Photodegradation of 17α-ethynylestradiol in dissolved humic substances solution: Kinetics, mechanism and estrogenicity variation

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ABSTRACT

17α-Ethynylestradiol (EE2) in natural waters may cause adverse effects on organisms due to its high estrogenic potency. Laboratory studies were performed to study the effects of a local humic acid (LHA), fulvic acid (LFA) and Aldrich humic acid (AHA) on the photochemical behavior and estrogenic potency of EE2. Here photolytic experiments demonstrated that pure aqueous EE2 could undergo direct and self-sensitized photodegradation at a global rate of 0.0068 hr⁻¹. Photodegradation rate of EE2 in 5.0 mg/L dissolved humic substances (DHS) was determined to be 0.0274, 0.0296 and 0.0254 hr⁻¹ for LHA, LFA and AHA, respectively. Reactive oxygen species (ROS) and triplet dissolved humic substances (3DHS*) scavenging experiments indicated that the promotion effect of DHS on EE2 photodegradation was mainly aroused by the reactions of HO• (35%–50%), 1O2 (<10%) and 3DHS* (22%–34%). However, the photodegradation of EE2 could also be inhibited when DHS exceeded the threshold of 10 mg/L. Three hydroxylation products of EE2 were identified using GC–MS and their formation pathways were also proposed. In vitro estrogenicity tests showed that EE2 was transformed into chemicals without estrogenic potency. These findings could extend our knowledge on the photochemical behaviors of steroid estrogens in sunlit natural waters.

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Introduction

Steroid estrogens, including estrone, 17β-estradiol, estriol and 17α-ethynylestradiol (EE2), have attracted increasing concerns in recent years due to their widespread presence in the environment (Rao et al., 2013) and their detrimental effects on both human and animal endocrine systems (Liu et al., 2015; Wolff et al., 2015). As an active ingredient of oral contraceptives, EE2 has been extensively used and discharged into natural waters (Zhang et al., 2014a, 2014b; Huang et al., 2013). EE2 was reported to be not only more stable than natural estrogens in the environment (Robinson and Hellou, 2009; Zuo et al., 2013) but also be of the strongest estrogenic potency among the steroid estrogens (Sumpter and Johnson, 2008). Therefore, knowledge on the fate of EE2 in natural aquatic systems is essential for ecological risk assessment.

EE2 can undergo direct photodegradation due to its light absorption band overlapping with the solar spectrum in 280–320 nm (Mazzelier et al., 2008), but the direct photolysis of EE2 is very slow even under the irradiation of a 250 W high pressure mercury lamp (Liu et al., 2003). It is interesting that EE2 was found to be photodegraded with a half-life of less than 2 days in natural waters (Zuo et al., 2006, 2013), and the fast degradation was proposed to be related with the...
ubiquitous occurrence of dissolved organic matter (DOM) and other aquatic components. DOM mainly includes dissolved humic substances (DHS), such as humic acid (HA) and fulvic acid (FA), which are the important sunlight absorbers in natural waters (Wang et al., 2015; Zhang et al., 2015). Two alternatives of DHS affecting the phototransformation of most organic pollutants in aqueous solutions could be anticipated: (1) photodegradation rates of contaminants would be increased by DHS acting as photosensitizers (Canonica, 2007) and generating reactive oxygen species (ROS), such as hydroxyl radical (·OH), singlet oxygen (¹O₂), superoxide radical (O₂−/HO₂−), hydrogen peroxide (H₂O₂), hydrated electron (e⁻aq) and peroxyl radical (ROO•) (Blough and Zepp, 1995; Liang et al., 2015; Song et al., 2012; Zhang et al., 2014a, 2014b); and (2) photodegradation rates of contaminants would be decreased by DHS competing the incident light with pollutants, scavenging ROS, and reducing the oxidized intermediates of pollutants (Brame et al., 2015; Janssen et al., 2014; Sempéré et al., 2015; Wenk and Canonica, 2012).

The knowledge on DHS affecting EE2 photodegradation is still limited, although the scientific effort devoted to study the occurrence and fate of estrogens in natural waters has never been stopped (Caupos et al., 2011; Chen et al., 2013; Leech et al., 2009). The photodegradation of EE2 was reported to be promoted by DOM, but no assertive conclusions were obtained about the photodegradation pathways and mechanisms (Grzybowski and Szydlowski, 2014). HO• photogenerated by DHS and triplet dissolved humic substances (3DHS) were reported to be the main contributors to the dissipation of organic pollutants from sunlit natural waters (Bodhipaksha et al., 2015; Liang et al., 2015; Song et al., 2012), and other photogenerated ROS, such as O₂−/HO₂−, H₂O₂, e⁻aq and ROO• played a minor role (Boule et al., 1999). However, the role of DHS in photogenerating reactive species and mediating the photodegradation of EE2 remains unclear, and the changes in estrogenic potency of EE2 induced by DHS in sunlit surface waters are also unknown.

Thus, this work was primarily conducted to investigate the photodegradation of EE2 in the absence and presence of a local humic acid (LHA) and a local fulvic acid (LFA) which was extracted from the Dianch Lake sediments. Well established ROS and 3DHS scavenging experiments were employed to qualitatively identify the reactive species produced by the LHA, LFA and Aldrich humic acid (AHA), and to quantify the contribution of the photogenerated ROS and 3DHS to EE2 degradation. Furthermore, this work aimed to identify the main photodegradation products of EE2 and assess the estrogenic potency variation of EE2 in the irradiated DHS containing waters. These results could extend our knowledge on the photochemical behaviors of EE2 and the mechanisms responsible for DHS mediating organic pollutants photo-transformation in natural waters.

1. Materials and methods

1.1. Origin of chemicals

Pyridine, Amplex Red, horseradish peroxidase, 1,4-benzoquinone (BQ), sorbic acid (SA), chromatographic grade 1-ProOH and EE2 standard sample were purchased from Sigma Aldrich with the highest purity available. Selected properties and optimized 3-dimensional structure of EE2 are displayed in Appendix A Table S1 and Fig. S1, respectively. Chromatographic grade acetonitrile, acetonitrile, ethyl acetate and n-hexane were purchased from Merck Co. (Germany). Trimethylchlorosilane and N,O-bis (trimethylsilyl) trifluoroacetamide were purchased from Fluka (USA) and used as the derivatization reagents. LHA and LFA were extracted from the collected sediments using a traditional method shown in Appendix A Scheme S1, and the information about the sediments collection is displayed in Appendix A Fig. S2. Commercial AHA was purified using the method as LHA extraction and used as a reference. Inorganic reagents of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Milli-Q water (electric resistivity >18 MΩ cm) was used throughout this study.

1.2. Solution preparation

EE2 working solution was prepared freshly every week by dissolving (3.00 ± 0.05) mg EE2 into 1 L Milli-Q water. The solution was continuously stirred for 24 hr at 25°C to ensure a maximum dissolution, and then it was filtrated by 0.45 μm glass fiber filters (GF/F, Millipore Corp.) prebaked at 450°C for 4 hr. The filtrate was stored in a brown reagent bottle wrapped with aluminum foil and placed in refrigerator at 4°C.

DHS stock solutions were obtained by dissolving 500 mg LHA or AHA into 300 mL NaOH solutions (0.05 mol/L) and 500 mg LFA into 300 mL Milli-Q water, respectively. All the solutions were oscillated for 24 hr, centrifuged at 2327 g for 20 min and filtrated by prebaked GF/F. The pH of the filtrates was adjusted to 7.8 ± 0.1 using 1 mol/L NaOH and 1 mol/L H₂SO₄, and then they were quantified the total organic carbon (TOC) content (MicroCube, Elemental). The prepared DHS stock solutions were stored in polyethylene containers and kept at 4°C in dark for use within 3 weeks. No significant change in the TOC content was observed during the storage period.

1.3. Photodegradation experiments

All the photodegradation experiments were performed on an XPA-7 merry-go-round photochemical reactor (Appendix A Fig. S3) with 50 mL cylindrical quartz tubes (Φ = 15 mm) and magnetic stirrers (150 r/min). A 500 W xenon arc lamp placed at the axial centre of the reactor was used as a light source which was equipped with air mass global filters (AM1.5). The irradiance spectrum of the light source is shown in Appendix A Fig. S4, and the light intensity at the surface of the reactor was determined to be 601.7 W/m² using a visible light irradiation detector (FZ-A, Photoelectric Instrument Factory, Beijing Normal University, China).

To explore the photochemical behaviors of EE2 induced by DHS, batch experiments were conducted as follows: (1) dark controls in Milli-Q water and DHS solutions, (2) photolyzing EE2 in Milli-Q water, (3) photolyzing EE2 in aqueous and D₂O/H₂O (V/V, 9/1) solutions with 5 mg/L DHS, (4) photolyzing EE2 in solutions with DHS ranging from 0 to 20 mg/L, (5) photolyzing EE2 in 5 mg/L DHS solutions with different scavengers, i.e., 0.26 mol/L 1-ProOH, 1 mmol/L SA,
2 mmol/L NaN₃ and 0.06 mmol/L BQ, (6) degrading EE2 in the presence of 20 μmol/L H₂O₂ or 100 μmol/L KO₂ under the condition of dark and irradiation, respectively. The initial concentration of EE2 in all experiments was fixed at 1.46 mg/L, and the pH value of the solutions was adjusted to 7.8 ± 0.1 followed by 1 hr equilibration under dark condition. All the sample tubes were fixed to allow air flow in and maintained at (25 ± 0.5)°C using a recirculating cooling water bath. Aliquots of 500 μL samples were withdrawn at suitable time intervals to quantify the residual EE2. Photodegradations and controls were carried out in duplicate at least.

1.4. Procedures for EE2 quantification

EE2 was quantified by a high performance liquid chromatography (HPLC, Agilent Technologies 1260, USA) equipped with a Waters symmetry-C18 reversed phase column (5 μm, 4.6 mm × 250 mm) and a fluorescence detector. The mobile phase was 60:40 (V/V) of acetonitrile and Milli-Q water containing 0.1% trifluoroacetic acid. EE2 was eluted from the column by the mobile phase with a constant flow rate of 1.0 mL/min and quantified at 236/310 nm. The retention time of EE2 was 5.0–5.1 min. No apparent change in EE2 response was observed when added with DHS. The quantification limit of EE2 (LOQ) was 0.03 mg/L, and the relative standard deviations for all samples were within 5%.

1.5. Photodegradation products identification

EE2 was irradiated in the presence of different DHS for 18 hr, which was designed to investigate the main photodegradation products of EE2. Targets were obtained by using the Oasis HLB cartridges (Milford, MA, USA) and analyzed by using a gas chromatography-mass spectrometry (GC–MS) according to prior reported methods (Zuo and Zhang, 2005). Briefly, 30 mL irradiated samples were extracted using the preconditioned cartridges at a flow rate lower than 4 mL/min. The targets loaded cartridges were then dried under vacuum for 180 min with 2 × 5 mL methanol/water (V/V, 1/9) prewashing. After that, analytes were eluted by ethyl acetate and evaporated to dryness with high purity nitrogen. Prior to GC–MS detection, the dried analytes were derivatized by adding 50 μL N,O-bis (trimethylsilyl) trifluoroacetamide containing 2% trimethylchlorosilane and 50 μL pyridine at 70°C for 30 min.

GC–MS analysis of the derivatized samples was performed on a Trace GC (Thermo Fisher Scientific, USA) equipped with an auto-sampler Triplus AS and a DSQ quadrupole mass spectrometer. The DB-5 MS capillary column (Thermo Fisher Scientific, USA) with a 0.25 mm inner diameter, 30 m length, and 0.25 mm film thicknesses was used as a separation column. The column temperature for separating the targets was programmed as previously reported (Huang et al., 2013). Ultra high purity helium carrier gas was maintained at a constant rate of 1 mL min⁻¹. Injector temperature was held at 280°C, and the injection volume was 1.0 μL in a splitless mode. The temperature of the MS transfer line and ion source was maintained at 300 and 250°C, respectively. The energy of the ionizing electrons was kept at 70 eV. Mass spectra were scanned in a full scan mode from 50 to 600 m/z mass range for qualitative analysis.

1.6. Yeast-based estrogenicity test

Saccharomyces cerevisiae carries human DNA estrogen receptor sequence and can encode the enzyme β-galactosidase when exposed to the chemicals with estrogenic potency. Therefore, the yeast estrogen screen (YES) bioassay was performed as a previously reported method with minor modifications to assess the variation of EE2 estrogenic potency aroused by photodegradation (De Boever et al., 2001). Briefly, aliquots of 20 μL irradiated EE2 solutions were taken out at each kinetics testing point and mixed with 180 μL fresh YES media in a round-bottom 96-well plate. One abiotic and one negative well containing no targets were performed on the plate as controls. The plate was incubated at 30°C for 48 hr, and it was intermittently shaken at 50 r/min for 2 min every 8 hr to intermix and disperse the growing cells. Aliquots of 50 μL cycloheximide (17 μmol/L) chloroprophol red galactopyranoside (10 mg/L) solutions were then added to stop the cell growth and visualize β-β-galactosidase, and all the positive wells were denoted by a red color. A plate reader (LT-4000MS, Labtech) was used to detect the optical density at 580 nm (OD₅₈₀) which was then subtracted by the background OD, and the differences were regarded as the proxy for estrogenic potency. Normalized OD/OD₀ values were used to evaluate the changes in EE2 estrogenic potency.

1.7. Other characterizations of DHS

LHA, LFA and AHA were characterized to obtain the information on their elemental compositions, UV–vis light absorbing characteristics, functional group composition, relative molecular weight, and fluorescent properties. The results are shown in Appendix A Table S2 and Figs. S4–S7, respectively. The yield of H₂O₂ generated by DHS over an irradiation period of 40 min was determined by a modified spectra method (Sharpless et al., 2014; Zhou et al., 1997) which is detailed in Appendix A Text S1.

2. Results and discussion

2.1. Photodegradation of pure aqueous EE2

Dark controls of EE2 pure aqueous solutions were carried out for 144 hr by covering the quartz tubes with aluminum foil. As shown in Fig. 1a, no significant decrease in the concentration of EE2 was observed, which indicates that the hydrolysis, biodegradation, thermolysis, volatilization and adsorption of EE2 onto reactor walls could be neglected during its photodegradation processes.

Pure aqueous EE2 could be degraded at a rate of 0.0068 ± 0.0002 hr⁻¹ (± standard errors represented at 0.95 confidence level) when it was exposed to the simulated solar light. Fig. 1b shows that 4% of EE2 was lost over the irradiation period, which was attributed to the weak emission intensity of the light source in the range of 280–320 nm and the weak light absorption ability of EE2 (Appendix A Fig. S4). Compared to the photodegradation of estrone (Caupos et al., 2011), 17β-estradiol (Leech et al., 2009), estriol (Chen et al., 2013) and other phenolic endocrine disrupting chemicals (Zhu and Zuo, 2013) in pure aqueous
solutions, EE2 is a relative photo recalcitrant steroid estrogen. All the adjustable determination coefficients ($R^2_{adj}$) for the linear regressions of $\ln(C/C_0)$ versus the reaction time exceeded 0.95 (Appendix A Table S3), which demonstrates that the photodegradation of EE2 in Milli-Q water follows the pseudo first-order kinetics. The photodegradation rate of EE2 can be obtained by Eqs. (1) and (2).

$$C = C_0 \exp(-k_{obs}t)$$  \hspace{1cm} (1) \\

$$\ln(C/C_0) = -k_{obs}t$$  \hspace{1cm} (2)

where $C_0$ (mg/L) and $C$ (mg/L) are the concentrations of EE2 at the time zero and $t$ (hr), respectively; $k_{obs}$ (hr$^{-1}$) is the observed pseudo-first-order rate constant. The half-life ($t_{1/2}$) of EE2 could be calculated by Eq. (3).

$$t_{1/2} = \ln 2/k_{obs}$$  \hspace{1cm} (3)

Upon addition of i-PrOH and NaN$_3$ into EE2 solutions, the degradation rate of EE2 was decreased (Appendix A Table S3, Fig. 1b). It has been reported that i-PrOH could selectively scavenge HO· at a rate of $1.9 \times 10^9$ (mol/L)$^{-1}$ sec$^{-1}$ (Buxton et al., 1988). NaN$_3$ could react with both HO· and 1O$_2$ and the scavenger HO· at a rate of $2.7 \times 10^8$ (mol/L)$^{-1}$ sec$^{-1}$ (Ge et al., 2010). Therefore, self-sensitization of EE2 was involved in its photodegradation in Milli-Q water. The mechanisms and pathways responsible for EE2 photodegradation were proposed as the Photoreaction (4)-(11) according to a previous study (Feilberg and Nielsen, 2000). Photolysis of EE2 in 100 nmol/L phenol was performed to confirm the function of phenolic hydroxyl group in EE2 photodegradation. As shown in Fig. 1b, half-life of EE2 in the illuminated phenol solution (88 hr) was significant shorter than that in Milli-Q water (102 hr). Also, these results corroborated the mechanisms responsible for phenolic compounds accelerating the photolysis of PAHs (Wu et al., 2015). Thus, the observed dissipation of EE2 in Milli-Q water was aroused by direct photodegradation and self-sensitization via HO· oxidation.

$$EE2 + hv \rightarrow ^1EE2^+ \rightarrow ^3EE2^+$$  \hspace{1cm} (4) \\

$$^3EE2^+ + O_2 \rightarrow EE2^+ + O_2^-$$  \hspace{1cm} (5)

Photodegradation of EE2 could be significantly accelerated by DHS compared to that in Milli-Q water (Fig. 2), and the degradation rate constant of EE2 in LHA, LFA and AHA solutions was determined to be $0.0274 \pm 0.0004$, $0.0296 \pm 0.0005$ and $0.0254 \pm 0.0005$ hr$^{-1}$, respectively. Acceleration efficiency (AE) was calculated to be 4.03 for LHA, 4.35 for LFA and 3.74 for AHA according to Eq. (12). Photoinductive activity of FA was also reported to be stronger than that of HA in mediating the photodegradation of dicarbollidimide (Hustert and Moza, 1997) and fenuron (Aguer et al., 2002). In order to explore the reasons responsible for the photo-reactivity discrepancy of the DHS in accelerating EE2 degradation, DHS were subjected to the characterization of elemental composition, fluorescent property, molecular weight and functional group. LFA was found to be of higher oxidation degree ($O/C = 0.77$, as shown in Appendix A Table S2) and richer phenolic components (stronger response of C–O and O–H vibration in Fourier Transform Infrared spectroscopy, as shown in Appendix A Fig. S5) than LHA and AHA. Oxygen containing groups, especially the phenolic and quinonoid fractions of DHS, were reported to be related with the photosensitization activity of DHS (Caupos et al., 2011; Ou et al., 2008; Sharpless et al., 2014). LFA was also characterized to be smaller than LHA and AHA by size exclusion chromatography (Appendix A Fig. S6), which was corroborated by the fluorescent characteristics of DHS (Appendix A Fig. S7). Intra-molecular interactions in larger molecules and aggregates can quench more singlet species and inhibit the formation of the triplets which was
responsible for ROS formation and contaminants transformation (Richard et al., 2004; Sharpless and Blough, 2014). Thus, the stronger photosensitization potency of LFA was a coupling effect of molecular weight and oxygen-containing functional groups.

\[ AE = \frac{k_{EE2 + DHS}}{k_{EE2}} \]

where \( k_{EE2 + DHS} \) and \( k_{EE2} \) are the rate constants for EE2 photodegradation in the presence and absence of 5 mg/L DHS, respectively.

To further explore the mechanisms by which DHS promotes the photodegradation of EE2, ROS scavenging experiments were conducted with reactive species scavengers (i-PrOH, SA and NaN₃) and DHS. Fig. 2 shows that all the scavengers induced a retardation of EE2 photodegradation in the DHS solutions, and the decreased degradation rate ratios were calculated by Eqs. (13)–(15).

\[ R_{HO} = \frac{k_{EE2 + DHS} - k_{EE2 + DHS + i-PrOH}}{k_{EE2 + DHS}} \]

\[ R_{1O2} = \frac{k_{EE2 + DHS + i-PrOH} - k_{EE2 + DHS + NaN3}}{k_{EE2 + DHS}} \]

\[ R_{3DHS*} = \frac{k_{EE2 + DHS} - k_{EE2 + DHS + SA}}{k_{EE2 + DHS}} \]

where \( R_{HO} \), \( R_{1O2} \) and \( R_{3DHS*} \) are the contribution ratios of reactions via HO·, ¹O₂ and ³DHS*, respectively; the \( k_{EE2 + DHS} \), \( k_{EE2 + DHS + i-PrOH} \), \( k_{EE2 + DHS + NaN3} \) and \( k_{EE2 + DHS + SA} \) are the photodegradation rate constants of EE2 in the presence or absence of i-PrOH, NaN₃ and SA, respectively.

Table 1 shows that HO· and ³DHS* contributed 38%–52% and 22%–34% to EE2 photodegradation in different DHS solutions, respectively. However, the contribution of ¹O₂ was 5% for LHA, 1% for LFA and 9% for AHA. Similarly, HO· photogenerated by HA was also found to be the main ROS dominating the photodegradation of cylindrospermopsin (Song et al., 2012), estrone (Caupos et al., 2011) and 17β-estradiol (Leech et al., 2009). The photodegradation of EE2 induced by the photogenerated ³DHS* also could be corroborated by prior studies (Canonica, 2007; Bahnmüller et al., 2014). In order to confirm the minor role of ¹O₂, EE2 was photodegraded in D₂O/H₂O (V/V, 9/1) with 5 mg/L DHS, by which a longer life of ¹O₂ could be expected (Song et al., 2012). Compared to the photodegradation of EE2 in aqueous solutions, the degradation rates of EE2 in D₂O/H₂O solutions were only increased by 9%, 7% and 11% for LHA, LFA and AHA, respectively. Therefore, ¹O₂ indeed gave a minor contribution to EE2 photodegradation in aqueous solutions. Formation pathways for HO· were detected by a battery of ROS scavenging and positive controls. O₂⁻/HO₂ were identified by using BQ as scavenger (Appendix A Fig. S8), but they were determined to be

<table>
<thead>
<tr>
<th>Systems</th>
<th>( R_{HO} ) (%)</th>
<th>( R_{1O2} ) in H₂O (%)</th>
<th>( R_{1O2} ) in D₂O/H₂O (V/V, 9/1) (%)</th>
<th>( R_{3DHS*} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EE2 + LHA</td>
<td>46</td>
<td>5</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>EE2 + LFA</td>
<td>38</td>
<td>1</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>EE2 + AHA</td>
<td>52</td>
<td>9</td>
<td>20</td>
<td>27</td>
</tr>
</tbody>
</table>

inactive towards EE2 (Appendix A Fig. S9). Addition of 1 mmol/L BQ into DHS solutions could decrease the production of H$_2$O$_2$ by 60% (Appendix A Fig. S10), i.e., the photogenerated H$_2$O$_2$ was mainly formed by O$_2^-$ reacting with H$^+$ or H$_2$O, which was responsible for the most HO$^.$ formation in the solutions. Finally, the mechanisms responsible for HO$^.$ formation and EE2 degradation in the irradiated DHS solutions were proposed as the Reactions (16)-(25).

$$\text{DHS} + \text{hv} \rightarrow \text{DHS}^+$$ (16)

$$1\text{DHS}^+ \rightarrow 2\text{DHS}^+$$ (17)

$$3\text{DHS}^+ \rightarrow \text{DHS}$$ (18)

$$3\text{DHS}^+ + \text{O}_2 \rightarrow 3\text{O}_2^+ + \text{DHS}$$ (19)

$$3\text{DHS}^+ \rightarrow \text{DHS}^- + \text{e}_{aq}^- + \text{DHS}^+$$ (20)

$$\text{DHS}^- + \text{e}_{aq}^- + \text{O}_2 \rightarrow 2\text{O}_2^- + \text{DHS}^+$$ (21)

$$2\text{O}_2^- + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O}_2 + \text{O}_2$$ (22)

$$2\text{O}_2^- + 2\text{H}_2\text{O} \rightarrow 2\text{OH}^- + 2\text{OH}^+ + 2\text{O}_2$$ (23)

$$\text{H}_2\text{O}_2 \text{hv} \rightarrow 2\text{HO}$$ (24)

$$\text{EE2} + \text{HO}^- + 2\text{OH}^- \rightarrow \text{EE2H}^+ \rightarrow \text{Products}$$ (25)

2.3. Dependence of the acceleration effects on DHS concentration

Although reactive species could be formed by the photolysis of DHS, the observed acceleration efficiencies of DHS were contrary to their UV-vis light absorption ability (Appendix A Fig. S4). It indicates that DHS might compete with EE2 for the incident light and photogenerated ROS (Bahnmüller et al., 2015). The dependence of EE2 photodegradation on DHS concentration could be typically attributed to the following mechanisms: (1) the light attenuation efficiency increased with DHS concentration increasing (Chowdhury et al., 2011), which could decrease the probability of incident photons interacting with EE2. As a result, the direct photodegradation of EE2 was inhibited; (2) intra-molecular interactions and the interactions between aggregations would be increased with DHS increase, which aroused a decrease in the production of reactive species; (3) ROS scavenging could be increased by the increased DHS concentration, which then inhibited the indirect photodegradation of EE2 (Janssen et al., 2014; Vione et al., 2006).

2.4. Formation pathways of EE2 hydroxylation products

To further shed light on the mechanisms for EE2 depletion in the irradiated DHS solutions, the photodegradation products of EE2 were determined by GC-MS. Three hydroxylation products were detected and displayed in Appendix A Fig. S11 and Table 2.

HO$^.$ typically behaves as an electrophile and reacts with organic compounds by following three competitive pathways, i.e., (1) addition to unsaturated carbon including aromatic ring, carbon–carbon double and triple bond, (2) hydrogen abstraction from saturated carbon, such as the methyl and methylene groups, (3) electron abstraction from aromatic ring, carbon–carbon double bond or carboxylate (Buxton et al., 1988; Shah et al., 2015). HO$^.$ addition to electron rich structures is usually faster than hydrogen and electron abstraction when it reacts with organic compounds (Melloni et al., 1981). Therefore, HO$^.$ is more likely to attack the benzene ring at C10, C2 and C4 where there are of higher charge density compared to other positions in EE2 (Appendix A Fig. S12) due to the electron-donating effect of the –OH at C3. Similar degradation mechanisms were also found in the photodegradation of estrone (Caupos et al., 2011), bisphenol A (Zhan et al., 2006) and microcystin-LR (Antoniou et al., 2008). Quinone methide derivative was reported to be one of the photodegradation products of EE2 in Milli-Q water (Mazellier et al., 2008), while it was not found in this work. In contrast, a carbon–carbon double bond between C9 and C11 formed, giving a molecular ion at 294 uma (2 uma with respect to EE2) and a retention time of 28.37 min for the GC separation.

Scheme 1 shows the formation pathways and mechanisms responsible for the three identified hydroxylation products which were triggered by HO$^.$ attacking the aromatic ring followed by loss of H$_2$O (pathway A), reaction with oxygen (pathways A, B and C) and release of hydroperoxyl radicals (pathways A, B and C). As a result, one hydrogen atom was substituted by a hydroxyl group (pathway B and C) and two hydrogen atoms were abstracted from C9 and C11 (pathway A). Compared to the photodegradation of 17β-estradiol in aqueous solutions (De Boever et al., 2001), the absence of EE2 photodegradation products arising from C17 may be attributed to the presence of the ethynyl group. The rate constant of ethynyl group reacting with HO$^.$ was reported to be of the same magnitude of that between HO$^.$ and alkene group (Melloni et al.,...

**Fig. 3** – The dependence of EE2 photodegradation rate constant on DHS concentration.
and these reaction rates were slower than that between HO· and aromatic ring (Buxton et al., 1988). Thus, HO· would preferentially attack the electron rich aromatic ring in EE2. It is notable that more products and intermediates, including those formed by HO· reacting with ethynyl group, may be produced in sunlit natural waters due to the influence of coexistent compounds and irradiation conditions.

### Table 2 – List of gas chromatography mass spectrometry (GC-MS) information on EE2 and its photodegradation products.

<table>
<thead>
<tr>
<th>Molecular structure (MW)</th>
<th>Molecular structure of derivatives</th>
<th>Characteristic ions (m/z)</th>
<th>Retention time (min)</th>
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<tr>
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<td>27.77</td>
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<tr>
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<td><img src="294" alt="image" /></td>
<td>423/438</td>
<td>28.37</td>
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<tr>
<td><img src="312" alt="image" /></td>
<td><img src="312" alt="image" /></td>
<td>513/528</td>
<td>30.86</td>
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<tr>
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<td><img src="312" alt="image" /></td>
<td>513/528</td>
<td>31.83</td>
</tr>
</tbody>
</table>

2.5. Estrogenicity changes in EE2-DHS solutions

Three hydroxylation products of EE2 were detected, but other products bound to DHS could not be separated and identified. Furthermore, GC-MS cannot detect the volatiles and usually is unable to detect the unstable species such as organic peroxides which usually decompose on the column or the GC.
Therefore, in vitro estrogenicity tests are essential to assess the changes in estrogenic potency of the irradiated EE2-DHS solutions. Fig. 4 shows the evolution of estrogenic potency of EE2-DHS solutions during the photodegradation period. A good agreement between EE2/EE20 and OD/OD0 indicates that the photogenerated intermediates of EE2 were not estrogenic, i.e., EE2 completely lost estrogenicity when it was photodegraded in the solutions presence of DHS. This result corroborated the previous studies which identified the formation of intermediates and loss of estrogenic potency for 17β-estradiol and EE2 upon occurrence of photodegradation in aquatic solutions (Whidbey et al., 2012). However, this conclusion was not suitable for estrone which was reported to be photodegraded into another potential estrogen, lumiestrone (Trudeau et al., 2011). Thus, the ubiquitous occurrence of DHS in natural waters can effectively reduce the adverse effects of EE2 on aquatic ecosystems.

3. Environmental implications

Since the irradiation intensity and the time used in this work were similar to the exposed sunlight density of natural waters, the results of EE2 photodegradation can well reflect the photodegradation induced by DHS in sunlit natural waters. Although EE2 is a refractory chemical for hydrolysis and direct photolysis, the photodegradation rate of EE2 could be significantly enhanced by DHS. The promoted photodegradation was mainly aroused by the reactions of photogenerated reactive species, including HO· (32%–52%), 3DHS· (22%–34%) and 1O2 (<10%). DHS, at a concentration higher than 10 mg/L, could also inhibit the photodegradation of EE2 by attenuating incident sunlight and scavenging ROS and other active species. Compared to EE2 biodegradation, in which the half-life of EE2 varied from 5 to 108 days (Zuo et al., 2013), photodegradation is one of the fast and effective pathways for EE2 dissipation from natural waters because the estrogenic potency of EE2 was found to be decreased in line with its degradation.

Although the concentration of EE2 in this study exceeded “natural” levels by three to six orders of magnitude, this laboratory work can be reasonably extrapolated to recognize the photochemical behaviors of EE2 in ambient waters by taking the influence of coexistent substances and aquatic parameters into consideration. For example, both the direct and indirect photodegradation of EE2 would be inhibited in deep and DHS-rich waters. The photodegradation procedure could also be incorporated into management and engineering scale application to minimize the hazards of EE2 to aquatic ecosystem. The ideal practice is applying that to remove estrogens from the effluents of sewage treatment plants which were identified as one of the main sources of estrogens discharge (Huang et al., 2013).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.03.002.

Fig. 4 – Evolution of optical density (OD580) and EE2 concentration during the irradiation period.
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