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Gas-permeable liquid-core waveguide coupled to LC-MS for studying the influence of oxygen on photodegradation processes

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ABSTRACT

Light-induced degradation (LID) strongly depends on experimental conditions, among which the presence of oxygen is a major parameter. Elucidating LID processes is of high concern to many areas of interest, e.g., for the conservation of cultural heritage, safety and shelf life of food, and UV-disinfection methods for water purification. Recently, we presented a new, fully automated tool to study molecular photodegradation in solution. The tool employs a gas-permeable liquid-core waveguide (LCW) as a light-exposure cell with in-situ absorption spectroscopy for real-time monitoring, coupled on-line to liquid chromatography (LC) with diode-array (DAD) and mass spectrometry (MS) for characterization of the photodegradation products. The current work reports on the assessment of the potential of the LCW in a tube-in-tube geometry for studying the role of oxygen in photodegradation processes, using Riboflavin and Eosin Y as model compounds. The LID results obtained for Riboflavin and Eosin Y using the LCW set-up were in line with reported data obtained with conventional approaches. On-line LC-MS analysis allowed semi-quantitative monitoring of LID differences underoxic and anoxic circumstances. An increase in degradation by 9% and 30% for Riboflavin and Eosin Y, respectively, was observed after 10 min under anoxic conditions. Moreover, for the first time, the fully debrominated species of Eosin Y (i.e., fluorescein), was identified as a photodegradation product in solution. The presented set-up can be highly useful for the investigation of photodegradation mechanisms and kinetics in solution, including the role of oxygen while increasing analytical efficiency and reducing time spent in the lab.

1. Introduction

The interaction between light and matter is essential to life as we know it. Unfortunately, these interactions may not always be beneficial. In many cases, they lead to the loss of physical and/or structural properties of molecules. These degradations must be revealed to understand and avoid adverse effects on products and processes. Elucidation of photodegradation mechanisms can be challenging as it may involve many parameters, such as the applied wavelength range, light dose, solution pH, and the presence of oxygen [1]. Especially the latter plays a significant role in photodegradation mechanisms. Dissolved oxygen enhances the photodegradation of many compounds and, for example, is important for the production of superoxide in heterogeneous catalysis [1–5]. On the other hand, for some compounds, the triplet excited state is quenched by oxygen, so that it returns to the ground state before reacting [6,7]. For such compounds, the photodegradation rate actually decreases when oxygen is present, as many photodegradation reactions involve the relatively long-lived triplet excited state.

Clearly, oxygen is an important parameter to include when studying photodegradation. The effect of oxygen is generally studied by irradiating aerated and deaerated sample solutions in a beaker or cuvette for a specific amount of time and comparing the resulting compositions after analysis by, e.g., liquid chromatography coupled to mass spectrometry (LC-MS) [8–11]. Although straightforward, this approach can be quite...
tedious and also prone to error as it often requires manual subsampling and degassing the set-up to perform kinetic studies.

This paper presents an alternative approach for studying the influence of oxygen on light-induced degradation (LID) processes using a recently developed platform [8,9]. The fully automated set-up employs a gas-permeable liquid-core waveguide (LCW) as a light exposure cell with in-situ absorbance spectroscopy for real-time monitoring of the photodegradation reactions during irradiation. The LCW is coupled online to LC with diode-array detection and quadrupole time-of-flight MS (LC-DAD-QTOFMS) allowing characterization and selective detection of degradation products. The analytical performance of the LC set-up was assessed and demonstrated in previous studies [12,13]. Here, we evaluate the suitability of the gas-permeable LCW for both oxic and anoxic LID studies, using two colored test compounds (Riboflavin and Eosin Y) of which the photodegradation is well documented.

Riboflavin (RF; Fig. 1), known as the water-soluble vitamin B2, present in a wide variety of foods and also used as a food dye (E101), has been widely studied for its limited photostability and degradation in aqueous and organic solvents [14-20]. It participates in important biological processes, such as the metabolism of carbohydrates, proteins, and fats, hemoglobin synthesis, and the functioning of the eye [21]. Many factors influence the photodegradation of RF, among which oxygen is an important parameter [15,20-22]. Fig. S1 presents a simplified scheme for the proposed photodegradation mechanisms of RF. The reported photodegradation products formed upon irradiation of RF by UV and visible light are cyclodextrinobiloflavine (CDRF), formylmethylflavin (FMF), lumiflavin (LF), lumichrome (LMC), and carboxymethylflavin (CMF) [23-25]. In addition, the quinoxaline derivatives 1,2-dihydro-1-methyl-2-keto-3-quinazoline carboxylic acid (KA) and 1,2,3,4-tetrahydro-1-methyl-2,3-dioxo-quinazoline (DQ), have been described as a result from ring cleavage of FMF in alkaline solutions [18,26,27]. Sheraz et al. [17] proposed a general scheme for the photodegradation of RF, indicating that LMC is formed through the singlet excited state of RF (\( ^1 \text{RF} \)), as well as through photolysis of FMF, which is an intermediate formed via the triplet excited state (\( ^3 \text{RF} \)). FMF is further degraded into LF and photo-oxidized to CMF. According to several studies [17,22], oxygen decreases the photodegradation rate of RF by quenching \( ^1 \text{RF} \) to generate singlet oxygen (\( ^1 \text{O}_2 \)). Anoxic conditions induce enhanced photodegradation of RF along a different degradation route as the photooxidation product CMF cannot be formed. The absorption spectra of the aforementioned degradation products, except for LMC, are virtually identical to that of RF. Therefore, MS is indispensable for the identification of these products.

Eosin Y (EY) is a synthetic red dye (Fig. 2) that has been studied widely due to its low light fastness [8,28-33]. EY was used by famous artists, such as Vincent van Gogh, who was aware of its low light stability and therefore had ‘all the more reason boldly to use them too raw, time will only soften them too much’, as he wrote in a letter to his brother Theo [34]. EY has not only been applied as a pigment in art objects but is also used as a staining agent for biomedical microscopy [35] or as a metal-free photocatalyst [36]. Photodegradation studies with EY have been performed in, e.g., alkaline methanolic solutions [28,29], in water [8] and DMSO [31], on textiles [37], and in paints or paints simulations [30,32,33]. The photodegradation of EY in solution follows consecutive bromine losses (EY-Br, EY-2Br, etc.), similar to those reported on substrates. Fig. S2 presents a simplified scheme for the proposed photodegradation mechanisms of EY. Alvarez-Martín et al. [8] found that under anoxic conditions the degradation rate of EY is lower, but that discoloration occurs faster as compared to anoxic conditions. They proposed two different degradation mechanisms: (i) in the presence of oxygen, the degradation mechanism is an oxidative process where the breakdown of the chromophore of the first debromination product (EY-Br) causes total discoloration; (ii) in the absence of oxygen, debromination takes place while the chromophore remains essentially intact. Ultimately, the fully debrominated product, fluorescein, is expected to be formed [8,29], however, it has never been identified as a photodegradation product. Pirok et al. [31] detected fluorescein in EY samples by LC-MS but assigned it to a sample impurity (i.e., byproduct in the synthesis of EY) which was already present before irradiation.

In this study, we evaluate the usefulness of a LID cell based on a gas-permeable LCW as a potential tool to efficiently study the effect of oxygen on photodegradation rates and processes. First, we assess the rate of oxygen diffusion into the LCW using the well-known methylene blue (MB) reaction. Subsequently, using the LCW we present a new approach for on-line deaeration of the irradiated sample, reducing the number of required manual steps. To investigate the applicability of the system, we conducted LID experiments for RF and EY under oxic and anoxic conditions and measured degradation time profiles by in-situ absorbance monitoring of the irradiated sample in the LCW and LC-MS analysis of the photodegradation products.

2. Materials & methods

2.1. Chemicals

Aqueous solutions were prepared using ultrapure deionized water (MQ; R = 18.2 MΩcm) (Millipore Simplicity Simpak 2, USA.). Methanol (MeOH) and acetone triturate (ACN) were both UPLC/MS grade and purchased from Biosolve ( Valkenswaard, The Netherlands). Formic acid (FA; ≥99%) was purchased from VWR Chemicals ( Darmstadt, Germany). Ethanol (EtOH) (99%), ammonium formate (97%), potassium hydroxide ( KOH; 90%), D-glucose (99.5%), methylene blue ( MB; ≥97%), riboflavin (RF; pharmaceutical secondary standard), and Eosin Y (EY; 99%) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Instrumental

2.2.1. Light exposure cell set-up

The set-up used for all photodegradation experiments is shown in Fig. S3 and was described in great detail in a previous publication [13]. Briefly, the 12-cm long LID cell consists of a tube-in-tube design (Fig. S4)
of which the inner tube is a gas-permeable Teflon AF2400 LCW (Cambridge Reactor Design, UK; i.d., 800 μm; o.d., 1000 μm; volume, 60 mL). The outer tubing made of polytetrafluoroethylene (PTFE or Teflon), has an inlet and outlet for the supply of a continuous flow of gas that surrounds the LCW. In this study, air (≈170 mL min⁻¹) or nitrogen (≈300 mL min⁻¹) was used. The gases can reach the liquid core by diffusion through nanopores in the LCW wall without the formation of bubbles. The LID cell is attached to two PEER connection pieces that contain a quartz optical window that allows in-coupling of light from the Xenon short-arc fiber-coupled irradiation source (Thorlars SLS205, Newton, NJ, USA; 240–1200 nm), and out-coupling of the transmitted light at the distal end of the LCW. The transmitted light, which was converted into absorbance spectra, was measured in real-time by a fiber-coupled CCD spectrometer from Thorlars with a spectral range of 350–700 nm (CCS100/M). The PEER combination pieces were both attached to a filter wheel for the potential addition of optical filters to study the effect of selected wavelengths; these were not used in the current study. The combination pieces contain fluid connections for sample introduction by a Multi-Purpose Sampler (MPS) from Gerstel (Mülheim an der Ruhr, Germany), and for transfer of the LCWs content to a 6-port valve (VICI, Houston, TX, USA) with a 20 μL sample loop, connected to an LC system (Agilent 1100 series, Waldbronn, Germany). Transfer of the irradiated sample was performed by adding 50 μL of ‘flushing solvent’ (75% MeOH in MQ) to the LID cell, which ‘pushed’ the middle section of the LID cell’s content to the sample loop. The LC system was coupled to a DAD (Agilent) and Agilent 6520 Q-TOF mass spectrometer and was triggered by the MPS to start the analysis after the sample loop was filled. After starting the LC-DAD-MS analysis, the LID cell was automatically cleaned by flushing with 300 μL of 75% MeOH in MQ, followed by 300 μL of MQ to be prepared for the next experiment.

2.2.2. LC-DAD-QTOFMS

The LC system was comprised of a quaternary solvent pump, a reversed-phase ZORBAX Eclipse RRHD C18 column (2.1×150 mm; particle size, 1.8 μm) of Agilent, and a security guard column (2.1×5 mm) with the same C18 phase, a column oven set at 40 °C, and a DAD providing UV/Vis absorbance spectra (250–800 nm) of the column effluent with a spectral resolution of 1 nm at a sampling rate of 1 Hz. Mobile phases A and B consisted of 95–2.5–2.5 (v/v/v) and 5–47.5–47.5 (v/v/v) MQ-MeOH-ACN, respectively. Both A and B contained 0.1% FA and 20 mM ammonium formate. The LC gradient ran at a flow rate of 120 μL min⁻¹ starting at 5% B for 1.5 min, increasing from 5% to 95% in 15 min, followed by an isotropic step at 95% B for 5 min, then decreasing to 5% B within 2 min, to finally equilibrate the column for 5 min at 5% B to be ready for the next LC analysis. MS data were recorded using electrospray ionization (ESI) in the positive ion mode in the 150–2000 m/z range at a data sampling rate of 1 Hz. The settings of the ESI source were: gas temperature, 300 °C; drying gas, 5 L min⁻¹; nebulizer gas pressure, 35 psi; capillary voltage, 3500 V. The QTOF settings for the analysis of RF were: fragmentor, 100 V; skimmer, 75 V; octopole 1 RF Vpp, 200 V. For the analysis of EY, the fragmentor was 200 V, and the octopole 1 RF Vpp was 300 V. All MS data were processed with MassHunter software.

2.3. Determination of gas-diffusion rate into the LCW

A solution of 1 g L⁻¹ MB in EtOH was prepared, and 1.6 g KOH and 2 g glucose were added to 60 mL of MQ. Then, 1 mL of the MB solution was added to the KOH/glucose solution. The latter was diluted with MQ to give a solution that exhibited an absorbance of about 1.5 AU when measured in the LID cell; this solution was then used for the experiments. Prior to sample introduction, the system and MB solution were deaerated by purging with nitrogen for 30 min. Then, 70 μL of MB solution was injected into the LCW while the nitrogen flow surrounding the LCW was still on to keep the system free of oxygen. After injection (t = 0 s), the absorbance was measured every second. At t = 40 s the nitrogen flow was substituted by a flow of air. Between t = 105 and 115 s the nitrogen flow was turned on again. When the absorbance stabilized again, the measurement was stopped. The LID system was cleaned and a fresh MB solution was injected. This process was carried out in five-fold.

2.4. Removal of oxygen from samples and solvents

To study photodegradation under anoxic conditions, oxygen must be removed from all samples and solvents. This was first done conventionally (‘external purging’) by bubbling a flow of nitrogen gas through the samples in an LC vial for 5 min. Solvents that were used for cleaning the LID cell were also purged for 30 min before use. With our new approach (‘in-situ purging’), the sample was introduced in the LID cell and then purged with nitrogen via the LCW wall, while the light source was turned off. To compare the effectiveness of the in-situ and external purging methods, aqueous solutions of RF (2 mg L⁻¹) were purged inside the LID cell for 1, 2, 3, 4, 5, 7.5, and 10 min in triplicate. Then, the purged samples were irradiated for 10 min with a continuous flow of nitrogen inside the outer tubing of the LID cell, followed by their LC-DAD analysis.

2.5. Photodegradation of Riboflavin and Eosin Y

For photodegradation experiments using the LCW set-up, solutions of 10 mg L⁻¹ (2.7 μM) RF and 1 mg L⁻¹ (0.15 μM) EY were prepared in MQ. The method of in-situ purging of the samples with nitrogen was applied to create anoxic conditions before irradiation. RF was irradiated for 10 min under anoxic conditions, i.e., with a continuous air or nitrogen flow, respectively, through the outer tubing. This was done in triplicate (n = 3). For EY, a photodegradation time profile was created by irradiating samples for 5, 10, 15, 20, and 30 min under anoxic and anoxic conditions, which was performed in duplicate (n = 2). In-situ absorption spectra were recorded every 2 min throughout all experiments. After irradiation, the samples were online transferred to and analyzed by LC-DAD-QTOFMS. Extracted-ion chromatograms (EICs) for the m/z’s of the compound ions of interest (±0.01 m/z) were constructed and peak areas were established and used for quantitative interpretation.

3. Results and discussion

3.1. Gas-diffusion rate into LCW

It is important to know how fast gas diffuses through the LCW capillary wall into the liquid core of the LID cell to prevent oxygen depletion during irradiation and to effectively introduce or remove oxygen from the sample inside the LCW. Similarly to what we presented in an earlier study [12], the redox reaction of MB involving glucose and oxygen was employed to study the diffusion rate through a color change. Under anoxic conditions, the reduced form of MB (colorless) will be present, which is transformed to the oxidized form (dark blue) when oxygen is introduced into the solution. The color change was monitored by absorbance measurements at 668 nm in real-time. The absorbance increase is an indication of the rate at which oxygen diffuses through the 100-μm thick LCW wall into the liquid core.

The experiment was started under anoxic conditions. A nitrogendrugged MB solution was injected at t = 0 s into the LCW while nitrogen gas was flushed through the outer tube so that MB was mainly present in its reduced, i.e. colorless, form (MBred). Subsequently at t = 40 s, air was introduced to the outer tube, and immediately a strong rise in absorbance at 668 nm was observed, indicating the formation of oxidized MB (MBox) in the LCW. Under these oxygen-rich conditions, a maximum absorbance (1.4 AU) was reached in only 20 s (at t = 60 s). When nitrogen was flushed through the outer tube again, the absorbance decreased within 60 s to the starting level, showing MBred is reconverted to MBred. This experiment convincingly shows that oxygen can be present in its reduced, i.e. colorless, form (MBred). Subsequently at t = 40 s, air was introduced to the outer tube, and immediately a strong rise in absorbance at 668 nm was observed, indicating the formation of oxidized MB (MBox) in the LCW. Under these oxygen-rich conditions, a maximum absorbance (1.4 AU) was reached in only 20 s (at t = 60 s). When nitrogen was flushed through the outer tube again, the absorbance decreased within 60 s to the starting level, showing MBred is reconverted to MBred. This experiment convincingly shows that oxygen can be
rapidly introduced to and removed from the solution in the core of the LCW. In line with previous observations [8], the full removal of oxygen seems to take more time than introducing it. This might be due to the lower diffusion rate of nitrogen through the pores [38,39], while the fact that only a little oxygen is needed to induce a significant color change, might also play a significant role here. Although the experiment does not quantitatively determine the concentration of dissolved oxygen inside the LCW, it adequately indicates the rate at which oxygen can be added or removed, exhibiting a valuable merit of the LID set-up.

3.2. In-situ vs. external purging with nitrogen

The LCW-LC-DAD-QTOFMS set-up represents a fully automated system to study photodegradation in solution. To investigate compounds under anoxic conditions, oxygen should be removed before exposure to light, which is conventionally done by bubbling samples with nitrogen (‘external purging’), requiring additional manual steps and related equipment. This would have to be done or repeated right before the start of every new experiment to prevent oxygen from diffusing back into the sample, which is rather laborious. Considering the LCW wall is permeable for nitrogen gas, oxygen can be removed from a sample by purging with nitrogen via the outer tube after it has been introduced to the LCW (‘in-situ purging’). This approach of oxygen removal was tested and compared to conventional external deoxygenation employing the photodegradation of RF. Oxygen decreases the LOD rate of RF (see also below). Therefore, a higher percentage of native RF will remain if oxygen is not properly removed from the system. The extent of LOD of RF in samples deoxygenated by conventional external purging with nitrogen for 5 min was compared with samples deoxygenated by in-situ purging with nitrogen for 1–10 min. After purging, both types of deoxygenated samples were irradiated by light from the Xenon source for 10 min while maintaining a continuous stream of nitrogen through the outer tube to sustain an anoxic environment. To further assess the effectiveness of in-situ deoxygenation, also samples that were not pre-purged with nitrogen and irradiated while having a continuous stream of air in the outer tube, were analyzed. The overall results of these experiments are shown in Fig. 3.

On the left in the figure, the remaining percentages of intact RF (as determined by LC-DAD) are presented for the samples irradiated under oxic conditions (i.e. without prior purging; blue bar) and under anoxic conditions after external purging with nitrogen (green bar). On the right, the remaining percentages of intact RF are shown for samples irradiated during in-situ purging with nitrogen for different time intervals (orange bars). Already after 1 min of in-situ purging with nitrogen, a significant difference in RF percentage as compared to the sample irradiated under oxic conditions is observed, indicating fast removal of oxygen from the core of the LCW. Furthermore, in-situ deoxygenation results in similar RF percentages as obtained after external purging of samples, with a slight overall decrease in RF percentage with increasing in-situ purging time (up to 10 min). In addition, standard deviations seem to decrease with longer in-situ purging times. Considering that in-situ obtained RF percentages are not significantly different from the RF percentage obtained with the external method, we conclude that deoxygenation in the core of the LCW before irradiation is just as effective as conventional nitrogen purging by sample bubbling. The new approach, however, is much more straightforward requiring fewer manual interventions, and, therefore, was applied to all further experiments.

3.3. Photodegradation studies under oxic and anoxic conditions in LCW

It is well known that oxygen has a significant effect on the photodegradation kinetics as well as the formed products of RF and EY [8,15,20,22]. Both the photodegradation mechanisms as well as the kinetics and k-values have been studied extensively for RF and EY [8,25,26,40]. These compounds were therefore chosen to assess the potential of the gas-permeable LCW for studying photodegradation processes in the presence and absence of oxygen. The assessment is based on the in-situ absorbance spectra measured during irradiation of the RF and EY solutions, and on the UV/Vis spectra and peak areas derived from EICs obtained during LC-DAD-MS analysis of the irradiated samples (Figs. S9 and S10, Figs. S16 and S17). Assignment of the photodegradation products was based on matching measured m/z values with molecular masses of degradation products of RF and EY reported in the literature (Tables 1 and 2).

3.3.1. Photodegradation of Riboflavin

Aqueous solutions of 10 mg L⁻¹ RF were irradiated (Xenon source, 240–1200 nm) for 10 min (n = 3) in the LCW under oxic and anoxic conditions while monitoring their absorbance. Subsequently, the irradiated samples were analyzed by LC-DAD-QTOFMS. The in-situ absorbance spectra measured in real-time (Fig. 4A and B) already indicate differences in irradiated sample composition, and thus the photodegradation process, when applying oxic or anoxic conditions. In Fig. 4B the faster decrease of absorbance at 450 nm (i.e. the absorption maximum of RF; Fig. 1) can be assigned to the higher degradation rate of RF under anoxic conditions. Under oxic conditions (Fig. 4A), the absorption at 450 nm also decreases due to the light-induced conversion of RF, however, the absorbance between 350 and 400 nm hardly decreases. This may be related to the formation of relatively large quantities of LMC (absorbance spectrum in Fig. S8) in those samples.

The results obtained by LC-DAD-QTOFMS nicely confirm the observations made from the in-situ absorption measurements. LC-DAD chromatograms of RF before and after irradiation under oxic and anoxic conditions are reported in the SI (Figs. S5-S7). Quantitative interpretation of the results was done by establishing and comparing the peak areas in the EICs for the parent and degradation products obtained under oxic and anoxic conditions, as shown in Table 1 and Fig. 4C.

The remaining amount of RF (m/z 377.142; Fig. S11A) after irradiation is higher in the oxic samples, again indicating that the photodegradation rate of RF is decreased in the presence of oxygen, which is in line with the literature [22]. The slower photodegradation of RF under oxic conditions is a result of RF being quenched by oxygen, leading to the return to the ground state of RF and the formation of ¹O₂. This mechanism has been well documented in the literature [41–43]; no
attempt was made to detect singlet oxygen inside the LCW. This also contributes to the differences in sample composition between theoxic and anoxicirradiated samples. The photooxidation product CMF (m/z 301.087; Fig. S11B) was only observed in aerated samples. According to the proposed degradation scheme by Sheraz et al. [17], FMF is formed and anoxic irradiated samples. The photooxidation product CMF (contributes to the differences insample composition between the oxic attempt was made to detect singlet oxygen inside the LCW. This also

Table 1
Overview of the compounds detected during LC-DAD-MS of RF solutions exposed to light in the LCW under oxic and anoxic conditions with observed m/z (for [M + H]+) and maximum absorption wavelength (λmax). Assignment based on degradation products as reported in the literature (references indicated). Compounds that were not detected are indicated by ‘nd’.

<table>
<thead>
<tr>
<th>RT (min)</th>
<th>ID</th>
<th>Molecular formula</th>
<th>Exact mass (Da)</th>
<th>Observed m/z</th>
<th>Observed λmax (nm)</th>
<th>Oxic</th>
<th>Anoxic</th>
<th>Also reported by</th>
</tr>
</thead>
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<tr>
<td>11.47</td>
<td>RF</td>
<td>C15H28N8O8</td>
<td>376.138</td>
<td>377.142</td>
<td>371; 447</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>–</td>
<td>CDRF</td>
<td>C15H28N8O8</td>
<td>376.138</td>
<td>–</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>11.13</td>
<td>CMF</td>
<td>C15H28N8O8</td>
<td>300.086</td>
<td>301.087</td>
<td>368; 448</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>11.79</td>
<td>FMF(1)</td>
<td>C15H28N8O8</td>
<td>284.091</td>
<td>285.093</td>
<td>375; 440</td>
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<td>✓</td>
<td>Smith et al. [20]</td>
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<tr>
<td>12.87</td>
<td>FMF(2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>371; 447</td>
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<td>✓</td>
<td>Ahmad et al. [14-16]</td>
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<td>257.098</td>
<td>377; 447</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>369; 444</td>
<td>✓</td>
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<tr>
<td>13.76</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>14.13</td>
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<td>242.080</td>
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<tr>
<td>–</td>
<td>KA</td>
<td>C13H28N8O8</td>
<td>232.085</td>
<td>–</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
<td>Ahmad et al. [18,26,27]</td>
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<tr>
<td>–</td>
<td>DQ</td>
<td>C13H28N8O8</td>
<td>204.090</td>
<td>–</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
<td>Ahmad et al. [18,26,27]</td>
</tr>
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</table>

Fig. 4. In-situ absorbance spectra of 10 mg L⁻¹ RF samples, measured every 2 min during irradiation under (A) oxic and (B) anoxic conditions. (C) Peak areas for RF and identified degradation products derived from EICs obtained during LC-MS analysis of RF solutions irradiated for 10 min under oxic (air, blue) and anoxic (N₂, green) conditions (n = 3). Note that for the sake of readability, the values for CMF are scaled by a factor of 10 with respect to the left y-axis, and the values for LMC are scaled against the right y-axis.

Table 2
Overview of the compounds detected during LC-DAD-MS of EY solutions exposed to light in the LCW under oxic and anoxic conditions with observed m/z (for [M + H]+) and maximum absorption wavelength (λmax). Assignment based on degradation products as reported in the literature (reference indicated). Compounds that were not detected are indicated by ‘nd’.

<table>
<thead>
<tr>
<th>RT (min)</th>
<th>ID</th>
<th>Molecular formula</th>
<th>Exact mass (Da)</th>
<th>Observed m/z</th>
<th>Observed λmax (nm)</th>
<th>Oxic</th>
<th>Anoxic</th>
<th>Also reported by</th>
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<td>20.01</td>
<td>EY</td>
<td>C28H30BrO5</td>
<td>647.706</td>
<td>648.716</td>
<td>531</td>
<td>✓</td>
<td>✓</td>
<td>Alvarez-Martin et al. [8,30]</td>
</tr>
<tr>
<td>19.58</td>
<td>EY-Br*</td>
<td>C28H30BrO5</td>
<td>567.798</td>
<td>568.806</td>
<td>524</td>
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<td>✓</td>
<td>Pirok et al. [31]</td>
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<td>18.62</td>
<td>EY-2Br*</td>
<td>C28H30BrO5</td>
<td>489.835</td>
<td>490.895</td>
<td>519</td>
<td>✓</td>
<td>✓</td>
<td>Alvarez-Martin et al. [8,30]</td>
</tr>
<tr>
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<td>nd</td>
<td>✓</td>
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<td>C28H30O5</td>
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<td>333.078</td>
<td>nd</td>
<td>nd</td>
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</table>

in anoxic samples, while final degradation products LF (m/z 257.098; Fig. S11D) and LMC (m/z 243.084; Fig. S11E) are present in lower concentrations compared to oxic conditions. Indeed, under oxic conditions, the concentration of LMC is expected to be higher as this product can be formed through both ¹⁸RF and ¹⁹RF. However, it was not expected that LF would be formed in higher abundance under oxic conditions as it is reported that FMF is more light-sensitive than RF [15,20]. From our results, it appears that FMF is less efficiently transformed into LF and LMC when oxygen is removed, which may indicate that the degradation of FMF is accelerated by oxygen or by other reactive species formed through the reaction of singlet oxygen with water, e.g., H₂O₂ or OH⁻ [18]. Several other degradation products reported in the literature, such as CDRF, KA, and DQ, were not detected in any of the irradiated samples. This may be because their concentrations were below the detection limits and/or because in the other studies, alkaline conditions were used.
3.3.2. Photodegradation of Eosin Y

Aqueous solutions of 1 mg L$^{-1}$ EY were irradiated in the LCW for 0, 5, 10, 15, 20, and 30 min ($n=2$) under oxic and anoxic conditions while monitoring their absorbance (Fig. 5A and C), and subsequently subjected to LC-DAD-QTOFMS analysis. LC-DAD chromatograms of EY before and after irradiation under oxic and anoxic conditions can be found in the SI (Figs. S12-S14). Table 2 presents the assignment of EY and degradation products based on their measured m/z values and bromine isotope patterns in the corresponding mass spectra (Figs. S16–S18). The quantitative interpretation was done by establishing and comparing the LC-MS peak areas in the EICs for the parent and degradation products obtained under oxic and anoxic conditions (Fig. 5B and D, respectively).

Under oxic and anoxic conditions, a rapid decrease in absorbance is observed for EY within 30 min of irradiation. The LC-MS results confirm these observations showing a decrease in the peak area of EY (m/z 648.716; Fig. S18A; Fig. 5B and D). The LC-MS data also show that EY-Br (m/z 568.806; Fig. S18B) and EY-2Br (m/z 490.895; Fig. S18C) were already present in the non-irradiated EY samples ($t=0$ min) and that their levels seem significant. However, the LC-DAD chromatograms show much lower relative peak areas for EY-Br and EY-2Br (Fig. S12). Since it is expected that the absorption coefficients of the debrominated species do not differ greatly from that of EY (Fig. S15), we conclude that these products were present in rather low amounts in the original EY sample. Possibly, the ionization efficiency of the debrominated products is higher than that of EY, resulting in relatively high relative peak areas for EY-Br and EY-2Br in the LC-MS chromatograms. Nevertheless, as the ionization efficiency per product is constant, it was still possible to monitor the change in the concentration of each degradation product over different irradiation times based on the obtained MS peak areas.

EY degraded much slower under oxic conditions, as follows from the comparison of the results obtained for aerated and deaerated samples, and also has been described in the literature [8]. The slower degradation under oxic conditions most likely results from the quenching of the triplet excited state of EY by oxygen to form $^{1}\text{O}_2$. This mechanism has been well documented in the literature [8,44,45]; no attempt was made to detect singlet oxygen inside the LCW. The LC-MS results indicate a gradual decrease in the concentration of EY, EY-Br, and EY-2Br over time in the presence of oxygen. The rate at which EY-2Br decreases (49%) during the first 5 min is higher than for EY (35%) and EY-Br (38%). This may indicate that EY first loses a single bromine, followed by chromophore breakdown. If EY-2Br would have been formed through EY-Br, a slower decrease of the former compound should have been observed. The triply and fully debrominated products (EY-3Br and fluorescein, respectively) were not detected in any of the aerated samples. This result is in line with the mechanism under oxic conditions (i.e. breakdown of the chromophore) as proposed by Alvarez-Martin et al. [8]. Unfortunately, no EY fragments related to the breakdown of the structure by photooxidation could be found in the obtained LC-MS data. This may be due to their low concentrations and potentially low ionization efficiencies. However, the in-situ absorbance spectra (Fig. 5A) do support the chromophore breakdown indicated by a decrease in absorbance at the maximum absorbance wavelength of EY. Similar to Alvarez-Martin’s reports, the aerated samples’ absorbance spectra do not show obvious shape changes, indicating that no significant levels of differently absorbing compounds are being formed.

A different trend is observed in the absorbance spectra measured under anoxic conditions (Fig. 5C). In addition to a decrease in absorbance intensity, a gradual shift in the absorbance maximum is observed over time. The recorded absorption spectra are very similar to the spectra of debrominated EY species reported by Kimura et al., who studied the photodegradation of EY in deaerated samples [28]. After about 8 min of irradiation of EY, the absorption spectrum gets a broader shape and shifts to that of fluorescein ($\lambda_{\text{max}}\approx 490$ nm). This observation can be directly correlated to the composition of the photodegraded samples as determined by LC-MS (Fig. 5D). Already after 5 min of

Fig. 5. In-situ absorbance spectra measured of 1 mg L$^{-1}$ EY samples over time under (A) oxic and (C) anoxic conditions. Peak areas of EY and its degradation products derived from EICs obtained during LC-MS analysis of 1 mg L$^{-1}$ EY samples after 0, 5, 10, 15, 20, and 30 min of irradiation under (B) oxic and (D) anoxic conditions ($n=2$).
irradiation under anoxic conditions, fluorescein (m/z 333.078; Fig. S18E) is formed in relatively high abundance. Next to fluorescein, also the formation of EY-3Br was observed after 5 min of irradiation in the absence of oxygen, indicating a different photodegradation mechanism as observed under oxic conditions. Between \( t = 0 \) and \( t = 5 \) min, a concentration decrease of 75% is observed for EY, 79% for EY-Br, and 68% for EY-2Br. The somewhat lower decrease of EY-2Br in the first minutes under anoxic conditions indicates that this compound may be formed through EY-Br losing another bromine, followed by the loss of a third bromine to form EY-3Br (m/z 410.987; Fig. S18D). The concentration of EY-3Br rapidly decreases after 5 min of irradiation, while the presence of fluorescein increases further. This process of consecutive bromine losses leading to fluorescein would support the mechanism proposed by Alvarez-Martín et al. [8] and Pirok et al. [31]. To obtain more information about the first stages of the photodegradation of EY, experiments with irradiation times below 5 min should be conducted. After 30 min of irradiation a decrease in fluorescein concentration is observed, indicating that eventually, this ‘final’ photodegradation product decomposes even further. No products related to the degradation of fluorescein were found, which again might be due to the low concentrations at which these products are formed and/or their low ionization efficiency.

The results presented here, as well as for Riboflavin, are broadly in line with reports from the literature, which affirms that the set-up is a valuable addition to the existing methods for studying photodegradation under oxic and anoxic conditions.

4. Conclusions

This study shows the potential of a gas-permeable LCW for studying LID processes under both oxic and anoxic conditions. The tube-in-tube design provides sufficiently high diffusion rates for oxygen and nitrogen to purge the samples under study. In-situ deaeration of samples in the LCW shows to be as effective as conventional oxygen removal by bubbling with nitrogen. This offers a convenient and straightforward means of deoxygenation, reducing the number of manual interventions and associated error sources. The feature of in-situ absorbance spectroscopy as provided by the used LID set-up has shown to be helpful. In general, it allows real-time monitoring of the irradiated sample and, more specifically, it supported the hypothesis of the chromophoric breakdown of EY under oxic conditions. The decrease and change in the absorption spectra during irradiation also correlated nicely with the sample compositions as determined by LC-DAD-MS. Interestingly, fluorescein was identified for the first time as a degradation product in irradiated samples of EY. Importantly, the results of the photodegradation experiments obtained with LCW, essentially follow the results reported in the literature, meaning that the current LID cell set-up can be applied to study the effect of oxygen on photodegradation processes. Although not utilized in this study, the set-up also offers the option to employ optical filters to investigate the wavelength dependence of photodegradation. We expect that the system will be useful for a wide audience covering many application areas. For example, in the food industry, knowledge about the influence of oxygen may impact the type of packaging material to increase shelf life. In museums, the obtained information will help to decide how vulnerable items should be stored and protected. Even in the pharmaceutical industry, tests have to be employed where the influence of, e.g., light and oxygen on drugs are studied. We believe that, with the automated system presented here, this can be done in a fast and straightforward manner, while providing high repeatability.

CRediT authorship contribution statement

Iris Groeneveld: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. Freek Ariese: Writing – review & editing, Supervision, Conceptualization. Govert W. Somsen: Writing – review & editing, Supervision, Conceptualization. Maarten R. van Bommel: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maarten R. van Bommel reports financial support was provided by Dutch Research Council.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jphotochem.2023.114685.

References


