were measured against Ag/AgCl reference electrodes but are reported relative to the standard hydrogen electrode.

We conducted the FIA measurements and prepared the reagent and redox standard stock solutions inside an anoxic glove box (\( N_2 \) atmosphere, \( O_2 < 2.3 \) ppm) with anoxic water. Water and buffer solutions were sparged with \( N_2 \) for two hours before transferring them into the glove box. The DOM samples were sparged for 15 min with \( N_2 \) prior to being transferred into the glove box. The DOM samples were diluted outside the glovebox two- to four-fold in concentrated phosphate buffer (200 mM) in order to match the carrier buffer (100 mM phosphate).
3. Results and discussion

3.1 Performance assessment of the SEC-EDC system

3.1.1. Size separation of DOM on the SEC column

We assessed size separation of the three model DOM isolates (i.e., SRFA, NNOM and PLFA) on the SEC column in the SEC-EDC system by continuously monitoring the absorbance of the SEC column eluates at 254 nm. Figure 2 shows representative area-normalized absorbance traces that resulted from the injections of the three DOM isolates (injection volumes of 2.5 mL of solutions with DOM concentrations of 2 mgC·L⁻¹). All three DOM isolates eluted between 30 or 35 and 65 min after injection in the form of broad peaks. SRFA and NNOM started to elute from the SEC column approximately 30 min, whereas PLFA eluted at approximately 35 min after injection. This finding suggests that the largest molecules in PLFA were smaller than those in SRFA and NNOM. The broad chromatographic peaks of all three DOM isolates are consistent with the wide molecular size distributions of DOM (Chin et al. 1994, Huber et al. 2011) and demonstrated their separation on the SEC. A detailed discussion of the separation is provided in the Supplementary Material in which we compare the SEC-EDC system to a previously published SEC system (Huber et al. 2011).

(Figure 2)

3.1.2. Linearity in the response on SEC-EDC system

The SEC-EDC system quantifies the oxidation states of injected DOM by determining the extent to which electron-donating moieties in the DOM reduce ABTS⁺⁺ to ABTS, which results in a solution absorbance loss at 405 nm. We established linearity in the absorbance loss at 405 nm with increasing injected
concentrations of the model DOM isolates. Figure 3a shows the baseline-corrected absorbance loss peaks, which increased in size as the injected amount of SRFA increased from 1.25 to 7.5 µg C (i.e., 2.5 mL of SRFA solution standards with concentrations from 0.5 to 3.0 mg C·L⁻¹). Similarly, injections of increasing amounts of NNOM and PLFA resulted in increasing sizes of the absorbance loss peaks (Figure S2 and S3 (SM) for PLFA and NNOM, respectively). For all three DOM isolates, the areas of the absorbance loss peaks increased linearly with increasing injected amounts of DOM (Figures 3b for SRFA and S2 and S3 for PLFA and NNOM, respectively; the R²-values of linear regression fits were 0.994, 0.985 and 0.992 for SRFA, PLFA and NNOM, respectively).

We converted the areas of the absorbance loss peaks to number of electrons transferred to ABTS²⁺ and, subsequently, to the EDC of SRFA, NNOM, and PLFA (Equation 1). The obtained EDC values were positively correlated to the EDC for the same DOM isolates reported determined by MEO (Aeschbacher et al. 2012). At the same time, the EDC values quantified by SEC-EDC were approximately 43% of the values determined by MEO (Figure S4). These smaller values reflected the relatively short reaction time of 3 min between DOM and ABTS²⁺ in the reaction coil of the SEC-EDC system. It has previously been demonstrated that EDC values increase with increasing reaction times between DOM and ABTS²⁺ in a reaction coil and that reaction times >20 min are needed to approach EDC values obtained by MEO (Walpen et al. 2016). While the reaction time on the SEC-EDC system can be increased by increasing the length of the reaction coil (or by decreasing the volumetric flow rate), such measures would increase the time for analysis per sample. Also, we subsequently report and discuss decreases in the EDC of DOM during chemical oxidation in relative instead of in absolute terms by normalizing EDC of
treated samples to the EDC of the respective untreated DOM sample (see sections 3.3 and 3.4). This normalization allows comparing the two analytical methods with regards to the trends in the EDC values of treated samples.

The absorbance loss peaks in Figure 3a and Figures S2a and S3a indicate that the limit of quantification (LOQ) for EDC on the SEC-EDC system was below 1.25 µgC. At an injection volume of 2.5 mL, this amount corresponds to solutions with a concentration of 0.5 mgC·L⁻¹. We therefore expected that the sensitivity of the SEC-EDC system was sufficiently high to allow quantifying changes in DOM redox states during chemical oxidation in real water samples, as demonstrated in section 3.4.

(Figure 3)

3.2 Performance assessment of the FIA-EDC system

In the FIA-EDC system, the DOM sample is directly injected into a carrier stream, which is continuously mixed with a reagent solution containing ABTS⁺. Reduction of ABTS⁺ to ABTS by DOM in the reaction coil lowers the Eh(ABTS⁺/ABTS) in solution relative to the Eh applied to the working electrode of the electrochemical flow-through detector, thereby giving rise to an oxidative current response. Figure 3c shows oxidative current peaks that resulted from successive injections of increasing amounts of SRFA into the FIA-EDC system. Both the peak heights (not shown) and areas (Figure 3d) increased linearly with increasing injected amounts of SRFA.

To quantify the absolute EDC value of SRFA from this data, we calibrated the FIA system using the oxidative current responses obtained from injecting different amounts of the redox standard ascorbate with EDC= 2.00 mol_e⁻·mol_ascorbate⁻¹ (current responses also shown in Figure 3c). This calibration was necessary because the
working electrode in the electrochemical flow cell only senses the solution in close
proximity to its surface. Using equation 2, we converted the linear regression line in
Figure 3d to an EDC of SRFA of 5.75 ± 0.19 mmol_e·g_C⁻¹. This value and the EDC
values of PLFA (2.15 ± 0.06 mol_e·g_C⁻¹) and NNOM (4.15 ± 0.13 mol_e·g_C⁻¹)
quantified on the FIA-EDC system were in very good agreement with the EDC of the
same DOM isolates previously quantified by MEO at pH 7 and an applied reduction
potential of $E_H = 0.71$ V (Aeschbacher et al. 2012) (see Figure S4). The good
agreement of EDC quantified by FIA-EDC and MEO show that the 22 min reaction
time of the model DOM isolates with ABTS•⁻ in the reaction coil of the FIA-EDC
system was sufficiently long to approach the EDC determined in MEO with an
approximate reaction time of 40 min (Aeschbacher et al. 2012).

We previously determined a limit of quantification (LOQ) on the FIA-EDC
system of approximately 10 to 50 ng_c for model DOM isolates (depending on their
EDC) (Walpen et al. 2016). This LOQ range is in good agreement with the oxidative
current peak in Figure 3c in response to the smallest injected amount of SRFA of 190
ng_c (well above the LOQ). With an injection volume of 100 µL, the LOQ of 10 to 50
ng_c corresponds to solutions with DOM concentrations of 0.1 to 0.5 mgC·L⁻¹. This
range is at the lower end of DOM concentrations for many natural water samples,
suggesting that the FIA-EDC system allows quantifying changes in DOM redox states
during chemical oxidation of natural DOM water samples, as demonstrated in section
3.4.

3.3 Effects of ozonation and chlorination on redox states of model DOM isolates

Figure S5 shows the changes in the absolute EDC values of the model DOM
isolates upon treatment with ozone and chlorine, as quantified by FIA-EDC. In the
following discussion, we report the EDC of DOM treated at a specific oxidant dose
relative to the EDC of the respective untreated DOM \((EDC_0)\) (i.e., \(EDC \cdot (EDC_0)^{-1}\)).

This normalization allows comparing results from the SEC-EDC and FIA-EDC systems, despite the different absolute EDC that these systems quantify for the same DOM sample (Figure S4).

3.3.1 Effect of ozonation on EDC of model DOM isolates

We exposed all three model DOM isolates to increasing specific ozone doses (from 0.05 to 0.5 mol\(_\text{ozone}\)·mol\(_\text{C}^{-1}\)), followed by quantifying the resulting changes in EDC of the treated DOM samples. On both the SEC-EDC and FIA-EDC systems, the EDC·(EDC\(_0\))\(^{-1}\) of the treated DOM decreased with increasing specific ozone doses (Figure 4a, b). At the highest specific ozone dose of 0.5 mol\(_\text{ozone}\)·mol\(_\text{C}^{-1}\), the EDC values had decreased to between 20 and 5% of the EDC of the untreated DOM samples. The pronounced decreases in EDC·(EDC\(_0\))\(^{-1}\) demonstrate that reaction of the added ozone with the DOM resulted in the loss of electron-donating moieties, most likely activated aromatic moieties, including phenols. These results are consistent with previous studies which reported that ozonation of DOM samples decreased their EDC (Chon et al. 2013, Wenk et al. 2013).

The decrease in EDC·(EDC\(_0\))\(^{-1}\) with increasing specific ozone doses was approximately linear at the lower doses but leveled off at non-zero values for higher ozone doses (Figure 4a, b). Per unit of ozone added, more electron-donating moieties in the DOM were therefore lost at the lower than at the higher specific ozone doses. This dose dependency in the EDC indicated that a small fraction of the electron-donating moieties was resistant to reaction with ozone and thus remained in the DOM even at the highest tested ozone doses. (Figure 4)
3.3.2 Effect of chlorination on EDC of model DOM isolates

Consistent with the results of ozonation, analysis of chlorinated model DOM isolates on both the SEC-EDC and the FIA-EDC systems showed decreasing EDC·(EDC$_0^{-1}$) for the three DOM isolates with increasing specific HOCl doses (Figure 4c, d). This finding is consistent with earlier work which used MEO and more concentrated DOM solutions to show that chlorination of three model DOM isolates decreased their EDC (Wenk et al. 2013). At the highest chlorine dose of 0.2 mol$_{\text{HOCl}}$·(mol$_{\text{C}}$)$^{-1}$, only the responses to injections of SRFA, the model DOM with the highest initial EDC, were sufficiently large on both the SEC-EDC and FIA-EDC systems to allow for an accurate quantification of residual EDC.

On both analysis systems, the EDC·(EDC$_0^{-1}$) of all three model DOM isolates appeared to decrease in a more linear fashion with increasing specific HOCl dose as compared to the decreases in EDC·(EDC$_0^{-1}$) during ozonation. The more linear decrease in EDC·(EDC$_0^{-1}$) during chlorination may have reflected increasing extents of chlorination of phenolic moieties in the DOM with increasing specific HOCl doses (Gallard and von Gunten 2002, Wenk et al. 2013). Such (poly)chlorinated phenolic moieties form by electrophilic attack of HOCl on the phenolic moiety of the DOM. The attack initially starts at the ortho- and para-position of the phenols, and continues with a gradual increased chlorination of the phenol moieties in the DOM. It can be expected that formed (poly)-chloro-phenols are less oxidizable by ABTS$^{++}$ and thus led to decreasing EDC·(EDC$_0^{-1}$).
3.4 Effects of ozonation and chlorination on redox states of real water DOM samples

3.4.1 Quantification of EDC of DOM in real water samples

We first quantified the absolute EDC values of the DOM in the three real water samples (at concentrations of 2.5, 2.5 and 3.85 mgC·L⁻¹ for SLW, VLW and WWE, respectively) using the FIA-EDC system. The specific EDC values decreased in the order SLW (4.86 mmolₑ·(gₑ)⁻¹) > WWE (3.72 mmolₑ·(gₑ)⁻¹) > VLW (1.17 mmolₑ·(gₑ)⁻¹). The high EDC value of SLW likely reflects that the lake from which the sample was collected contained (poly)phenolic DOM from the nearby peatland. We previously showed that DOM from peatlands has exceptionally high EDC as compared to aquatic and terrestrial model DOM isolates (Walpen et al. 2018, Walpen et al. 2016). By contrast, VLW had a lower EDC than any other DOM we previously analyzed ((Aeschbacher et al. 2012 Walpen et al. 2018, Walpen et al. 2016). This finding suggests that the DOM of VLW had a very low concentration of oxidizable, activated aromatic moieties consistent also with its very low SUVA₂₅₄ value (Table S3) as compared to previously analyzed aquatic model DOM isolates (Walpen et al. 2016). While the SUVA₂₅₄ of WWE was even smaller than that of VLW, it had a much higher EDC, indicating that this DOM had a comparatively high content of hydroxylated aromatic rings.

3.4.2 Effect of ozonation on EDC in real water samples

Ozonation of real water samples showed decreasing EDC·(EDC₀)⁻¹ on both the SEC-EDC and the FIA-EDC systems for increasing specific ozone doses (Figure 5a, b). This finding implies that (i) ozonation resulted in a loss of electron-donating moieties also in DOM in real water samples (and not only from model DOM isolates...
dissolved in the laboratory, as shown in sections 3.3.1 and 3.3.2) and (ii) both systems can be used to measure these losses. Similar to the observation for model DOM isolates, the $E_{DC} \cdot (E_{DC_0})^{-1}$ decreased linearly with increasing specific ozone doses at low doses but leveled off at higher doses.

On the SEC-EDC system, the $E_{DC} \cdot (E_{DC_0})^{-1}$ of WWE and VLW decreased to <20% for the sample with the highest specific ozone dose. The abatement of these moieties appeared less efficient in SLW, for which $E_{DC} \cdot (E_{DC_0})^{-1}$ decreased to only about 40% for the highest ozone dose. Analyses of the same samples on the FIA-EDC system showed comparable relative EDC decreases with increasing specific ozone doses for SLW and WWE. However, the measured decreases in EDC for VLW upon ozonation were much smaller on the FIA-EDC system. We will discuss potential causes for this apparent discrepancy between the two analytical systems in section 3.5.

3.4.3 Effect of chlorination on EDC in real water samples

Similar to the effects of ozonation, SEC-EDC and FIA-EDC analyses of the chlorinated real water samples showed decreasing $E_{DC} \cdot (E_{DC_0})^{-1}$ with increasing specific HOCl doses (Figure 5c, d), demonstrating that (i) HOCl removed electron donating moieties in the DOM of all three real water samples and (ii) that both analytical systems were capable of monitoring HOCl-induced oxidation of DOM in real water samples. With the exception of VLW on the FIA-EDC system, the decreases in $E_{DC} \cdot (E_{DC_0})^{-1}$ as a function of the specific HOCl doses again appeared to be more linear than the decreases in $E_{DC} \cdot (E_{DC_0})^{-1}$ with increasing specific ozone doses. Over the entire range of specific HOCl doses, both analytical systems showed
that chlorination resulted in higher EDC decreases for VLW and WWE than for SLW, suggesting that the latter contained a higher fraction of the total electron donating moieties that did not readily react with HOCl.

The two analytical systems showed a large difference in the decreases in EDC·(EDC₀)⁻¹ for VLW (Figure 5c, d). On the SEC-EDC system, the EDC of VLW decreased continuously with increasing specific HOCl dose to approximately 15% of its initial value at the highest tested chlorine dose of 0.2 mol HOCl·(mol C)⁻¹. Conversely, the EDC values of the same samples measured on the FIA-EDC system decreased more readily with increasing HOCl doses to negative values (i.e., the electrochemical cell yielded reductive instead of oxidative current responses) above a specific HOCl dose of 0.04 mol HOCl·(mol C)⁻¹. Furthermore, the errors in replicate analyses were larger, as shown by the relatively large error bars on the VLW data. These apparent differences in the results generated by the two analytical systems will be discussed in section 3.5.

3.5 Comparison of results from SEC-EDC and FIA-EDC systems

3.5.1 Analyses of ozonated and chlorinated model DOM isolates

To compare the results obtained from the two analytical systems, we plotted the decreases in EDC·(EDC₀)⁻¹ of the three model DOM isolates during ozonation and chlorination determined by FIA-EDC (y-axis) versus the EDC·(EDC₀)⁻¹ decreases of the same samples determined by SEC-EDC (x-axis). Figure 6a shows that the results from the two analysis systems were in very good agreement for ozonated model DOM isolates (i.e., the data scattered around the 1:1 line). This finding implies that ozonation of the model DOM isolates resulted in similar relative decreases in the contents of DOM moieties that readily donate electrons to ABTS⁺ during the 3 min in
the reaction coil of the SEC-EDC system and of the moieties that reacted with
ABTS'•+ over 25 min in the reaction coil of the FIA-EDC system.

While the results from the two systems were also in good overall agreement
for chlorinated model DOM isolates (Figure 6b), the data of SRFA and PLFA
showed more systematic deviations from the 1:1 line. In the case of SRFA, the
slightly smaller decreases in EDC·(EDC₀)⁻¹ on the FIA-EDC than the SEC-EDC
system may have resulted from the longer reaction time of the DOM with ABTS'•+
in the FIA-EDC system. Chlorination of SRFA likely formed chlorinated phenolic
moieties, which had slower reactivities with ABTS'•+ than the corresponding non-
chlorinated moieties. Monochlorinated phenols, which are formed for small chlorine
doses, may have been oxidized by ABTS'•+ in the FIA-EDC system with longer
reaction times but not in the SEC-EDC system with shorter reaction times, which
explain the observed larger decreases in EDC·(EDC₀)⁻¹ on the SEC-EDC than the
FIA-EDC system. With higher chlorine doses, where (poly)-chlorinated phenols may
be formed, there is no reaction with ABTS'•+ anymore, and both systems measured
low EDC·(EDC₀)⁻¹. By increasing the length of the reaction coil and therefore the
reaction time of ABTS'•+ with DOM in the SEC-EDC system this potential cause for
the deviation could possibly be eliminated.

The slightly smaller decreases in EDC·(EDC₀)⁻¹ of chlorinated PLFA on the
SEC-EDC than the FIA-EDC system (Figure 6b) may indicate that PLFA contains a
larger fraction of electron donating moieties that are only slowly oxidized by ABTS'•+.
3.5.2 Analyses of ozonated and chlorinated real water samples

Similar to the model DOM isolates, analysis of the ozonated and chlorinated SLW and WWE samples on the SEC-EDC and the FIA-EDC systems showed very similar changes in EDC·(EDC₀⁻¹) (Figure 6c, d). The agreement between results from the two systems was again very good for the ozonated samples (Figure 6c). Slight deviations of data points from the 1:1 line for the chlorinated SLW and WWE (Figure 6d) likely resulted from shorter ABTS⁺⁻ reaction times in the SEC-EDC than the FIA-EDC system, as rationalized above for SRFA (Figure 6b).

An overall good agreement could be observed for three model DOM isolates and two real water samples. Only the analysis of ozonated and chlorinated DOM in VLW showed larger differences between the two systems. The decreases in EDC·(EDC₀⁻¹) of VLW with increasing oxidant dose were smaller on the FIA-EDC than on the SEC-EDC system for ozonated samples (Figure 6c) but larger for the chlorinated samples (Figure 6d). While the cause for this apparent discrepancy between the systems (as well as the very different responses on the systems) remains unidentified, a number of factors likely contributed. First, the DOM in VLW had an exceptionally low EDC, resulting in comparatively small spectrophotometric and amperometric responses close to the LOQ on the SEC-EDC and FIA-EDC systems, respectively. These small responses further decreased with increasing chemical oxidation. Second, it is conceivable that inorganic ions in VLW samples interfered with the electrochemical detection on the FIA-EDC system but not with the spectrophotometric detection on the SEC-EDC system. Finally, it remains a possibility that the differences in the reaction times in the reaction coils of the SEC-
EDC and the FIA-EDC system contributed to the apparent discrepancy in the results obtained by the two systems.

3.6 Implications for water treatment and process research

3.6.1 Monitoring chemical oxidation of DOM in water treatment facilities

The SEC-EDC and the FIA-EDC systems are capable of detecting changes in the concentrations of electron-donating moieties in DOM, both in samples prepared from model DOM isolates as well as in real water samples, during its chemical oxidation by ozone and chlorine. The sensitivity of analyses on both systems is sufficiently high to be applicable to samples in water treatment facilities with very low DOC concentrations (0.5 - 1 mgC·L⁻¹). Furthermore, both systems allow for automated sample analyses. The presented analytical systems therefore overcome the two major analytical challenges of mediated electrochemical oxidation, the approach previously used to monitor chemical DOM oxidation (Wenk et al. 2013: insufficient sensitivity for low DOM samples and manual sample injection. With the previously applied method, quantification of EDC by MEO required approximately four-fold higher DOM concentrations (i.e., 10 mgC·L⁻¹) and, at the same time, sample volumes of 5 to 7 mL, corresponding to 50 to 70 µgC per analysis. Both the SEC-EDC and the FIA-EDC systems require much smaller amounts of DOM (i.e., < 1 µgC; see sections 3.1 and 3.2), which highlights their superior sensitivity over MEO and thus their applicability to real water samples with low DOC concentrations. In addition to their higher sensitivity, the SEC-EDC and FIA-EDC systems provide automated sample injection, whereas MEO requires manual sample addition to electrochemical cells. Taken together, both SEC-EDC and FIA-EDC have potential to become central analytical tools to determine changes in the concentrations and reactivity of electron-donating moieties in DOM during chemical water treatment.
For routine EDC analysis in water treatment, we recommend using spectrophotometric rather than electrochemical detection to quantify ABTS$^{•+}$ reduction by DOM. While spectrophotometric measurements are less sensitive, they are insensitive to variations in the conductivity of injected sample solutions and thus more robust than amperometric measurements. The use of an SEC column allows separating DOM from inorganic sample matrix constituents that may interfere with the analysis of the redox state of the DOM (e.g., VLW water). However, the use of an SEC column increases the analysis time (90 min) and thus decreases overall sample throughput. The frequency of sample injection and thus overall sample throughput on a FIA system is limited by sample peak broadening, which increases with the reaction time in the system. The FIA-EDC system used herein had a ten-minute injection interval and a total sample analysis time of 25 min. This is typically sufficient for oxidation processes in water treatment, because the dynamics of changes of raw water quality are in the order of hours to days. For applications that require more frequent sample injections, we recommend segmented-flow analysis coupled to spectrophotometric detection. Such systems would allow for a close-to-real time assessment of the chemical oxidant demand of specific water samples and of changes in the oxidation states of DOM during chemical oxidation.

3.6.2 Mechanistic insights into chemical oxidation of DOM

Changes in DOM oxidation state during ozonation and chlorination are currently inferred from decreases in the DOM absorbance, typically at 254 nm. While these absorbance measurements are a semi-quantitative measure of changes in the contents of aromatic moieties in the DOM, they do not provide (i) information on the contents of activated aromatic moieties that readily react with the chemical oxidants and (ii) further insights into the reaction pathways by which the oxidants react with
the activated aromatic moieties. This information is available when complementing absorbance measurements with EDC measurements. We plotted the decreases in the EDC·(EDC₀⁻¹ of the three model DOM isolates with increasing chemical oxidant doses versus the concurrent decreases in the absorbance at 254 nm, A₂₅₄, normalized to the absorbance of the untreated sample, A₂₅₄₀ (Figure 7). Ozonation of the DOM isolates resulted in large decreases in both EDC·(EDC₀⁻¹ and A₂₅₄·(A₂₅₄₀⁻¹, consistent with the electrophilic addition of ozone to activated aromatic moieties under ring opening. Conversely, chlorination resulted in comparable decreases in EDC·(EDC₀⁻¹, while the decreases in A₂₅₄·(A₂₅₄₀⁻¹ were much smaller than determined for ozonation. This finding suggests that electron-donating activated aromatic moieties were oxidized under comparatively small losses of aromatic units in the model DOM. Similar distinct effects of ozonation and chlorination on DOM were also previously reported when using MEO to quantify EDC values (Wenk et al., 2014). Figure 7 also shows that each of the two chemical treatments resulted in comparable trends of EDC·(EDC₀⁻¹ versus A₂₅₄·(A₂₅₄₀⁻¹ decreases for the three different DOM isolates, suggesting that the observed distinct reactivity patterns of ozone and chlorine apply to DOM from different sources and with different physicochemical properties. A more detailed, mechanistic discussion of changes in EDC and A₂₅₄ of the DOM upon ozonation and chlorination is part of a forthcoming publication (Önnby et al. 2018).

(Figure 7)

4. Conclusions

This study presents two analytical approaches, SEC-EDC and FIA-EDC, to quantify changes in the EDC of DOM isolates and DOM in real water samples during...
ozonation and chlorination over a range of specific oxidant doses. The major conclusions are:

- The high sensitivity of SEC-EDC and FIA-EDC and linearity in the analytical response allows quantifying changes in the EDC values of DOM in aqueous solutions at concentrations as low as 1 mg C·L⁻¹. The two analytical systems can thus be used to quantify the EDC of DOM in most water samples from natural and engineered systems that typically contain larger DOM contents.

- For five out of the six DOM samples studied, analysis of the treated DOM samples on the two systems yielded comparable decreases in the relative EDC with increasing specific oxidant doses. The reduction of ABTS•⁺ by DOM can thus be quantified either spectrophotometrically or electrochemically. For one real water sample with an exceptionally low absolute EDC, the results of the two analytical systems deviated from each other and highlighted superior robustness of the spectrophotometric over the electrochemical detection.

- Quantifying changes in the EDC of DOM during chemical oxidation, particularly when combined with monitoring changes in UV absorbance, provides a direct means to monitor loss of electron-donating aromatic moieties in DOM during chemical oxidation and provides insights into the distinct chemical pathways by which DOM is oxidized by different chemical oxidants. In water treatment facilities, monitoring of EDC will help to adjust oxidant doses to variations in the contents of activated aromatic moieties in the DOM and hence the oxidant demand.
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Appendix A. Supplementary Material

Supplementary Material related to this article can be found at […].

References


electrodialysis treatment followed by size-exclusion chromatography with nitrogen detection. Water Research 47(14), 5381-5391.


**Figure 1.** Schematic designs of the two analytical system setups used to quantify the changes in the electron donating capacity (EDC) of model DOM isolates and real water samples during ozonation or chlorination: the SEC-EDC (top) is based on size exclusion chromatography (SEC) and the FIA-EDC (bottom) is based on flow-injection analysis.

**Figure 2.** Absorbance measured at 254 nm for the three DOM isolates (SRFA, PLFA, and NNOM) eluting from the size exclusion chromatography (SEC) column on the SEC-EDC system. The concentrations of the analyzed DOM solutions were 2 mgC·L⁻¹ for all three DOM samples. The absorbance of each DOM is normalized to the total area of its elution peak.

**Figure 3.** Linearity in the absorbance and oxidative current response on the SEC-EDC and FIA-EDC systems, respectively, for injections of the model DOM isolate SRFA. (a) Increases in the heights of the absorbance loss peaks at 405 nm on the SEC-EDC system with increasing injected amounts of SRFA (injected carbon masses from mₐₙₐ₄= 1.25 to 7.5 µgC). The absorbance loss peaks resulted from the reduction of ABTS⁺⁺ by electron-donating (i.e., oxidizable) moieties in SRFA. (b) Linear increase in the areas under the negative absorbance peaks on the SEC-EDC system (shown in panel a) with increasing injected amounts of SRFA. (c) Oxidative current responses on the FIA-EDC system for injections of increasing amounts of SRFA (injected carbon masses from mₐₙₐ₄= 0.19 to 0.93 µgC) and of decreasing injected amounts of the redox standard ascorbate (electron donating capacity of EDC= 2.00 molₑ⁻·molₐscorbate⁻¹) used to calibrate the FIA-EDC system response for absolute EDC quantification of DOM samples. (d) Linear increases in the areas of the oxidative current peaks (shown in panel c) with increasing amounts of SRFA injected into the FIA-EDC system. The linear regression curve corresponds to an EDC of SRFA of 5.75 ± 0.19 mmolₑ⁻·g⁻¹.

**Figure 4.** Changes in the electron donating capacities (EDC) of the DOM isolates SRFA (blue squares), PLFA (red circles), and NNOM (green triangles) upon chemical oxidation at different specific doses of (a, b) ozone and (c, d) chlorine (HOCl), analyzed on (a,c) the SEC-EDC system and (b, d) the FIA-EDC system. All EDC of DOM isolates reacted with a specific oxidant dose are reported relative to the EDC of the respective untreated DOM, EDC₀. The DOM samples were chemically oxidized in pH 7 buffer (10 mM phosphate) and in the presence of t-BuOH (100 mM; for both ozonation and chlorination). The concentration of all three model DOM isolates was 2.5 mgC·L⁻¹ (corresponding to 0.208 mmolC·L⁻¹). The EDC of PLFA and NNOM samples treated with the highest specific HOCl dose are not shown because their responses were too small to be accurately analyzed.
Figure 5. Changes in the relative electron donating capacities (EDC) of the DOM from natural and engineered aquatic systems, SLW (purple diamonds), VLW (orange triangles), and WWE (brown inverted triangles) upon chemical oxidation at different specific doses for (a, b) ozone and (c, d) chlorine (HOCl), analyzed on (a, c) the SEC-EDC system and (b, d) the FIA-EDC system. All EDC of DOM samples reacted with a specific oxidant dose are reported relative to the EDC of the respective untreated DOM samples, EDC₀. The DOM samples were chemically oxidized in pH 7 buffer (10 mM phosphate) and in the presence of t-BuOH (100 mM; for both ozonation and chlorination). The DOC concentration of all three real water samples was 2.5 mgC·L⁻¹ (i.e., 0.208 mmolC·L⁻¹).

Figure 6. Comparison of the relative decreases in the electron donating capacities (EDC) of DOM samples measured by the FIA-EDC system (y-axis) and the SEC-EDC system (x-axis). All EDC of DOM samples reacted with a specific oxidant dose are reported relative to the EDC of the respective untreated DOM samples, EDC₀. The decreases in EDC are shown for (a, b) model DOM isolates and (c, d) real water DOM during both (a, c) ozonation and (b, d) chlorination. The data is replotted from Figures 4 and 5 above. The grey dashed line represents the 1:1 line (i.e., same relative decreases in EDC for FIA-EDC and SEC-EDC).

Figure 7. Changes in the relative electron donating capacities (EDC) of the DOM isolates SRFA (blue triangles), PLFA (red circles) and NNOM (green squares) quantified on the FIA-EDC system plotted versus the relative changes in the absorbance values at 254 nm (A₂₅₄) of the same isolates during ozonation and chlorination with different specific oxidant doses (oxidant dose increases as indicated by the arrows in the figure). All EDC and A₂₅₄ values of DOM samples treated with a specific oxidant dose are reported relative to the EDC and A₂₅₄ values of the respective untreated DOM samples, EDC₀ and A₂₅₄,₀, respectively.
Separation Detection
Mixing and Reaction

Absorbance 254 nm

Sample Selection and Injection
Solution Delivery

Reagent Solution (ABTS•–)
Carrier Solution

Auto-Sampler

LC-Pump

Pneumatic Pump

Syringe Pumps

10-Port Selector Valve & Samples

Reagent Solution (ABTS•–)

Carrier Solution

Injection Valve

10-Port Selector Valve & Samples

Absorbance 405 nm

Mixing Tee

Reaction Coil 3 min

Reaction Coil 22 min

Oxidative Current

Mixing Tee
Area-Normalized Absorbance $A_{254}$

Elution Time (min)

SEC-EDC

- SRFA
- PLFA
- NNOM
**Graph a.** SEC-EDC

- $\Delta A_{254} (\text{mAU} \cdot \text{cm}^{-1})$
- Time (min)
- $m_C (\mu g_C)$
- 0
- 1.25
- 2.5
- 3.75
- 5
- 6.25
- 7.5

**Graph b.**

- Area $= 0.31 \cdot m_C + 0.06$
- $R^2 = 0.994$
- Mass carbon injected $m_C (\mu g_C)$
- Peak Area (min $\cdot$ mAU $\cdot$ cm$^{-1}$)

**Graph c.** FIA-EDC

- Oxidative Current (nA)
- Time (min)
- $m_C (\mu g_C)$
- Amount ascorbate injected (nmol)
- ascorbate
- SRFA

**Graph d.**

- Area $= 38.78 \cdot m_C + 0.45$
- $R^2 = 1$
- Mass carbon injected $m_C (\mu g_C)$
- Peak Area (μC)
a. **SEC-EDC**

- DOM isolate
- SRFA
- PLFA
- NNOM

b. **FIA-EDC**

- DOM isolate
- SRFA
- PLFA
- NNOM

c. **SEC-EDC**

- DOM isolate
- SRFA
- PLFA
- NNOM

d. **FIA-EDC**

- DOM isolate
- SRFA
- PLFA
- NNOM

Specific $O_3$ Dose ($mol_{Ozone} \cdot mol_{C}^{-1}$)

Specific $HOCl$ Dose ($mol_{HOCl} \cdot mol_{C}^{-1}$)
a. Ozone

b. HOCI

c. Ozone

d. HOCI

DOM isolate

- NNOM
- PLFA
- SRFA

real water DOM

- SLW
- VLW
- WWE
Increasing specific ozone dose

Increasing specific HOCl dose

$$A_{254} \cdot (A_{254, 0})^{-1}$$

$$EDC \cdot (EDC_0)^{-1}$$

HOCl-treated
- NNOM (green triangles)
- PLFA (red circles)
- SRFA (blue squares)

Ozone-treated
- NNOM (green triangles)
- PLFA (red circles)
- SRFA (blue squares)

Increasing specific ozone dose

Increasing specific HOCl dose
Highlights

- Determination of electron-donating capacity (EDC) in DOM by two analytical methods
- EDC is a quantitative measure of activated aromatic moieties in DOM, including phenols
- High sensitivity of methods allows analysis of dilute DOM samples (<1 mg·L⁻¹)
- Ozonation and chlorination result in dose- and oxidant-dependent EDC decreases
- EDC provides a means to assess oxidant reactivity with DOM and adjust oxidant dosing
Monitoring Chemical Oxidation of Dissolved Organic Matter (DOM)

Untreated DOM

Specific HOCl or Ozone Dose

Extent of Chemical Oxidation...

... quantified by the Electron Donating Capacity (EDC) to ABTS•+

DOM

Chemically oxidized DOM

ABTS•+

ABTS

ABTS•+

ABTS

e−

e−

e−