Reactions of amines with ozone and chlorine: Two novel oxidative methods to evaluate the N-DBP formation potential from dissolved organic nitrogen

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1. Introduction

Dissolved organic matter (DOM) is a complex mixture of heterogeneous chemical structures. It is ubiquitous in all water types, from source water (natural organic matter, NOM) to wastewater effluents (effluent organic matter, EOM) (Leenheer and Croué, 2003; Shon et al., 2006). Its electron-rich moieties such as phenolic compounds, alkenes and amines are recognized as the principal consumers of chemical oxidants (e.g., ozone and chlorine) applied in drinking water and wastewater treatment (von Gunten, 2018), and react through various pathways (Deborde and von Gunten, 2008; Lim et al., 2019; Tekle-Röttering et al., 2020; von Sonntag and von Gunten, 2012). As a consequence, disinfection by-products (DBPs) are formed during primary disinfection/oxidation and final disinfection during distribution of drinking water (Rosario-Ortiz et al., 2016) or before discharge/reuse of wastewater. Many DBP classes have been studied, such as trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), haloacetalddehydes, N-nitrosamines, halonitromethanes (HNMs), etc., each having specific
molecular structures and toxicities (von Gunten et al., 2010; Krasner, 2009; Krasner et al., 2006; Li and Mitch, 2018; Mitch and Sedlak, 2002; Plewa et al., 2004; Richardson et al., 2007). In particular, nitrogenous DBPs (N-DBPs) are of increasing concern. Research suggests that some halogenated N-DBPs during ozonation with knowledge gaps about (i) the DON composition (serving as N-DBP precursors) and (ii) the mechanisms of reactions between DON moieties and ozone (leading to the formation of N-DBPs).

The heterogeneous nature of the DON constitutes a vast array of N-DBP precursors. DON is a mostly unresolved heterogeneous mixture, mainly composed of compounds originating from excretions of organisms or degradation (Jørgensen, 2009). Amino acids have been the most studied DON compounds and they generally represent 10–20% of the freshwater DON (Dotson and Westerhoff, 2009; Sipler and Bronk, 2015; Westerhoff and Marsh, 2002). Other organic compounds such as amino-sugars (in peptidoglycan or chitin), dissolved nucleotides from RNA/DNA strands (containing purine), and their metabolites (urea, humic substances, D-amino acids, diaminopimelic acid, glucosamine and methylamines) also represent a significant fraction of the freshwater DON (Jørgensen, 2009; Jørgensen et al., 2003; Pehlivanoglu-Mantas and Sedlak, 2006). It is expected that the DON concentration in drinking water sources will increase due to eutrophication, run-off events, and potable water reuse (Council, 2012; Delpla et al., 2009; Gerrity et al., 2013; Graeber et al., 2015; Marron et al., 2019; Pehlivanoglu-Mantas and Sedlak, 2006; Sedlak, 2014). To this end, the chemical composition of wastewater effluent DON has been investigated, with concentrations generally in the range of 47–350 µM as N (Liu et al., 2012; Pehlivanoglu-Mantas and Sedlak, 2006). About 10–20% are composed of amino acids, <5% of chelating agents (e.g., EDTA, NTA), 10% of humic substances from source water, and <1/10% of other N-containing micropolutants (Parkin and McCarty, 1981; Pehlivanoglu-Mantas and Sedlak, 2006; 2008). Overall, only a small fraction of the DON compounds has been identified (Pehlivanoglu-Mantas and Sedlak, 2006).

Ozone reacts with electron-rich amine moieties (Lim et al., 2019; Tekle-Röttinger et al., 2020) by an initial addition at the lone electron pair of the nitrogen to form an ozone adduct R⁻N⁻-O-O⁻-O⁻, which rapidly decomposes, mainly by losing O₂⁻ (Lim et al., 2019; von Sonntag and von Gunten, 2012). For primary and secondary amines, the corresponding alkylhydroxylamine is then formed, which is rapidly further oxidized to nitro-products (Lim et al., 2019; Shi and McCurry, 2020). Nitromethane has been identified during ozonation of various model amine compounds, DOM isolates and wastewater (McCurry et al., 2016; Shi and McCurry, 2020). For tertiary amines, the main end products are N-oxides (Lim et al., 2019; Muñoz and von Sonntag, 2000; Zimmermann et al., 2012). It has also been demonstrated that ozonation of some aliphatic amines, DOM isolates and wastewater effluents forms nitrate (Song et al., 2017; de Vera et al., 2017), which is the endpoint of nitrogen oxidation. However, so far it is inconclusive which factors influence the nitrate formation yields. Comparatively, organic primary and secondary amines in their neutral form react with chlorine to quickly form the corresponding chloramines by electrophilic substitution (Deborde and von Gunten, 2008; Heeb et al., 2017; Wajon and Morris, 1982). At pH 7, organic amines exert significant second-order rate constants for the reactions with chlorine (10³–10⁴ M⁻¹s⁻¹) (Heeb et al., 2017). These chloramines can be detected by photometric methods targeting the total chloride concentration (Aleta et al., 1984; Pinkernell et al., 2000), which possibly enables a quantification of the total amine concentration in DOM.

An assessment of the formation of N-DBPs during ozonation is therefore dependent on the understanding of the ozonation mechanisms and the nature of the precursors. To overcome these difficulties, new methods are required to identify and quantify the precursors of N-DBPs in DON. The aim of this study was to develop two novel assays using controlled oxidation conditions: (i) free available chlorine (FAC) titrations at pH 9.2 with the formation of chloramines and (ii) DON oxidation to nitrate by ozonation at pH 7.0. These methods rely on the pH dependance of the apparent second-order rate constants (k_app) for the reaction of chlorine and ozone with the major functional groups in DOM (Fig. 1a and b). Controlling the oxidation conditions can narrow down the diversity of target functional groups that react with the two selected oxidants. Chloramines and nitrate are two independent and easy to quantify parameters that are formed during the proposed controlled oxidations. They gauge the potential of N-DBP formation from the reactions of DON with ozone. These methods were optimized and tested with numerous model compounds, four NOM/EfOM isolates and two wastewater samples. Furthermore, a kinetic and mechanistic assessment of the nitrate formation during reactions of amines with ozone was carried out with 30 selected model compounds.

2. Material and methods
2.1. Chemicals and NOM/EfOM isolates

Chemicals used in this study are listed in Table S1 of the Supporting Information (SI), together with their suppliers and purities. Standard DOM isolates (i.e., Suwannee River Natural Organic Matter (SRNOM), Suwannee River Fulvic Acid (SRFA) and Upper Mississippi River Organic Matter (UMROM)) were obtained from the International Humic Substances Society (IHSS). Additionally, a wastewater effluent organic matter isolate (EfOM-CH) was obtained from the Université de Poitiers. Relevant physico-chemical characteristics of these isolates are provided in Tables S2 and S3 (SI).

2.2. Water samples

Synthetic water samples were prepared by dissolving a model compound or a standard NOM/EfOM isolate in ultra-pure water (Milli-Q water, Millipore, Merck, Germany; resistivity: 18.2 MΩ-cm at 25°C; TOC: 2 ppb). Before each experiment, the DOC concentration of a synthetic water containing a standard NOM/EfOM isolate was spectrophotometrically standardized with the procedure described in Text S1 (SI). Wastewater samples were obtained from the wastewater treatment plant “ARA Thunsee” (Thun, Switzerland). Samples were collected in amber glass bottles at two different treatment stages: (i) after the biological treatment (WWTA, including nitrification); (ii) after treatment for micropollutant removal (WWTB, powdered activated carbon and sand filtration (PAC + SF)). On the same day, they were filtered on a 2.7 µm glass microfiber filter (Whatman) and a 0.45 µm nylon membrane (Membrane Solutions), and stored in a refrigerator before use. Table S4 (SI) summarizes their main water quality parameters.

2.3. Preparation of ozone and chlorine stock solutions

For kinetic and batch dosage experiments, ozone gas was produced by a CMG 3–5 ozone generator (Innovatec) supplied with O₃ gas (Alphagas 2 O₃ 99.9995%) at 12 and 13 L/h. The gas was passed through a phosphate buffer trap (0.5 M, pH 6), before continuous sparging through 500 mL ultra-pure water chilled in an ice bath until ozone saturation (1.1–1.2 mM). The ozone stock solution was spectrophotometrically standardized prior to use, by measuring the absorbance of a mixture of ozone stock (1 mL) and phosphoric acid (50 mM, 2 mL) in a 1 cm quartz cell (ε_O₃: 260 nm = 3·200 M⁻¹cm⁻¹ von Sonntag and von Gunten, 2012). For continuous ozonation, ozone gas was produced by a LABZB ozone generator (Triogen) supplied with O₂ gas (Alphagas 2 O₂ 99.9995%).

A 40 mM stock solution of FAC was prepared from a commercial sodium hypochlorite solution in ultra-pure water. The commercial sodium hypochlorite solution and the FAC stock solution were...
spectrophotometrically standardized prior to use, in basic conditions and in a 1 cm quartz cell (ϵ\text{OCl}_{292\text{nm}} = 350 M^{-1}cm^{-1} (Hand and Margerum, 1983)).

2.4. Nitrate formation during ozonation

2.4.1. Kinetic experiments with model compounds

The kinetics of NO\textsubscript{3} formation were studied for 4 model compounds: benzylamine (primary amine), N-methylbenzylamine (secondary amine), L-alanine (α-amino acid) and β-alanine (β-amino acid) by following (1) the ozone decay and (2) the NO\textsubscript{3} formation, for 90 min, in 20-fold stoichiometric molar excess of ozone, at pH 7. A solution of model amine (20 µM) and tert-butanol (t-BuOH, a hydroxyl radical scavenger; 20 mM) in phosphate buffer (10 mM, pH 7) was added to a 250 mL glass bottle equipped with a Dispensette III BottleTop dispenser (BrandTech) (Hoigne and Bader, 1994). The ozone stock solution was added below the liquid level through Teflon tubing ([O3]\text{t}=0s ≈ 400 µM), using a glass syringe, and the solution stirred for 60 s. At specified time intervals (but not before 60 s), a 1 mL sample was taken and added to a vial containing indigo (0.25 mL, 2 mM) and phosphate buffer (0.25 mL, 20 mM, pH 2), to measure ozone by the indigo method (Section 2.6.3). After a delay of 5 s, a second 1 mL sample was transferred to a vial containing 10 µL 3-buten-2-ol (136 mM) to rapidly quench residual ozone (Text S2, SI). This sample was used to quantify NO\textsubscript{3} by ion chromatography (IC, Section 2.6.1) without interference of indigo. The procedure was repeated in duplicate for each model compound.

2.4.2. Batch dosage experiments with model compounds for mechanistic investigations

Batch ozonation experiments were performed with 30 selected model compounds (Table S5, SI) to investigate the yields and mechanisms of NO\textsubscript{3} formation. They were conducted in amber borosilicate glass vials containing one of the model nitrogenous precursors (50 µM), phosphate buffer (5 mM, pH 7.0), ultra-pure water and t-BuOH (20 mM). Appropriate volumes of an ozone stock solution were added last using a glass syringe, to obtain specific molar ozone:amine ratios (between 0 and 20) in a final volume of 15 mL. The vials were sealed with Teflon-lined septum caps and left to react for 24 h at room temperature before sparging any potential residual ozone with N\textsubscript{2} gas. The quenched samples were analyzed for nitrate by IC-UV (Section 2.6.1). For 3 model amines (benzylamine, N-methylbenzylamine and N,N-dimethylbenzylamine), other expected transformation products (TPs) were also

Fig. 1. (a, b) Apparent second-order rate constants (k\textsubscript{app}) as a function of the pH, for the reactions of (a) ozone and (b) chlorine with model compounds: phenolic compounds (phenol, resorcinol, 4-hydroxybenzoic acid (4-HbA)), alkenes (sorbic acid (SA), butenol) and amines (glycine, dimethylamine (DMA) and trimethylamine (TMA)). (c) Parallel category plot for the ozonation of 5 selected model amine compounds (N-methylbenzylamine (MBA), benzylamine (BA), glycine, L-alanine and β-alanine), comparing their log10(k\textsubscript{app}) with their NO\textsubscript{3} yield (%) and the second-order rate constant of NO\textsubscript{3} formation (k\textsubscript{f}NO\textsubscript{3} (M\textsuperscript{-1}s\textsuperscript{-1}), Text S6, SI), at pH 7.0. The last two parameters were obtained from kinetic experiments for which the results and experimental conditions are reported in Figures S2 and S3 and Table S6 (SI). (d) Parallel category plot for the chlorination of 37 selected model amine compounds, comparing their pK\textsubscript{a} and log10(k\textsubscript{HOCl}) with the time needed to abate at least 99% of their initial concentration ([O3]\text{t}=0s). This last parameter was calculated by kinetic simulations performed with a software architecture described in Figure S1 (SI). Modeling conditions: pH 9.2, [HOCl]\text{t}=0s = 15 µM, [amine]\text{t}=0s = 10. Rate constants and pK\textsubscript{a} were obtained from (von Sonntag and von Gunten, 2012) and (Deborde and von Gunten, 2008) for O\textsubscript{3} and HOCl, respectively. Texts S5 and S9 (SI) describe the calculations for k\textsubscript{app}.
ozonation experiments were performed to determine (1) the NO\textsubscript{3} formation and (2) the maximum NO\textsubscript{3} formation under high ozone excess conditions. 250 mL of ultra-pure water with NOM/EfOM isolates (DOC \approx 10 mgC/L, spectrophotometrically standardized) or 150 mL of ultra-pure water with 100 mL of wastewater sample (dilution factor: 2.5), each containing phosphate buffer (10 mM, solution pH 7.00 \pm 0.05) and t-BuOH (100 mM) were continuously stirred in a GLS 80 glass reactor (Duran), equipped with a multiple gas-tight distributor PTFE cap, to accommodate 4 tubes (Fig. 2a). At t=0 s, an O\textsubscript{3}/O\textsubscript{2} gas mixture was continuously bubbled (0.5 L/min) in the sealed reactor through a Teflon TPE) tube (PharMed BPT NSF-51), which has excellent ozone resistance. A liquid circulation loop entirely made of Teflon tubes was mounted between the reactor and a 0.5 cm flow-through quartz cell to continuously monitor and record the total absorbance of the solution at \( \lambda = 260 \) nm of the sparged solution was separately measured in a 0.5 cm quartz cell and reported as (A\textsubscript{260}/A\textsubscript{260, max} lum), with a Shimadzu UV-1800 spectrophotometer. A peristaltic pump (flow rate: 6 mL/min, i.e., hydraulic residence time: 4 s in the cell) was placed after the spectrophotometer, and equipped with a thermoplastic elastomer (TPE) tube (PharMed BPT NSF-51), which has excellent ozone resistance. At specified time intervals, 500 \( \mu \)L of solution were collected for nitrate analysis (Section 2.6.1) with a gas-tight glass syringe through a T-piece and a valve installed on the circulation loop. Residual ozone was immediately quenched by an excess of sulfite ([SO\textsubscript{3}]) \geq 0.8 mM, Text S2, SI).

At less frequent reaction times, 2.5 mL of solution were collected to measure its absorbance at \( \lambda = 260 \) nm of the sparged solution was separately measured in a 0.5 cm quartz cell and reported as (A\textsubscript{260}/A\textsubscript{260, max} lum), i.e., the residual absorbance of the analyzed water not caused by ozone. Ozone exposures (OE, Ms) were calculated as

\[
OE = \int_{t=0}^{120 \text{ min}} A_{260,OE}(t) \cdot ((3200 \text{ L}/(\text{mol-cm}))\cdot(0.5 \text{ cm}))^{-1} \, dt
\]

with A\textsubscript{260,OE}(t) = A\textsubscript{260,OE}(t) - A\textsubscript{260,OE}(t), a fitted function describing the absorbance of ozone over time (0-120 min) as the difference between the total absorbance of the solution and the absorbance of the water matrix only, at 260 nm.

2.5. Chloramine formation during chlorination

2.5.1. Kinetic simulations

To evaluate the reaction time needed to transform amines to chloramines, 37 model amines were selected (with known species-specific second-order rate constant (k\textsubscript{HOC}) and pK\textsubscript{a} at 25 C from a database (Deborde and von Gunten, 2008). A software architecture linking Matlab with Kintecus (Figure S1, SI) was built to perform kinetic simulations to conveniently find the time needed to transform 99% of each amine to chloramines (t\textsubscript{99%}). Modeling conditions were chosen as follows: pH 9.2, [amine]\textsubscript{0} = 10 \mu M, [HOCI]\textsubscript{0} = 10, 12, 15, 20, 30 and 40 \mu M.

2.5.2. Oxidative FAC-titrations

To perform oxidative titrations (Fig. 2b), increasing chlorine doses ([FAC]\textsubscript{0} = 0 to 40 \mu M) were added to a series of batch reactors (10 mL) containing a model compound (10 \mu M, unless indicated otherwise), or a NOM/EfOM isolate (DOC = 5 mgC/L) or wastewater (Table S4, SI), and a 260 nm of the sparged solution was separately measured in a 0.5 cm quartz cell and reported as (A\textsubscript{260}/A\textsubscript{260,0} lum), i.e., the residual absorbance of the analyzed water not caused by ozone. Ozone exposures (OE, Ms) were calculated as

\[
OE = \int_{t=0}^{120 \text{ min}} A_{260,OE}(t) \cdot ((3200 \text{ L}/(\text{mol-cm}))\cdot(0.5 \text{ cm}))^{-1} \, dt
\]

with A\textsubscript{260,OE}(t) = A\textsubscript{260,OE}(t) - A\textsubscript{260,OE}(t), a fitted function describing the absorbance of ozone over time (0-120 min) as the difference between the total absorbance of the solution and the absorbance of the water matrix only, at 260 nm.

Fig. 2. Schematic representation of the experimental setups for (a) the continuous ozonation experiments and (b) the oxidative FAC-titrations. (a) Monitoring parameter A: the evolution of the reaction was continuously monitored by measuring the absorbance of the solution (\( \lambda = 260 \) nm, optical path length = 0.5 cm). Monitoring parameter B: at selected reaction times, 0.5 mL of solution was withdrawn with a glass syringe and NO\textsubscript{3} was quantified by IC-UV analyses. Monitoring parameter C: at less frequent reaction times, 2.5 mL of solution were collected to measure its absorbance at \( \lambda = 260 \) nm, after ozone purging with N\textsubscript{2}, (b) Monitoring parameters D and E: final concentrations of FAC and TC were spectrophotometrically quantified by the DPD method, for increasing HOCl doses (reactors 1 to n); the same procedure was applied with and without (BOC)\textsubscript{2}O derivatization.
carbonate buffer (5 mM, pH 9.2). After a selected reaction time (t<sub>app</sub>, determined by kinetic simulations), 2.5 mL of sample were immediately transferred to a 1 cm quartz cell containing 125 μL of a N,N-diethyl-p-phenylenediamine (DPD) solution and 125 μL of a phosphate buffer (0.22 M, pH 6.3). [FAC]<sub>t-trx</sub> and total chlorine ([TC] = [FAC]<sub>t-trx</sub> + [chloramines formed]<sub>t-trx</sub>) were quantified by the DPD colorimetric method (Section 2.6.2).

2.5.3. (BOC)<sub>2</sub>O-derivationization of primary and secondary amines

A derivatization procedure was adopted to selectively block the reactivity of primary and secondary amines towards chlorination, by transforming them into carbamates. It was based on a method using di-tert-butyl dicarbonate (BOC)<sub>2</sub>O as protecting agent (Ginhorn et al., 1991). The detailed procedure is described in Text S3 (SI).

2.6. Analytical methods

2.6.1. Quantification of amines and their TPs

Benzyline (BA), N-methylbenzylamine (MBA), N,N-dimethylbenzylamine (DMBA) and their ozonation TPs, i.e., N,N-dimethylbenzylamine N-oxide, nitromethylbenzene, benzaldeyde oxime and N-methyl-1-phenylmethanimine oxide were measured by high pressure liquid chromatography (HPLC) equipped with an UV detector. They were identified by retention time comparison with commercial standards. Nitrate was measured by ion chromatography (IC) with UV detection at 220 nm. Nitromethane was measured by gas chromatography and tandem mass spectrometry (GC–MS/MS) after a liquid-liquid extraction. Chromatographic conditions, calibrations and limits of quantification are provided in Text S4 (SI).

2.6.2. Quantification of FAC and total chlorine (TC)

FAC concentrations were measured by the DPD colorimetric method described elsewhere (Baird et al., 2017; Rodier et al., 2016). DPD is oxidized by FAC to the Würster dye (DPD<sup>+</sup>), which can be spectrophotometrically quantified at λ = 510 nm (ε<sub>DPD</sub>:510 nm = 19’800 M<sup>–1</sup>cm<sup>–1</sup>, Bader and Hoigné, 1988) in a 1 cm quartz cell. By the subsequent addition of potassium iodide (KI) crystals, total chlorine (TC = FAC + mono- + dichloramines) was also quantified. The LOD and LOQ of the DPD method were 0.75 μM and 2.28 μM, respectively.

2.6.3. Quantification of ozone

The indigo method was used to quantify the ozone concentration (Bader and Hoigné, 1981). Rapid reaction between excess indigotrisulfonate and residual ozone at pH = 2 (1:1 molar ratio) causes discoloration of the initial blue indigo solution, for which the absorbance was recorded at λ = 600 nm (ε<sub>Indigo:600 nm</sub> = 20’000 M<sup>–1</sup>cm<sup>–1</sup>, Bader and Hoigné, 1981) in a 1 cm quartz cell. This allowed quantification of ozone concentrations as a function of reaction time in kinetic experiments.

2.6.4. Quantification of DON in water samples

For the wastewater samples, DON was determined by size-exclusion chromatography coupled to UV organic carbon and nitrogen detection (SEC-UV-OCD-ND) (Chon et al., 2013). Samples were dialysed (cellulose ester dialysis tubes Biotech, diameter 15 mm, 0.1–0.5 kDa molecular weight cutoff, Spectrum Laboratories) prior to SEC-UV-OCD-ND to minimize interference from inorganic nitrogen (NO<sub>3</sub> and NH<sub>4</sub>). For the NOM isolates, the elemental nitrogen content published by the IHSS was used (Table S3, SI).

2.6.5. Quantification of other wastewater quality parameters

Alkalinity, DOC, total dissolved nitrogen (TDN), NO<sub>3</sub>, NO<sub>2</sub>, NH<sub>4</sub> and pH were determined at Eawag (Dübendorf, Switzerland) using methods described in Table S4 (SI).

3. Results and discussion

3.1. Assessment of the nitrate formation during ozonation of model compounds

Before measuring the nitrate formation during ozonation of DOM/EIO samples, the relevance and extent of nitrate as an oxidation end product had to be evaluated. To strengthen the current mechanistic knowledge, three aspects were studied with several model nitrogenous compounds: (1) the temporal evolution of NO<sub>3</sub> formation with 4 model amines (kinetic experiments); (2) the yields of NO<sub>3</sub> formation with 30 model amines (dosage experiments); (3) the mechanisms of NO<sub>3</sub> formation with 3 aliphatic amines (dosage experiments).

3.1.1. Kinetics of nitrate formation from model amine compounds

To study the evolution of nitrate during ozonation, kinetic experiments were performed with BA, MBA, L-alanine and β-alanine and the results were compared with previously published data for glycine (de Vera et al., 2017). These compounds were chosen after evaluation of their apparent second-order rate constants (k<sub>app</sub>, Text S5, SI) at pH 7, which ranged over 2 orders of magnitude (Table S6, SI).

O<sub>3</sub> was completely consumed within 90 min for each of the compounds (Figure S2, SI), comparable to glycine under similar conditions (de Vera et al., 2017). The ozone exposures were between 0.32 and 0.39 M s<sup>–1</sup> (256–312 mg min<sup>–1</sup> m<sup>–1</sup>), but higher for β-alanine (0.57 M s<sup>–1</sup> or 456 mg min<sup>–1</sup> m<sup>–1</sup>). The second-order rate constants for NO<sub>3</sub> formation (k<sub>NO<sub>3</sub></sub>), as defined in Text S6 and Figure S3, SI) and NO<sub>3</sub> yields were determined, based on the profiles of O<sub>3</sub> decay and NO<sub>3</sub> formation over time.

In Fig. 1c, k<sub>NO<sub>3</sub></sub>, NO<sub>3</sub> yields and k<sub>NO<sub>3</sub></sub> were compared in parallel for the five target compounds. Clearly, no correlation exists between k<sub>app</sub> and the NO<sub>3</sub> yields nor between k<sub>app</sub> and k<sub>NO<sub>3</sub></sub>. This suggests that the nitrate formation kinetics and yields are not controlled by the initial ozone attack but by further reactions involving intermediate transformation products. The chemical structures of these intermediates could be a key parameter controlling the kinetics of NO<sub>3</sub> formation and yields during ozonation.

3.1.2. Nitrate yields of model amine compounds during ozonation

Amino acids. Eight amino acids with different side chains were studied, seven of which showed >90% conversion to nitrate ([NO<sub>3</sub>]<sub>i</sub>:[amino acid]<sub>i</sub>) with a molar ratio [O<sub>3</sub>:[amino acid]<sub>i</sub> = 20 (Fig. 3). With one oxidant equivalent, an average NO<sub>3</sub> yield of ~14% was observed for 6 amino acids. This is only possible if some intermediate TPs are more reactive towards ozone than the precursors, because >1 molar equivalent of ozone is needed to transform an amine to nitrate. Exceptions were methionine and cysteine, containing an ozone-reactive sulfide or thiol group, respectively, which partially consume ozone via a fast reaction (von Sonntag and von Gunten, 2012). Since the sulfoxides are ozone- refractory, the sulfur reaction pathway of methionine is completed for 1 molar equivalent of ozone. Therefore, methionine showed a NO<sub>3</sub> yield of 59.8% for a molar ratio [O<sub>3</sub>:[methionine]<sub>i</sub> = 5, which is similar to an average value of 53% for the other tested amino acids, excluding cysteine. A higher ozone demand for cysteine (requiring more than 1 molar equivalent of ozone for completion) was observed with NO<sub>3</sub> yields of only 30.5% and 71.8%, for molar ratios [O<sub>3</sub>:[amino acid]<sub>i</sub> = 5 and 20, respectively.

Primary amines. NO<sub>3</sub> yields of nine structurally different primary amines were determined (Fig. 3). In contrast to amino acids, great variability was observed among the tested compounds. BA, 4-chlorobenzylamine and 4-nitrobenzylamine showed the highest NO<sub>3</sub> yields (~90% for [O<sub>3</sub>:[amine]<sub>i</sub> = 20). Ethanolamine, 2-phenylethylamine, α-methylamine and 3-phenyl-1-propylamine had intermediate yields (37–58%), while ethylamine and β-alanine had the lowest yields (<20%). Since ethylamine differed from L-alanine only by the absence of a carboxyl group on the α-carbon and β-alanine by a longer carbon...
chain, it indicates that the carboxyl group in α-position to the amino group might play an important role.

Secondary amines. Secondary amines overall displayed a lower conversion to nitrate compared to primary amines (Fig. 3), which could be due to the greater degree of dealkylation required, which is not the main ozone reaction (Lim et al., 2019). Diethylamine (N-methylated ethylamine) formed 16.6% NO$_3$ for a molar ratio [O$_3$]:[amine]$_0$ = 20, similar to ethylamine (19.2%). Under the same conditions, MBA (N-methylated benzylamine, 32.4%) and sarcosine (N-methylated glycine, 28.8%) displayed an augmented NO$_3$ yield compared to diethylamine (16.6%). NO$_3$ yields from secondary amines were equivocal and seemed to be structure dependent.

Tertiary amines. Three tertiary amines (N,N-dimethylbenzylamine, tramadol and trimethylamine) were ozonated, with all showing small NO$_3$ yields, even at high specific ozone doses (Fig. 3).

Other N-containing functional groups. Due to the abundance of proteins and peptides in DON, the contribution of amides to nitrate formation was also explored. Two amide-containing compounds were tested—acetamide and diglycine (Gly-Gly). For acetamide, 2% yield of nitrate was obtained with a molar ratio [O$_3$]:[acetamide]$_0$ = 20. Gly-Gly contains 2 moles of N per molecule (peptide link and NH$_2$-terminus). At a molar ratio [O$_3$]:[Gly-Gly]$_0$ = 20, a NO$_3$ yield of 50% was observed, suggesting that the terminal NH$_2$ group was fully converted to nitrate, with no significant contribution from the amide group. Further compounds which make up significant fractions of DON are urea and pyrrole. Urea, which contributes 0–65% (mean ± std: 6.9 ± 9.7% (mol/mol)) of the DON pool in natural estuarine environments and 6.4 ± 5.7% in riverine environments (Sipler and Bronk, 2015) formed almost no nitrate (Fig. 3). Pyrrole (abundant in waters due to its presence in chlorophyll, heme, porphyrins, histidine and tryptophan) (Tekle-Røttering et al., 2020), produced only 7% nitrate when oxidized with 20 molar equivalents of ozone.

3.1.3. Transformation products formation and mechanistic considerations

Our results suggest that the carbonyl group in α-position stimulates NO$_3$ formation from amino acids during ozonation. Consistent NO$_3$ yields for the tested amino acids indicate a similar ozone-oxidation pathway for all amino acids. The formation of nitrate from glycine has been previously studied (Berger et al., 1999; Lim et al., 2019; McCurry et al., 2016; Song et al., 2017; de Vera et al., 2017). Proposed mechanisms pass through several intermediates such as hydroxylamines, oximes or nitro(so)alkanes. Decarboxylation and hydrolysis were proposed as mechanisms forming nitrate. However, the mechanisms of NO$_3$ formation from primary, secondary and tertiary amines during ozonation are still unclear, and the observed variability in NO$_3$ formation was initially unexpected. To study these mechanisms in more detail, oxidation cascades of BA, MBA and DMBA were followed in batch dosages experiments.
BA yielded one of the highest NO$_3$ yields among the selected primary amines. Fig. 4a shows the abatement of BA and the evolution of its TPs at pH 7, for specific ozone doses from 0 to 20 mol$_{O_3}$/mol$_N$. The BA concentration decreased for molar ratios [O$_3$]:[BA]$_0$ < 10 and was entirely abated at 10 molar equivalents of ozone. For molar ratios [O$_3$]:[BA]$_0$ ≤ 5, the major TP was nitromethylbenzene (max. 76% of the abated BA), whereas NO$_3$ also rose rapidly, already reaching 32% of the abated BA at a molar ratio [O$_3$]:[BA] = 3. Nitromethylbenzene was a transient TP, completely oxidized to NO$_3$ with a yield of 91% at a molar ratio [O$_3$]:[BA]$_0$ = 20. This indirectly supports the amine oxide pathway (reactions (i), (j) and (g) in Fig. 4c) as the main oxidation mechanism. This pathway is also supported by sequentially increasing NO$_3$ yields for benzylamine and nitromethylbenzene at molar ratios [O$_3$]:[BA]$_0$ of 1 and 5 (Fig. 3). Benzaldehyde oxime (nitromethylbenzene tautomer) was part of the analytical method, but not observed (reaction (k) in Fig. 4c). The nitrogen mass balance was 89–103% for all ozone doses, which confirms that no major TPs were missed. Although not measured, NH$_4$ formation should consequently be minor, disqualifying the electron transfer pathway (reaction (h) in Fig. 4c). These results contrasted with a recent study showing that the only TP of ethylamine ozonation was nitroethane (tested molar ratios [O$_3$]:[ethylamine]$_0$: 0–3) and with a NO$_3$ yield of only 10% for a 20-fold ozone excess (Lim et al., 2019). Another study also reported a complete transformation of methylamine to nitromethane at ≥12 molar equivalents of ozone (McCurry et al., 2016). Batch dosage experiments demonstrated that nitromethylbenzene forms more NO$_3$ than nitromethane (Fig. 3), and we therefore hypothesize that electronic effects (induction, delocalization and resonance) play a decisive role in reactivity differences. The acid dissociation constant (K$_a$) is the most informative parameter related to electron withdrawing effects encountered by an amine group, as a
function of the chemical groups in its vicinity (Schwarzenbach et al., 2016). A strong positive correlation was observed between $K_a$ and NO$_3$ formation yields for 6 primary amines (Text S7, SI), supporting our hypothesis. The TP investigation for BA suggested that a C formation yields for 6 primary amines (Text S7, SI), supporting our approach may only cover part of the total pool of primary and secondary amines, even at high ozone doses. To overcome this potential understimation, we assessed a complimentary second approach based on a FAC-titration.

3.1.4. NO$_3^-$ formation potential as a proxy for N-DBP precursors

Based on these results, the NO$_3^-$ formation potential can be regarded as an estimate of the highly reactive species in DON, mainly activated primary and secondary amines, and amino acids. Under realistic conditions (e.g., in a wastewater treatment plant), the formation of N-DBPs (i.e., intermediate compounds in the N oxidation cascade) can be formed, whereas in our continuous ozonation system, a large fraction of these specifically targeted intermediates (such as nitro or nitroso compounds) will be further oxidized to nitrate, depending on their reactivity and susceptibility (see Figs. 3 and 4). Therefore, nitrate formation during ozonation could be a potential proxy for the pool of reactive N-DBP precursors. Similar approaches exist to estimate other DBP precursor concentrations (Baird et al., 2017; Mitch and Sedlak, 2004), however, our approach may only cover part of the total pool of primary and secondary amines, even at high ozone doses. To overcome this potential understimation, we assessed a complimentary second approach based on a FAC-titration.

3.2. Assessment of the chloramine formation with oxidative FAC-titrations

Chloramines are quickly formed by the reaction of chlorine with amines (Deborde and von Gunten, 2008). Therefore, one could indirectly quantify N-DBP precursors by quantifying chloramines with a non-selective method (DPD), after reaction with increasing doses of chlorine; this is the goal of the FAC-titrations. To this end, the oxidation conditions should maximize the formation of chloramines and minimize the consumption of chlorine by other reactive species. At pH 9.2, chlorine reacts fast with primary and secondary amines, as well as with activated phenolic compounds such as resorcinol (Fig. 1b). Optimal reaction times have to be chosen to satisfy the chlorine demand of amine model compounds or amine moieties in DOM, which was done by kinetic simulations of a simple reaction (amine + HOCl→ chloramine, with $k_{\text{app},\text{pH} 9.2}$ defined in Text S9, SI), at several initial conditions.

3.2.1. Kinetic simulations and reaction time

Fig. 1d shows the modeled time for a 99% abatement ($t_{99\%}$) of 37 model amine compounds during chlorination (FAC$_0$ = 15 $\mu$M) at pH 9.2. For 36 amines, $t_{99\%}$ was smaller than 60 s. For diisopropylamine, a combination of high $pK_a$ (11.07) and relatively low $k_{\text{HOCl}}$ (1.8 $\times$ $10^7$ M$^{-1}$ s$^{-1}$) leads to a longer $t_{99\%}$ of 139 s. Generally, secondary amines have lower $k_{\text{HOCl}}$ than primary amines but similar $pK_a$-values, leading to higher $t_{99\%}$. Amino acids have intermediate $k_{\text{HOCl}}$ values (9.54–10.10) and high $k_{\text{HOCl}}$ ((0.32–1.13) $\times$ $10^9$ M$^{-1}$ s$^{-1}$), yielding low $t_{99\%}$. Finally, peptidic structures enhance the reactivity of the terminal amine by decreasing the $pK_a$ (7.94–8.70), despite having $k_{\text{HOCl}}$ values ((0.6–1.26) $\times$ $10^7$ M$^{-1}$ s$^{-1}$) one order of magnitude lower than amino acids. Figure S5 (SI) compares the calculated $t_{99\%}$ of the 37 selected model amines for 6 FAC doses ranging between 10 and 40 $\mu$M, typically used in the oxidative FAC-titration experiments in this study. [FAC]$_0$ = [amine]$_0$ = 10 $\mu$M is the least favorable condition since 12 compounds (mostly secondary amines) had a $t_{99\%}$>120 s. With a FAC$_0$ = 12 $\mu$M, only diisopropylamine had a $t_{99\%}$>120 s and all amines had a $t_{99\%}$<120 s for a [FAC]$_0$ = 20 $\mu$M. Based on these observations, a reaction time ($t_r$) of 120 s seemed reasonable for complex mixtures such as DOM. For the FAC-titrations of model compounds (glycine, methylamine and dimethylamine), a $t_r$=60 s is sufficient, based on calculations with the same model.

3.2.2. (Boc)$_2$O derivatization efficiency

For primary and secondary amines, derivatization efficiencies were $\geq$97% (Figure S6, Table S9, SI). These results were comparable to previously reported efficiencies (Einhorn et al., 1991; McCurry et al., 2016). In contrast, derivatization had no significant effect on the measured concentrations of either four phenolic compounds and N, N-dimethylbenzylamine, a tertiary amine ($p$-value >0.05) (Figure S11,
amines to carbamates. For benzylamine, the formation of nature of phenolic moieties but only transforms primary and secondary and dimethylamine, [amine] comparison (HPLC-UV) with a commercial standard (data not shown).

**3.2.3. Oxidative FAC-titrations of model compounds**

Fig. 5a–c and Table S9 (SI) show the results of oxidative FAC-titrations for three amine model compounds (glycine, methylamine and dimethylamine, [amine]$_0$ = 10 µM), as a function of the chlorine doses ([FAC]$_0$ = 0–40 µM) at pH 9.2. Residual FAC measurements resulted in two distinct phases. The initial phase (for FAC doses <10µM) was characterized by a full FAC consumption. The second phase showed a linear increase (for FAC doses >10µM), with slopes of regression lines of about 1 (0.98–1.01) and intercepts close to 10 µM (9.10–10.91 µM), which corresponds to the initial amine concentration. In contrast, corresponding TC measurements were characterized by a single linear phase with slopes of about 1 (0.94–0.97) and intercepts not significantly different from zero (Table S9, SI). During the initial phase, the total applied FAC could be only measured as TC, meaning that the applied FAC was fully transformed to chloramine species. Thereafter, a constant slope of about 1 (0.97) and intercepts not significantly different from zero (Table S9, SI). Therefore, the difference between the TC and FAC regression model intercepts ($\beta_{\text{FAC}}$ and $\beta_{\text{TC}}$, respectively) corresponds to the initial total concentration of precursor amines: $\Delta \beta = \beta_{\text{TC}} - \beta_{\text{FAC}} = [\text{chloramines}]_{t=0s} = [\text{amines}]_{t=0s}$.

To confirm $\Delta \beta$ as a valid amine indicator, the same three model amines were FAC-titrated for the same conditions, but after (BOC)$_2$O-derivatization (Fig. 5d–f). In contrast to non-derivatized amines, no $\Delta \beta$ were observed (Table S9, SI), indicating that no chloramines were formed during chlorination. Identical slopes for FAC and TC close to 1 also confirmed that the added FAC remained unchanged in solution, in presence of carbamate.

Chlorine reactive phenolic compounds are present in DOM at much higher concentrations than nitrogen-containing species, therefore, their influence on oxidative FAC-titrations had to be tested. Phenol was selected as the simplest phenolic compound - its apparent second-order rate constant for the reaction with FAC under the conditions of the oxidative FAC-titrations at pH 9.2 ($k_{\text{app},\text{phen}0.2} = 78 \text{ M}^{-1} \text{s}^{-1}$ Deborde and von Gunten, 2008) is about 2’800 times lower than for glycine ($k_{\text{app},\text{gly}0.2} = 2.2 \cdot 10^3 \text{ M}^{-1} \text{s}^{-1}$ Deborde and von Gunten, 2008) (Fig. 1b). The oxidative FAC-titration of phenol resulted in slopes <1 for both regression lines (Figure S7a, SI). This indicates that phenol partially consumes the applied FAC dose, resulting, in this case, in a constant [FAC]$_{t=60s}$/[FAC]$_{t=0}$ ratio of about 2/3, independent of [FAC]$_{t=0}$. A kinetic simulation was performed, with the same conditions and based on known species-specific second-order rate constants for reaction of FAC with the phenolate ion and its chlorophenolate ions, which are formed upon chlorination (Table S10, SI). The kinetic simulation agreed well with the experimentally observed behavior of phenol during the oxidative FAC-titration (Figure S8, SI). In contrast, no FAC consumption was observed when 2,4,6-trimethylphenol was FAC-titrated since the three sites of possible FAC attack (in ortho- and para- positions) are blocked by methyl groups (Figure S7b, SI). Furthermore, experiments...
were performed with resorcinol (benzene 1,3-diol, Figure S7c, SI), a phenolic compound with significantly higher chlorine-reactivity than phenol \( (k_{\text{app,phen}}=2.0-10^8 \text{ M}^{-1}\text{ s}^{-1}) \) (Rebenne et al., 1996). A large initial phase of about 25 \( \mu \text{M} \) as well as a slope of about 2/3 were observed for a reaction time of 60 s, induced by both the rapid initial reaction of resorcinol with FAC and slower subsequent reactions between FAC and the ensuing products (Rebenne et al., 1996). An equivalent right shift should occur on the FAC and TC lines, having no influence on \( \Delta\beta \), since no chloramines are formed from these compounds. Therefore, non-zero \( \beta_{\text{TC}} \) are an indication of the presence of rapidly reacting phenolic-type moieties.

Based on these results, \( \Delta\beta \) is proposed as a novel reliable parameter, indirectly quantifying the total content of fast chlorine-reactive nitrogen compounds in aqueous solutions. This is a complementary indicator to the nitrate formation from ozonation discussed above.

### 3.3. Effects of controlled oxidative treatments on DOM properties

Four standard NOM/EfOM isolates (SRFA, SRNOM, UMRNOM & EfOM-CH) and two wastewater samples (WWTA and WWTB) were subjected to continuous ozonation and FAC-titration experiments. Changes in DOM bulk characteristics (nitrate and chloramine formation, and changes in optical properties) occurring as a function of ozone/chlorine treatment are discussed. Nitrate and chloramine concentrations were compared to the DON content of each water sample. WWTA and WWTB had a DON concentration of 1.30 ± 0.37 mgN/L (93 ± 26 \( \mu \text{M} \)) and 1.14 ± 0.37 mgN/L (80 ± 26 \( \mu \text{M} \)) (Text S10, SI). Compared to NOM isolates, wastewater samples had higher specific DON concentrations (Fig. 6c). These values are in the range of typical DOM concentration in wastewater effluents.

#### 3.3.1. Nitrate formation

An optimal DON transformation to \( \text{NO}_3^- \) requires a large ozone excess, i.e., > 20 mol \( \text{O}_2 \)/mol \( \text{O}_3 \) (Section 3.1.2). Practically, ozone exposures of about 0.32–0.57 M s (256–456 mg min/L) could be reached in batch experiments containing only one ozone-reactive compound (Section 3.1.1). Further increasing the ozone exposures in such systems is impracticable due to the maximum ozone concentration of the ozone stock solution for our setup (i.e., ~1.1 mM). In DOM containing solutions, other faster reacting moieties (e.g., phenolic compounds, Fig. 1a) can act as competing ozone consumers (Omby et al., 2018), lowering the ozone availability for nitrate formation. For these reasons, continuous ozonation experiments were developed in this study. In solutions containing DOM isolates or wastewater, the laboratory system (Fig. 2a) enabled high ozone exposures for a contact time of 120 min (i.e., 0.63–0.97 -Ms or 504–776 mg min/L, Figure S9, SI). These are much higher than ozone exposures typically obtained in laboratory batch experiments with model water or wastewater effluents (Lee et al., 2014; Song et al., 2017; de Vera et al., 2017).

Upon continuous ozonation, a gradual \( \text{NO}_3^- \) production was observed for the four DOM isolates, but with different production rates (Fig. 6a). The \( \text{NO}_3^- \) production was biphasic, with a short initial phase with a high rate followed by a second longer phase with a lower rate (Figure S9, SI). The maximum \( \text{NO}_3^- \) formation potential was defined as the specific \( \text{NO}_3^- \) concentration resulting after a reaction time of 120 min and represented 13–20% of the total DON content (Fig. 6c). In the wastewater samples, the background \( \text{NO}_3^- \) also contributed to the formation of \( \text{NO}_3^- \) (Text S11, SI). To assess the \( \text{NO}_3^- \) formation potential of the DON matrix only, the total \( \text{NO}_3^- \) formation potential was corrected for the initial \( \text{NO}_3^- \) concentration (Fig. 6b). The corrected \( \text{NO}_3^- \) production curves were also

![Fig. 6. Nitrate formation and relative change in absorbance at 260 nm for four isolates and two wastewater samples as a function of time, during continuous ozonation experiments. (a) Specific formation of \( \text{NO}_3^- \) for SRFA, SRNOM, UMRNOM and EfOM-CH (DO\( \text{O}_3 = 10 \text{ mg/L, spectrophotometrically standardized}), (b) formation of \( \text{NO}_3^- \) for WWTA and WWTB (Table S4, SI), (c) nitrate formation (continuous ozonation) compared to the chloramine formation (FAC-titration), in percent of the specific DON content, (d) normalized absorbance at 260 nm of the whole solution (inserts: zoom on the initial phase), (e) change in water matrix absorbance at 260 nm relative to the initial DOM absorbance, (f) zoom on the 10 first minutes of the continuous ozonation experiments with the four NOM isolates showing the formation of \( \text{NO}_3^- \) together with the normalized absorbance at 260 nm of the whole solution (colors refer to the same isolates shown in (a) and (d)). Dashed lines in (d) are corrected NO\( _3^- \) concentrations (i.e., total \( \text{NO}_3^- \) - \( \text{NO}_3^- \) from initial \( \text{NO}_3^- \) oxidation (Text S11, SI)). Error bars depict the standard deviations of duplicate experiments and lines in (d) are averaged signals of duplicate experiments. Experimental conditions: pH 7.00±0.05 (phosphate buffer, [PO\( _4^- \)]\(_{\text{tot}} = 10 \text{ mM})\), \([\text{BuOH}] = 100 \text{ mM, continuous } \text{O}_3/\text{O}_2 \) gas bubbling during 120 min, at room temperature (22±1 °C).](image-url)
characterized by two phases (Figure S9, SI), separated by an intermediate plateau, and finally plateaued at about 42–45% of their DON content (Fig. 6c).

### 3.3.2. Effects of continuous ozonation on DOM optical properties

Fig. 6d shows the normalized absorbance of the solution at 260 nm ($A_{260}/A_{260\text{,max}}$) as a function of time, as monitored during continuous ozonation experiments. This indicator is influenced by two factors: (i) the water matrix absorbance ($A_{260\text{,w}}$) and (ii) the absorbance of dissolved ozone ($A_{260\text{,O}_3}$). During the initial phase ($t_r < 4$ min), a V-shape evolution is visible (insert, Fig. 6d). Although a complete deconvolution of the signals is impossible (i.e., $A_{260\text{,w}}$ vs. $A_{260\text{,O}_3}$), it can be qualitatively interpreted: the descending branch is due to the initial decrease of DOM absorbance (between ~40 s and 120 s), whereas the ascending branch is attributed to the rapid ozone dissolution, reaching its maximum (i.e., $A_{260\text{,max}}$) after about 10 min. The inflection point occurs at the moment where $A_{260\text{,O}_3}$ outcompetes $A_{260\text{,w}}$. Overall, $A_{260\text{,max}}$ profiles are similar for all samples. The slowly decreasing phase observed after the maximum is due to the instability of the ozone generator, without having an influence on the experiments. Moreover, comparing $A_{260}/A_{260\text{,max}}$ at $t_r = 0$ s between isolates renders information about their relative initial absorbance: SRFA > UMRNOM > SRNOM > EfOM-CH, in agreement with their SUVA254 (Table S2, SI).

Fig. 5e shows the relative changes of absorbance at 260 nm of the water matrix ($\Delta A_{260}/A_{260\text{,w}}$) as a function of time, during the same continuous ozonation experiments. This parameter decreased over time, i.e., with increasing ozone exposure. For the NOM isolates, a decrease of 30% occurred at the end of the initial descending branch (120 s) before plateauing at about 40% (25% for SRFA) of the initial $A_{260\text{,w}}$ at $t_r > 10$ min. For WWTA and WWTB, the initial decrease was smaller (about 20%) and the final plateau occurred at about 50% of the initial $A_{260\text{,w}}$ value.

### 3.3.3. Chloramine formation

Oxidative FAC-titrations of the four DOM isolates (DOC=5 mgC/L) were carried out with a reaction time of 120 s. For SRNOM and UMRNOM (Fig. 7a, c), significant $\Delta \beta$ were observed (i.e., 0.68 and 1.32 µmolHOCl/mgC, respectively), indicating that 38–39% of their specific DON content formed chloramines upon oxidative FAC-titration (Fig. 6c).

The same oxidative FAC-titrations performed after derivatization showed no significant $\Delta \beta$ (Figure S10, SI), confirming that $\Delta \beta$ consists of chlorine reactive nitrogen-containing compounds, which form chloramines. For SRFA (the fulvic acid fraction of SRNOM) and EfOM-CH (the hydrophobic fraction of the sampled EfOM), no significant $\Delta \beta$ were observed (Fig. 7b and d). A FAC slope of 0.80–0.84 (i.e., <1, Table S9, SI) for the four tested isolates revealed the presence of slower HOCl consuming compounds, which did not form chloramines. For wastewater samples, the background NH$_2$/NH$_4^+$ concentration also contributed to the formation of chloramines (Text S12, SI) (Wolfe et al., 1984).

To assess the chloramine formation potential of the DON matrix only, the total chloramine formation potentials (Fig. 7e, f) were corrected for the initial NH$_2$/NH$_4^+$ concentrations. After correction, WWTA and WWTB showed significant $\Delta \beta$ of 1.31 (11% of the DON content) and 0.79 µmolHOCl/mgC (5%), respectively (Fig. 6c).

### 3.4. Characterisation of DBP precursors and oxidation mechanisms in DOM

Fig. 6f compares $A_{260}/A_{260\text{,max}}$ and the specific NO$_3$ formation for
the 10 first minutes of the continuous ozonation experiments, for the four isolates. The NO$_3^-$ formation started only after completion of the initial descending branch of ($A_{260}/A_{260,max}$)$_{tot}$. Similarly, two steps could be observed during FAC-titrations of the four isolates: (i) a complete HOCI consumption at lower doses (i.e., significant $\beta_C$, Table S9, SI) without formation of chloramines, and (ii) chloramines formation (i.e., significant $\Delta f$, Table S9, SI) and/or slow chlorine consumption (i.e., FAC and TC slopes $< 1$, Table S9, SI) at higher doses (Fig. 7). These observations experimentally confirm a step-wise oxidation of electron-rich moieties in DOM, which has been hypothesized by kinetic modeling (Buffe et al., 2006). From a kinetic point of view, this corresponds to two main steps occurring successively: (i) oxidation of phenolic compounds; (ii) oxidation of amine compounds (Fig. 1a, b).

Decreases in SUVA$_{254}$ have been correlated with the abatement of conjugated NOM structures, by cleavage of aromatic rings (Remucal et al., 2020; von Sonntag and von Gunten, 2012; Weishaar et al., 2003; Wenk et al., 2013). Therefore, the observed initial changes in optical properties (i.e., initial descending ($A_{260}/A_{260,max}$)$_{tot}$ branch and fast initial decrease in ($A_{260}/A_{260,0}$)$_{tot}$) are most likely caused by oxidation of phenolic moieties. Further decreases in ($A_{260}/A_{260,0}$)$_{tot}$ may be attributed to ring cleavages of other aromatic structures reacting more slowly with ozone, such as anisoles and polymethoxybenzenes, which are structures present in lignin (Mvula et al., 2009). By comparing the ($A_{260}/A_{260,max}$)$_{tot}$ profile of each isolate and the profile of a control solution containing no DOM, the initial ozone exposure consumed by phenolic compounds (i.e., $t_{0.5} \sim 40$–120 s) could be calculated, from which the concentration of abated phenolic compounds was estimated (Text S13, SI). This results in 0.74–1.09 mmolPhOH/mgC, that were abated during this first phase, independently of the total phenolic content (Figure S11, SI). $\beta_C$ observed during FAC-titrations are in the same range (0.5–1.2 mmolPhOH/mgC, Figure S11, SI), suggesting that both assays also provide estimates for highly reactive phenolic compounds serving as DBP precursors, mostly resorcinol, hydroquinones and catechols, as previously observed (Arnold et al., 2008; Jiang et al., 2020; Zhao et al., 2012).

The formation of nitrate reveals the presence of mineralizable nitrogenous moieties, such as amino acids and activated primary and secondary amines (Section 3.1.2). The biphasic nature of the nitrate formation during ozonation suggests that various types of nitrogenous moieties were mineralized. The first phase was systematically associated with a higher $k_{f(NO_3)}$ than the second phase (Figure S9, SI). According to the kinetic and dosage experiments with model compounds, the first phase may be attributed to compounds forming nitrate quickly (i.e., amino acids, phase 1 in Fig. 6c) with estimated second-order rate constants $k_{f(NO_3)}$ in the range of 2.5–6.5 M$^{-1}$s$^{-1}$, which is similar to L-alanine (7.1 M$^{-1}$s$^{-1}$). For instance, the estimated constant of fast reacting amine moieties in SRFA was 0.07 mmolC/mgC, which corroborates well with a previously reported amino acid concentration (0.08 mmolC/mgC) (Averett et al., 1994). For all isolates, the first phase represented less than 10% of their total DON content, which is lower than for WWTA and WWTB (25%, Fig. 6c). The second phase may be attributed to mineralizable primary and secondary amines (phase 2, Fig. 6c) with $k_{f(NO_3)}$ in the range of 0.1–0.3 M$^{-1}$s$^{-1}$, which is closer to $\beta$-alanine (0.1 M$^{-1}$s$^{-1}$) or N-methylbenzylamine (0.6 M$^{-1}$s$^{-1}$). Comparatively, the total content of amino-acids and primary and secondary amines is quantified during FAC-titrations. For SRNOM and UMRNOM, the chloramine formation (40% of DON, Fig. 6c) was higher than the nitrate formation (25% of DON) and the difference may be attributed to the fraction of primary and secondary amines which is not easily mineralized. Nevertheless, this difference is still within a factor of two, which is a reasonable overlap considering the different nature of the two assays. Although these moieties are not forming nitrate quantitatively (i.e., not quantified by continuous ozonation), they can still form N-DBPs such as nitroalkanes upon ozonation, as was demonstrated in this study and in previous investigations (Lim et al., 2019). For SRFA and EffOM-CH, a low nitrate formation was observed (i.e., $\leq 20\%$ of DON) together with an absence of measurable chloramine formation (Fig. 6c). For SRFA, the absence of measurable $\Delta f$ may be due to a too high LOQ for the FAC-titration method (0.5 mmolC/mgC) which is higher than the formation of nitrate measured during ozonation (0.19 mmolC/mgC). The kinetics of NO$_3^-$ formation for EffOM-CH were higher than for the other isolates (Fig. 6a), but closer to the WWTA/B samples. This suggests that other compounds could be responsible for it, such as partially oxidized biomolecules produced during biological wastewater treatment.

WWTA and WWTB had low chloramine formation yields (5–11%) and high NO$_3^-$ yields (42–45%). The initial nitrate formation kinetics is extremely high with $k_{f(NO_3)}$ in the range of 23–29 M$^{-1}$s$^{-1}$. These values are far above the $k_{f(NO_3)}$ for the probe compounds and suggest a direct formation from intermediate compounds. The second phase is then again comparable to $\beta$-alanine and N-methylbenzylamine. This suggests that 34–37% of their DON is composed of moieties which are ozone-reactive but not forming chloramines during chlorination. They are therefore not amino acids or amines, but could be partially oxidized nitrogenous compounds or other biomolecules formed during nitrification, where N-O-containing compounds could be formed. As a consequence, the corresponding nitrogen-containing moieties are not readily available for chlorination. Although being significant contributors to the N-DBP formation potentials, the exact nature of these species remains unknown. Nevertheless, the behavior is in agreement with, e.g., 4-nitrobenzylamine, which was efficiently transformed to nitrate upon ozonation.

Overall, the novel approaches applied in this study show that ozone and FAC-titrations provide complimentary information for the characterization of DOM in terms of potential N-DBP precursors quantification and characterization. In the tested samples, up to 40% of the DON is chlorine and/or ozone reactive and serves as N-DBP precursors. Furthermore, there was a reasonable agreement between ozone and FAC-titrations regarding the fast reactive moieties in NOM samples, which may lead to C-DBPs. Finally, our findings reveal clear differences between the selected NOM/EfOM isolates and the real water samples, however, no general conclusions can be drawn.

3.5. Practical implications

Previous laboratory-scale studies mostly focused on the yields of N-DBP formation from specific model compounds or standard NOM sources under excess oxidant conditions, which could lead to DBP overestimation under real oxidation conditions (Bond et al., 2012). In the proposed approach, a subtle change of perspective is proposed, by focusing on the specific quantification of common oxidation products (nitrate and chloramines) formed upon controlled oxidation experiments with ozone or chlorine. Nitrate is the end product of the nitrogen oxidation cascade and our study demonstrates that intermediate N-DBPs are formed at lower ozone doses. In that sense, the nitrate formation is an unambiguous indicator of the N-DBP formation potential, because nitrate is formed only after extensive ozonation via intermediate TPs. This suggests that the fraction of DON that can form undetected N-DBPs during ozonation is high. In WWTP including an ozonation step, moderate ozone doses are typically targeted (0.55–0.65 gO$_3$/gDOC) (Bourgin et al., 2018). It can be assumed, that phenolic moieties consumed 0.02–0.21 gO$_3$/gDOC during the initial phase of continuous ozonation experiments (Text S13, SI). Therefore, a significant part of the applied ozone dose could subsequently oxidize DON, forming various N-DBPs, even under realistic ozonation conditions. FAC-titrations offer complimentary information about the nature of the N-DBP precursors, as not all primary and secondary amines form nitrate readily upon ozonation. This permits to estimate the concentration of N-DBP precursors present in a water sample, also including primary and secondary amines which are only partially oxidized to e.g., nitroalkanes during ozonation.
4. Conclusion

In this study, kinetics and mechanisms of \( \text{NO}_3^- \) formation by the reaction of amine compounds with ozone have been studied with model compounds, DOM isolates and wastewater samples. Moreover, a combination of two novel oxidation assays was developed and applied to better characterize the nature of oxidant-reactive nitrogenous moieties in DOM. The following conclusions can be drawn:

- Molar \( \text{NO}_3^- \) yields of 30 nitrogenous model compounds have been determined in ozone dosage experiments, at pH 7. With 20 molar equivalents of ozone, amino acids and activated primary amines showed the highest yields (>90%), followed by other primary and secondary amines (20–50%) and tertiary amines (<15%). This information can serve as a basis for a better understanding of reactivity of nitrogenous moieties in DOM.

- Nitroalkanes are major intermediate TPs in the oxidation cascade of primary and secondary amines, e.g., nitromethylenbenzene (benzylamine and \( N \)-methylbenzyamine) and nitromethane (\( N \)-methylbenzyamine). For secondary amines, the formation of nitroalkane occurs by \( C-N \) bond cleavage on nitrite intermediates, e.g., \( N \)-methyl-1-phenylmethanimine oxide and \( N \)-phenylmethanimine oxide (\( N \)-methylbenzyamine).

- The nitrate formation potential of a nitrogenous compound is not correlated to its apparent second-order rate constant for the reaction with ozone, but it is related to its chemical structure. In particular, the presence of an electron-withdrawing group in the vicinity of the amine group promotes the formation of \( \text{NO}_3^- \) by decreasing the \( \alpha \)-value of the \( \alpha \)-carbon on intermediate nitroalkanes.

- The formation of \( \text{NO}_3^- \) in NOM/EfOM and wastewater samples during continuous ozonation is biphasic. The first phase is attributed to amino acids and the second phase to mineralizable primary and secondary amines, as kinetically determined. This is an effective way to characterize and quantify the DON fraction which is ozone-reactive. This fraction was higher for wastewater samples than for NOM/EfOM isolates (i.e., 40% vs. <20%). For wastewater samples, the formation of \( \text{NO}_3^- \) was also faster.

- FAC-titration is a complementary method used to reveal the formation of chloramines in NOM/EfOM or wastewater samples, as a proxy for the total primary and secondary amine content.

- For two NOM isolates (SRNOM and UMRNOM), the DON fraction which is chlorine-reactive was higher than the DON fraction which is ozone reactive (i.e., 40% vs. <20%). This reveals the presence of primary and secondary amines which are not forming \( \text{NO}_3^- \) quantitatively but that can potentially form nitroalkanes. In contrast, wastewater samples had higher ozone-reactive DON fractions than chlorine-reactive fractions (i.e., 40% vs. <11%), revealing the presence of partially oxidized nitrogenous compounds with a N-O bond, formed during nitrification. These observations highlight the good complementarity of both assays in deconvoluting the composition of the DON pool.

- Oxidant-reactive phenolic compound concentrations could also be estimated by both methods and were in the range 0.5–1.2 \( \mu \text{mol} \cdot \text{L}^{-1} / \text{mg} \) for the NOM/EfOM isolates.

- Overall, the tested samples had a significant oxidant-reactive DON fraction (up to 45%) which could lead to the formation of \( \mu \text{M}-\)levels of potentiometrically reactive ND-BPs such as nitro-compounds during ozonation of drinking water or wastewater. The presented assays can help to gauge this level for specific water samples. A post-chlorination could potentially lead to the formation of toxic halonitro- compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117864.

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