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Pirok, B.W.J.; Moro, G.; Meekel, N.; Berbers, S.V.J.; Schoenmakers, P.J.; van Bommel, M.R.

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Original article

Mapping degradation pathways of natural and synthetic dyes with LC-MS: Influence of solvent on degradation mechanisms

Bob W.J. Pirok [a,b,*], Giacomo Moro [a], Nienke Meekel [a], Sanne V.J. Berbers [a], Peter J. Schoenmakers [a], Maarten R. van Bommel [a,c]

[a] *University of Amsterdam, van 't Hoff Institute for Molecular Sciences, Analytical-Chemistry Group, Science Park, 904, 1098 XH Amsterdam, The Netherlands*
[b] *TI-COAST, Science Park, 904, 1098 XH Amsterdam, The Netherlands*
[c] *University of Amsterdam, Faculty of Humanities, Conservation and Restoration of Cultural Heritage, Johannes Vermeerplein, 1, 1071 DV, Amsterdam, The Netherlands*

ABSTRACT

To help conserve the vast array of (combinations of) dyestuffs and pigments encountered in cultural-heritage objects and application materials, a rapid and convenient method for dye-degradation research is required. In-solution degradation studies of dyes in a strong solvent, such as the commonly used dimethyl sulfoxide (DMSO), are potentially of interest, in addition to studies involving other solvents, such as water. The degradation of eosin and carminic acid under the influence of light was investigated in two solvents, i.e. in a mixture of DMSO and acetonitrile and in pure water. A liquid chromatography – mass spectrometry (LC-MS) method was developed for analysis of the degraded samples and identification of the individual components. The presence of DMSO generally facilitated faster degradation, which, in combination with its universal solvating properties are advantageous. However, different products were formed in the presence of DMSO. Degradation pathways for eosin and carminic acid in these solvents are proposed.

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1. Introduction

Whether applied as pigments or dyes, organic colorants in cultural-heritage objects are subjected to various external conditions throughout their lifetime. This often results in degradation. As a result, cultural-heritage objects can change appearance and the original intention of the artist or craftsman can be lost. Conservation of the object for future generations requires extensive research into the identity of the colorants and their degradation. The latter is particularly important, because the current state of a cultural-heritage object may no longer feature the originally applied colorants. Instead a mixture of degradation products may be present. Chemical analysis is conducted with the aims of:

- determining the original composition to understand the creation of the artwork;

- assessing the progress of degradation, which may result in a better understanding of the original appearance, and;
- elucidating the mechanism and state of degradation to allow protective measures to be taken.

The chemical analysis of thousands of – often impure – dyestuffs, used across millions of old and new cultural-heritage objects, poses an immense challenge. Moreover, the mechanism of degradation is intrinsically dependent on the external conditions to which the object is exposed (e.g. temperature, humidity, air composition, light) [1]. Degradation may also be greatly affected by the material to which the dye was applied [2,3]. Therefore, the number of potential cases that must be studied is colossal and rapid convenient methods for chemical analysis are required.

While a large number of non-invasive analytical methods exist to obtain useful information on the dyes applied in the object, crucial information is often missing. Indeed, non-invasive tools, such as Raman, UV-vis, FORS, and fluorescence spectroscopy [4–7], are useful to identify the overall composition of the applied dyes. However, the information is often distorted by signals arising from other materials present in the object, such as varnishes, fillers, additives and, most importantly, bulk materials, such as textile yarns, and binding media necessary to apply organic pigments as paint. In-situ

* Corresponding author at: University of Amsterdam, van 't Hoff Institute for Molecular Sciences, Analytical-Chemistry Group, Science Park, 904, 1098 XH Amsterdam, The Netherlands.
E-mail address: b.w.j.pirok@uva.nl (B.W.J. Pirok).

methods typically provide an averaged perspective with signals of all compounds present within the scanning domain confounded.

Alternative methods focus on the extraction of the dyes from the original material and subsequent analysis by separation techniques, such as liquid chromatography (LC) coupled with mass spectrometry (MS) [8–11]. While these methods allow detailed quantitative information to be obtained, there are a number of disadvantages. For example, the extraction procedure can show bias to interaction with specific groups of analytes, which may then be overrepresented in the results. Also, the state of the colorants and their degradation products may be altered by the extraction process

To understand the degradation of dyes, accelerated ageing on textiles or paintings needs to be carried out. This is normally conducted using so-called mock-ups. While the ageing can be accelerated using highly focussed light sources, it may still easily take several weeks before a significant fading is observed. Although this largely depends on the dye and wavelengths used, realistic case studies tend to be time consuming.

As an alternative, we previously investigated the possibility of dissolving dyes and age the solution instead of solid coloured materials [2]. This has the advantages that samples can be taken at regular time intervals and that no sample preparation is required. We observed that the degradation products found in solution match those found when the dye is applied on textile or as ink on paper. Unfortunately, many colorants, in particular organic pigments, are not soluble in water. Moreover, there is a possibility that hydrophobic degradation products are formed during degradation, and that these are excluded from the matrix. One relatively universal and popular extraction solvent is dimethyl sulfoxide (DMSO), which was found to be a fairly good solvent for a vast array of distinct dyes [10].

2. Research aim

In this work, the degradation is investigated of one natural dye, carminic acid, and one synthetic dye, eosin, aged in-solution in water, and acetonitrile with DMSO, under the influence of UV-light. In particular, the applicability and influence of the more-universal solvent DMSO is studied. Ultimately, we hope to establish a universal system for degradation studies using (multi-dimensional) LC-MS analysis.

3. Material and methods

3.1. Chemicals

Acetonitrile (LC-MS grade) was obtained from Biosolve (Valkenswaard, NL), deionized water (MS-grade) was procured from Merck (Darmstadt, Germany). Triethylamine ($\geq$ 99.5%), dimethyl sulfoxide (DMSO, $\geq$ 99%, SAFC) and formic acid ($\geq$ 96%) were obtained from Sigma-Aldrich (Darmstadt, Germany). The dyestuffs were obtained from the reference collection of the Cultural Heritage Agency of the Netherlands (RCE, Amsterdam, The Netherlands). For comparison, carminic acid was also obtained from Sigma-Aldrich.

3.2. Instrumentation

A Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, 254 nm, ca. 1–3 μW/cm^2) was used for degradation of the dyestuffs in-solution. The LC system used for LC-MS analysis comprised an Agilent 1100 series quaternary pump (G1311 A), an Agilent 1260 Infinity degasser (G1322 A), an Agilent Infinity 1290 diode-array detector (DAD, G4212 A) equipped with an Agilent Max-Light Cartridge Cell (G4212-6008, V_{det} = 1.0 μL) and an Agilent 1100 series autosampler (G1313 A). The injection volume was set to 20 μL and DAD data were recorded at several wavelengths at 160 Hz. The system was controlled by OpenLAB CDS ChemStation software (Edition Rev. C.01.04) [Build 35]. An Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT column (959941-902, 50 $\times$ 4.6 mm i.d., 1.8-μm particles) was used. The flow was split using a stainless-steel tee-connection (P/N: U- 428, IDEX, Lake Forest, IL, USA), with a 500 mm $\times$ 0.25 mm i.d. tubing to the DAD and 500 mm $\times$ 0.12 mm i.d. to the MicroTOF-Q mass spectrometer (S/N 228888.00132, Bruker Daltonik, Bremen, Germany). The MS was equipped with an electrospray-ionization source and configured to run in negative mode at an acquisition rate of 4 Hz. The system was controlled with Compass 1.3 for MicroTOF-SR1 (MicroTOF control Version 3.0, Build 53) from Bruker.

3.3. Procedures

3.3.1. Sample preparation
The dye samples were dissolved in:

- 0.5 mL acetonitrile/dimethyl sulfoxide 1:1 (v/v), and;
- water, in concentrations of about 200 mg/L and were put in 1.8-mL Clear-Glass grade vials obtained from Waters (Milford, MA, USA).

Acetonitrile was used as a part of the sample solution to enhance dye solubility and to improve compatibility of the sample solvent with the LC mobile phase. As a consequence, we cannot rule out that the presence of acetonitrile also affects the degradation (see also section 4). These vials were placed horizontally (with identification on the bottom) on a slat with notches in the UV cabinet. The samples were irradiated with 254-nm UV light for different durations (specified in section 3 below). Samples were taken at regular time intervals (at every 20 min from 0 to 160 min and after 330 min of degradation) and stored in darkness at –24 °C prior to analysis. The dye samples were also dissolved in water for reference, following the same procedure as described above.

3.3.2. Analytical methods
The LC separation was adapted from our earlier described fast ion-pair reversed-phase chromatography method [9]. A buffer containing triethylamine (5 mM) in water and formic acid (to obtain a pH of 3) was prepared. Mobile phase A consisted of buffer/acetonitrile 95:5 [v/v] and B of buffer/acetonitrile 5:95 [v/v]. The flow rate was 1.85 mL/min. The gradient used was as follows. 0 – 0.25 min, isocratic at 100% A; 0.25 – 8.25 min, linear gradient to 100% B, maintained at B for 0.5 min; 8.75 – 9.5 min linear gradient to 100% A. For MS and MS/MS the following operating conditions were used. End-plate offset −500 V; capillary voltage 3800 V (positive mode −4400V); nebulizer-gas pressure 2.0 bar; drying-gas flow 10 L·min^{-1}, source temperature 250 °C. RF1 and RF2 funnels operated at 250V, and the hexapole at 400V. The quadrupole ion energy was 4.0eV and the collision energy used for the different samples ranged from 1.5 to 35eV. The collision RF was at 1500V, the transfer time was 80 μs and the pre-pulse storage time 4 μs. For MS/MS analyses the mass of interest was set to pass the filter with a margin of 0.5 m/z. The isolation width was 8 up until a m/z of 500, then a linear gradient from 8 to 10 from 500 to 1000 m/z.

3.4. Data analysis

For exploratory purposes, the data were analyzed with the Data-Analysis Version 4.0 Sp 4 software (Build 281; Bruker Daltonik). Processing of the chromatograms and comparison of the LC-MS data was carried out using the in-house-written PIOTR program [12].

Fig. 1. Photographic image of the setup for exposure of the dye solutions to the UV light.

4. Results and discussion

4.1. Degradation setup and chromatographic method

To study the degradation pathway, both dyes were separately dissolved in:

- a mixture of dimethyl sulfoxide (DMSO) and acetonitrile (ACN) (1:1, v/v), and;
- water, in a Clear-Glass grade vial. The vial was placed sideways on a holder in the light chamber and subjected 254-nm light using vials that were partially transmittant at this wavelength (see Fig. 1).

The setup used was adequate, but not optimal, because:

- the curved side of the vial facing the lamp caused some scatter of the light;
- the closed-system environment prevented dissipation of heat generated by the irradiation, possibly resulting in thermal degradation (although runs where the system was supplied with exclusively thermal energy did not give rise to degradation products);
- the contents of the vial were not continuously homogenized, so that degradation in some regions (e.g. at the surface layer) may have progressed faster;
- oxygen was not continuously refreshed, except during sampling when the vial was opened, which is important as degradation in an anaerobic environment may lead to different pathways [13];
- the transmittance of the sample-holding vial could not be adjusted.

The latter was particularly important as we noted during preliminary experiments that for some dyes the degradation occurred almost instantly when subjected to the intensity of the UV source. For such dyes, we were not able to study the influence of exposure time. We therefore opted to use vials which only transmitted a very limited amount of light at 254 nm. Nevertheless, as can clearly be seen in Fig. 1, the solution responded to the incident light and fluorescence could be observed. In addition, degradation was observed, as will be discussed below. Therefore, the setup was deemed sufficient for this feasibility study to investigate the degradation pathways of the selected dyes with LC-MS.

At specific intervals (see section 2.3.1), UV-radiation was paused and the contents of the vial were sampled and analysed using LC-MS. The LC separation was adapted from our earlier developed ion-pair reversed-phase (IP-RPLC) method [9]. However, due to the limited compatibility of the tetramethylammonium salt with the MS, the more volatile triethylamine (which yields the triethylammonium ion, Et_3NH^+ at low pH) was selected as ion-pair. An ion-pair neutralizes the analyte charge in order to allow the hydrophobic carbon structure to interact with the stationary-phase surface, without being obscured by the charged moieties. Typically, methods using an ion-pair suffer from broad elution bands and are rather slow. We thus opted to use a dual gradient, with the ion-pair concentration decreasing as the organic modifier concentration increased to optimize peak shape.

One problem with these degradation studies is that some of the degradation products are present in trace concentrations only. As the amount of original material from the object is extremely limited, the sample concentration is difficult to adjust in practice. Because the degradation mechanisms was unknown, it was difficult to predict which analytes would be formed and at what concentration. Therefore, a relatively large injection volume of 20 μL was used at the risk of poor initial mixing of the DMSO with the aqueous mobile phase.

4.2. Degradation of carminic acid

Fig. 2 displays an overlay of several LC-DAD chromatograms recorded for photo-degraded samples in DMSO/acetonitrile solution obtained at every 20 min from 0 to 160 min and after 330 min of degradation. Changes in intensity of the different chromatographic peaks could be observed as compounds were formed and consumed during the degradation process. The chromatographic peaks were identified using UV-vis and mass spectra. The split chromato-
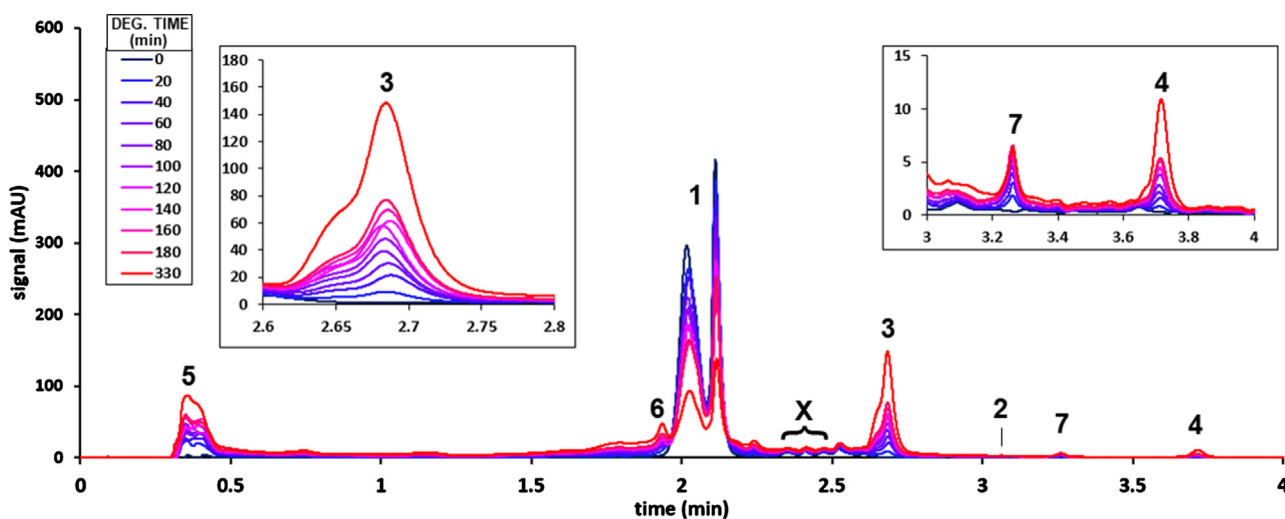

Fig. 2. Overlay of IP-RPLC-DAD chromatograms of carminic acid dissolved in DMSO/acetonitrile at every 20 min from 0 to 160 min and after 330 min of degradation. The numbers refer to the structures in Fig. 3. See Supplementary Material S-3 and S-4 for additional data. Detection wavelength: 254 nm.
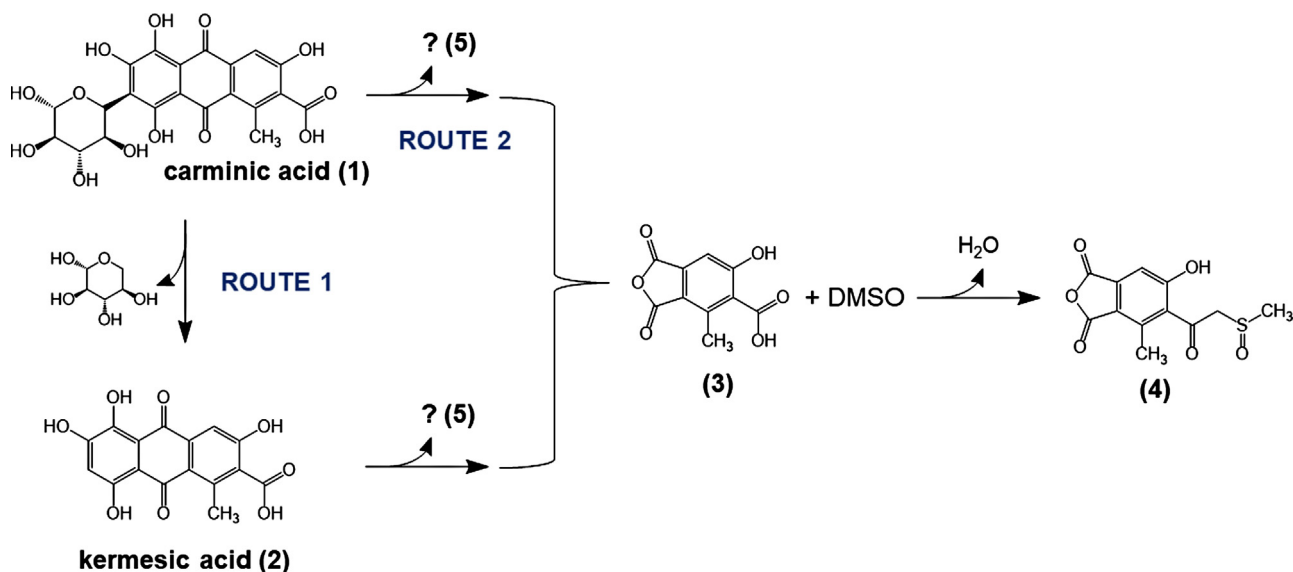
Fig. 3. Proposed degradation pathways of carminic acid in DMSO by UV-light at 254 nm.
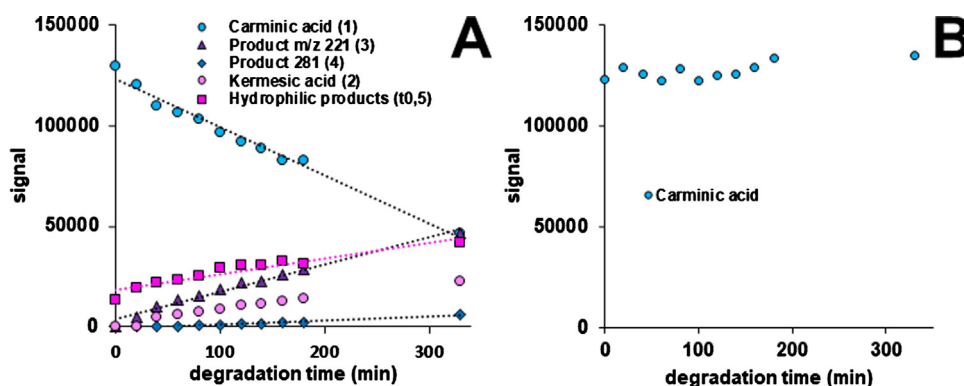

Fig. 4. Concentration trends of carminic acid and other species as a result of irradiation by light at 254 nm in (A) DMSO/acetonitrile and (B) water. Data represents LC-MS peak heights.

graphic bands of carminic acid and the main degradation product (Fig. 2, peaks 1 and 3, resp.) were indicative of detrimental mixing effects upon injection. This can easily be resolved by lowering the injection volume to, for example, 5 μL (see Supplementary Material Figure S-1 for an example where the carminic acid peak was no longer distorted, but kermesic acid was no longer observed).

Based on these data, tentative degradation pathways were constructed as shown in Fig. 3. In the following text, numbers between parenthesises refer to the chromatographic peaks from Fig. 2.

Two main routes of degradation were found to be plausible. First, the loss of the hydrophilic sugar moiety is observed, resulting in kermesic acid (Fig. 2, peak 2) with a mass of 330.08 Da. Next, the ring structure may collapse, leading to a degradation product with a mass of 222.02 Da (Fig. 2, peak 3). Alternatively, an immediate loss of the ring structure in carminic acid would also lead to this same fragment. The latter route may be supported by the formation of a relatively large and hydrophilic product (Fig. 2, peak 5, m/z 252.02 Da) in combination with the presence of kermesic acid at relatively low concentrations. Unfortunately, insufficient evidence was obtained to confirm either degradation pathway. However, the presence of the carboxylic acid group in the final degradation product (Fig. 3, peak 3, m/z 222.02 Da) could be confirmed by MS/MS data (see Supplementary Material section S-4). In combination with the (two-decimal) accurate mass this could be assigned to a molecular formula of $C_{10}O_6H_6$. This compound is regularly found in chromatographic analysis of historical samples of dyes containing cochineal [14].

Interestingly, an additional molecule (Fig. 2, peak 4) was observed, with a mass-to-charge ratio of 282.05, indicating the presence of a sulphur atom. This could only be explained through the reaction of DMSO with the carboxylate group and the subsequent release of water, which is in accordance with literature [15–19] (see Supplementary Material section S-2 for details on the mechanism). The more-hydrophilic degradation products (Fig. 2, peak 5) were difficult to separate using the reversed-phase LC method and eluted around the dead volume, along with calibrant used within the first section of the method. Other formed products were one with a mass of 219.01 Da (Fig. 2, peak 6) and a product of interaction of DMSO with carminic acid (Fig. 2, peak 7), which was also confirmed by MS/MS measurements (see Supplementary Material section S-3).

Fig. 4 displays the trends in concentration of the different degradation products of carminic acid and of carminic acid itself. While the trend for each compound is quantitative, the relative concentrations are not, because the electrospray-ionization interface is known to be more efficient for analytes that prefer the similarly-charged ionized state. As a result, a quantitative assessment of the analyte concentrations is difficult to establish. Nevertheless, the information from the mass spectrometer and the MS/MS data are invaluable for tentative identification of the analytes and peak
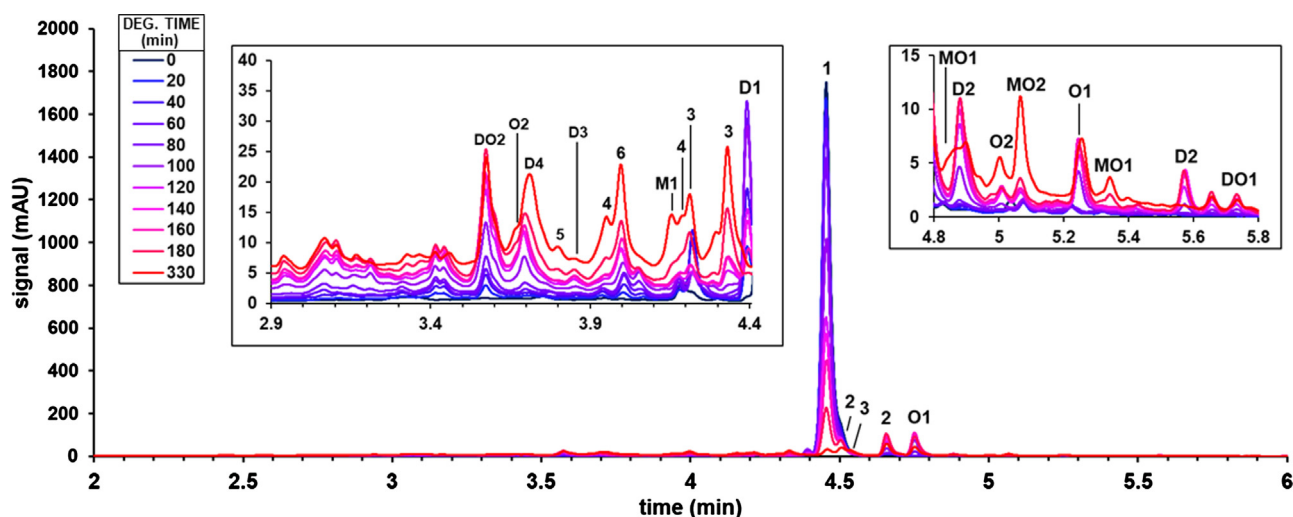
Fig. 5. Overlay of IP-RPLC-UV chromatograms of eosin dissolved in DMSO/acetonitrile after 0, 20, 40, 60, 80, 100, 120, 140, 160 and 330 min of degradation. The numbers refer to the structures in Fig. 6. See the Supplementary Material sections S-7, S-9 and S-10 for UV-vis spectra of several identified chromatographic peaks. Wavelength: 254 nm.

assignments. For the pursuit of quantitative information, the analyst may refer to the data obtained from the DAD if standards are available for calibration. Interestingly, the hydrophilic compounds were already present in the stock sample (at degradation time of 0 min). The figure also shows that, while present, the interaction with DMSO is a relatively slow process (series 4).

Although knowledge of the degradation pathway of carminic acid is useful from a perspective of conservation science, the interaction of caminic acid and its degradation products with DMSO is even more interesting. As discussed before, a DMSO is preferred in a number of extraction methods, because it is seen as a (near-) universal solvent for most dyes found in cultural-heritage objects. It is thus important to understand the consequences of this choice when conducting in-solution degradation experiments. The degradation of carminic acid in water was also investigated (Fig. 4B). While no degradation or interactions were observed, it is good to realize that the effect of the pH was not within the scope of the present study. Proteolytic interactions have been shown to affect photo-aging processes [20]. Nevertheless, the observation above does raise the question whether or not the observed degradation in DMSO/acetonitrile can be attributed to the presence of this solvent in presence of light.

4.3. Degradation analysis of eosin

The second dye of interest was the synthetic dye eosin. The degradation mechanism of eosin has been studied in the past under various conditions, including using catalysts [21]. Similar to the case with carminic acid, the eosin solution was sampled at different time points of degradation (at every 20 min from 0 to 160 min and after 330 min of degradation). The results as observed by DAD detection at 254 nm are displayed in Fig. 5. Eosin was found to respond strongly to the light source with a wide array of peaks appearing in the chromatogram. It should be noted that the ostensibly low signal intensities of the degradation products are misleading. As the molecular structure is altered by the degradation process, the chromophoric properties of the degradation products, such as the absorption maxima and extinction coefficients, may change drastically. It can thus be expected that as groups leave the aromatic, conjugated system, the electron density within the ring will be altered. Consequently, the optimal absorption wavelength of the dye can be expected to change as well. As a result, 254 nm is not the ideal wavelength for detection of all analytes and concentrations may be much higher than suggested by the chromatograms

shown in Fig. 5. In addition, it should be noted that the eosin used for this study was an authentic sample obtained from a reference collection and thus was not pure. As a result, degradants or impurities may have been present already. However, this does not affect the observed trends.

Again, a number of degradation pathways were observed, some of which were also reported in literature [22]. As expected, a cascade mechanism was found (Fig. 6,1–5), with consecutive bromine losses, ultimately leading to fluorescein (Fig. 6A,5). The loss of bromine under the influence of light has been well documented [13,23,24] and a clarification of the mechanism is provided in Supplementary Material section S-5. In addition, the MS/MS spectra of all degradation products of the bromine-loss pathway are provided in Supplementary Material section S-7. These spectra reflect the importance of the typical bromine isotope pattern.

Similar to carminic acid, the carboxylic-acid moiety of eosin was found to interact with DMSO as solvent and two possible structures were investigated and verified by MS/MS (see Supplementary Material section S-6). In general, DMSO was found to interact with the carboxylic-acid groups of all molecules (i.e. degradation products). In Fig. 5 and Fig. 6B, this process has been depicted with the notation "D" in combination with a number referring to the molecule from Fig. 6A. Presumably related to the interaction of DMSO with the dyes, methylation of the carboxylic acid was also found, indicated with the notation "M1" (Fig. 6B) [25]. MS/MS data are provided in Supplementary Material section S-8.

The main product, however, appeared to correspond to a mass of 414.86 Da (Fig. 6,6), a proposed structure is provided in Fig. 6C. The MS/MS data (Supplementary Material section S-9) revealed the presence of a ring system with two bromine atoms and the presence of a carboxylic-acid group. The LC-MS data yielded the molecular formula of $C_{14}H_7O_5Br_2$, which together with the retention information led to the proposed tentative structure. However, it should be noted that the identity of this molecule is unknown to the authors and that NMR analysis would be required to support the identification. A possible reaction mechanism is provided in Supplementary Material section S-12.

Another interesting interaction found was the addition of an oxygen atom (indicated with "O"). The fact that isomers were observed, as can be seen in Fig. 5 for species O2, indicates that the addition occurs at multiple sites in the molecule. For the MS/MS data of this analyte see Supplementary Material section S-10. A mechanism for this reaction is proposed in Supplementary Material section S-11.
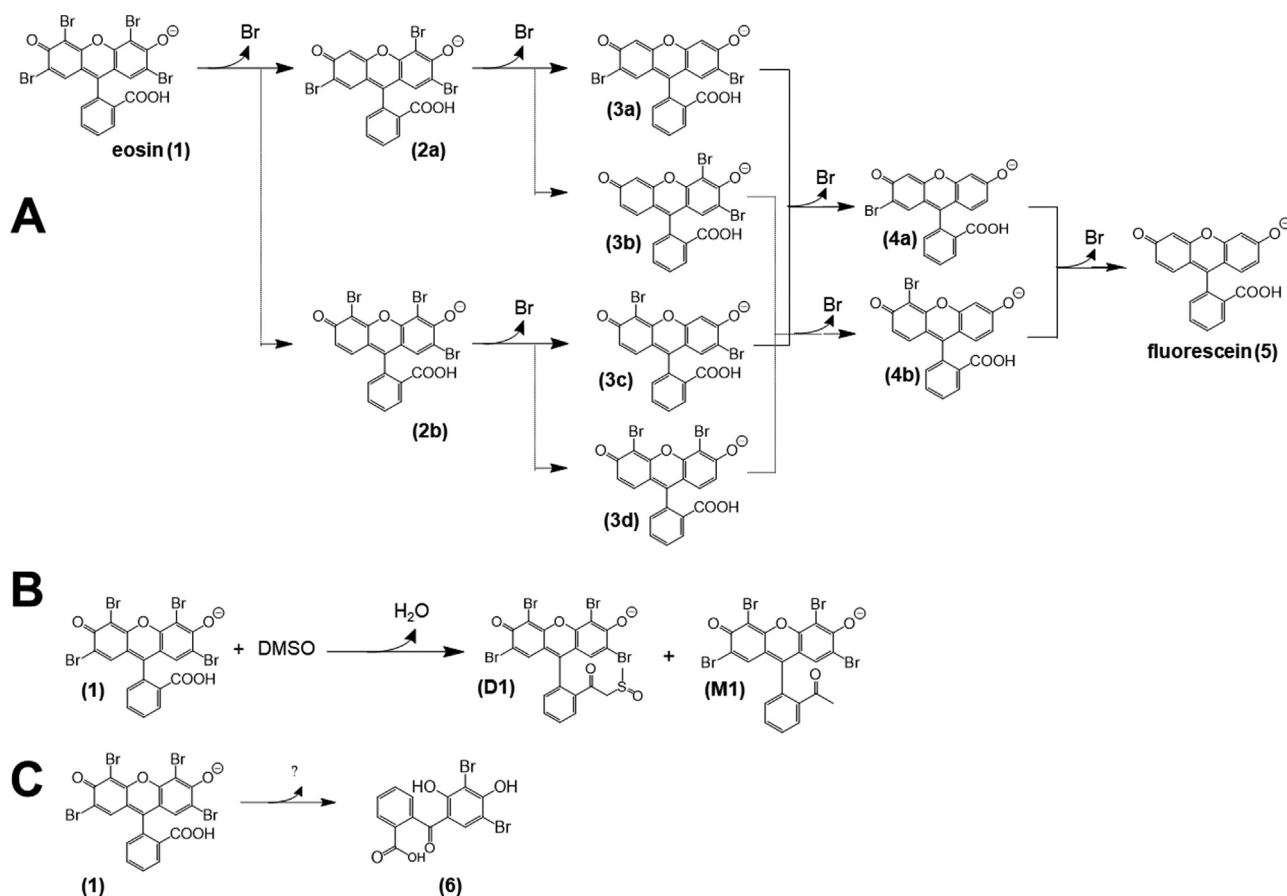
Fig. 6. Proposed degradation pathways of eosin in DMSO/acetonitrile by UV-light at 254 nm.

Fig. 7 shows the relative concentration trends for the various degradation products. Eosin itself (plotted on a different y-axis scale) initially responded rather slowly to the UV light and any decrease in concentration was too small to measure accurately. In Fig. 7A, the trends are shown for eosin and the two possible isomers after the loss of one bromine atom. Interestingly, one variant eosin-Br A, possibly a by-product of synthesis, was already present in the original sample and this immediately degraded significantly. However, after approximately one hour, the rate of degradation of eosin into eosin-Br A exceeds its degradation rate of the latter compound.

A similar trend is observed for the trace amount of eosin with two and three bromine atoms missing, but not for fluorescein (i.e. eosin with all four bromine atoms absent [26]). The latter compound was found to be present in significant concentrations in the starting dye. As far the other structures shown in Fig. 6A, fluorescein may also be formed as a result of degradation of eosin, but the trends in Fig. 7B suggest that this does not occurs within 60 min at the present irradiation conditions. Possibly, the formation of fluorescein is dependent on the presence of earlier-formed degradation products.

Fig. 7C displays the trends for some other degradation products. Two notable examples are the methylation of the carboxylic acid moiety of eosin (M1) and the interaction with DMSO (D1). This latter reaction is very fast initially, but the concentration of D1 decreases over time to nearly zero, even though all of the eosin has not yet been degraded at this time point. Most interesting is the formation of an unknown compound (Fig. 6, 6), which appears to be the end-product of one of the reaction pathways during degradation.

The degradation was also carried out in water. The observed degradation pathways were similar, although the mechanism as proposed in Fig. 6B was, unsurprisingly, not observed. In general,

the degradation of eosin in water was much slower and most degradation products were only observed at trace concentrations after hours of exposure to light. Again, it is good to note that effects of the pH were outside of the scope of the present study and that the pH was uncontrolled. At a different pH the degradation speed or pathway may be different [20].

Again, the degradation of eosin was also carried out in water, but during degradation precipitation of unknown degradation products was observed. A different pH might improve solubility, but this was not investigated in this study. The degradation products corresponded to eosin with one, two or three bromine atoms lost and a main product with $m/z = 414.86$ was observed. No DMSO interaction was discerned. In general, the degree of degradation was relatively low, with barely 10% of the originally present eosin degraded.

4.4. Comparison of in-solution and on-textile degradation for eosin

Of course, it is important to establish whether the degradation mechanism is similar in case the dye was applied in a cultural-heritage object. Therefore, a number of textiles were dyed with eosin and subjected to light degradation in a Xenotest instrument (see Supporting Material section S-13 for conditions) for 0, 20 60, 140 and 305 h, a test that is typically used in degradation studies in the field of cultural heritage. After degradation, extraction was performed with DMSO according to an established method [14]. It is worth noting that during the light degradation no DMSO was present.

The results are shown in Fig. 8. Three general observations can be made in the comparison between in-solution and on-textile degra-
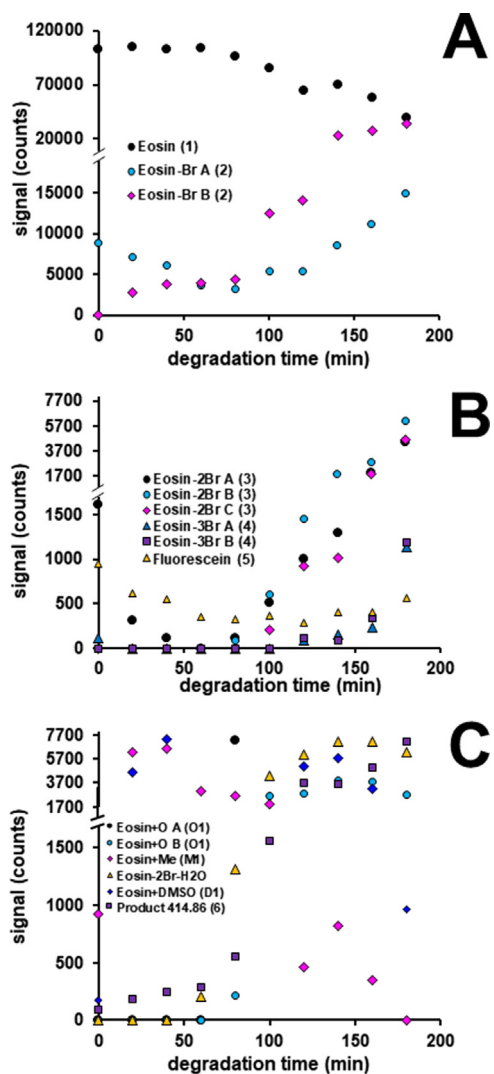
Fig. 7. Concentration trends of eosin and other species in DMSO/acetonitrile as a result of irradiation by light at 254 nm. Data represent LC-UV peak heights at a wavelength of 254 nm. Note the interruption of the y-axis on all figures.
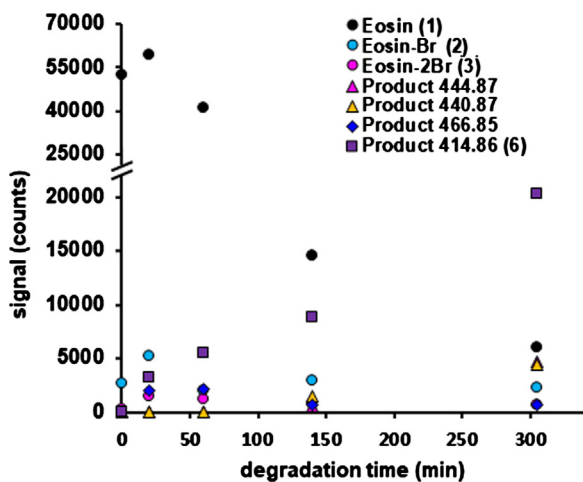

Fig. 8. Concentration trends observed for extracts of eosin-dyed textile after different durations of UV-light exposure at 254 nm. Data represents LC-MS peak heights.

dation. First and foremost, evidence for interaction with DMSO was not found, which confirms that reaction products are not formed by the mere presence of DMSO but require the combination of DMSO and light. However, an increase in the concentration of degradation products from the bromine-loss mechanism was observed. However, this occurred mainly for one of the variants for both the one-bromine-loss and two-bromine-loss cases, whereas the other variants were only detected in minor concentrations, as was fluorescein. This suggests that either the other variants were not readily formed or that these reacted away rapidly to the other degradation products.

Secondly, a number of unidentified degradation products with masses 440.87, 444.87 and 466.85 were found. These compounds were not observed for in-solution degradation. One disadvantage of on-textile degradation is that the extraction method can also extract material from the textile fibers, creating more possible options for the structure for these products and thus complicating identification.

Finally, as was the case with in-solution degradation (in DMSO/ACN and in water), the major degradation product had a mass of 414.86 (Fig. 6, 6). All of these observations suggest that, while there are some understandable differences in degradation mechanisms, the key degradation pathways are similar. Nevertheless, the interaction of eosin and some degradation products with DMSO may affect the kinetics of the main degradation pathways and this should be taken into account.

5. Conclusions

We have shown that in-solution degradation studies of dyes in a strong solvent, such as DMSO, can potentially be interesting in comparison with studies in other solvents, such as water. For both carminic acid and eosin, little to no degradation was observed after 5.5 h of exposure to 254-nm UV light in water, whereas significant degradation was found in the presence of DMSO with acetonitrile. Thus, the presence of DMSO accelerated the degradation. While the presence of acetonitrile may also contribute to acceleration and/or influence the degradation pathway, no evidence of this was found. A number of pathways were shown to be inherently linked to DMSO, thus proving the potential impact of the solvent.

From a comparison with data obtained from the analysis of extracts of textile dyed with eosin, we learned that a number of the degradation products, including the main one, were also observed with in-solution degradation of eosin in DMSO. This means that both systems are comparable. However, additional degradation mechanisms were identified, which exclusively occur in the presence of DMSO and light.

The use of LC-MS was indispensable, not only for the identification of the unknown degradation products, but also since it yielded a higher sensitivity for these components. LC-MS allowed for the identification of (partly) co-eluting components, whereas LC-UV provided limited discriminating power in this respect.

Considering the vast numbers of (combinations of) dyestuffs, pigments, degradation products and cultural-heritage objects, we believe that the presently described rapid in-solution degradation studies are indispensable for the fast, tentative identification of degradation products and for proposing degradation pathways, provided that the effects of the reaction conditions, such as the presence of DMSO, are properly taken into account.

To obtain truly comprehensive information on dye degradation, we believe that multi-dimensional separations will be indispensable. In comprehensive two-dimensional LC (LC × LC), all of the effluent from a first-dimension separation is divided in fractions by a modulation interface and transferred to a second-dimension column for additional separation [27–29]. While the increased sep-

aration and identification power are of great value, an exciting opportunity lies in the modulation interface. This typically comprises a valve equipped with storage loops, but by equipping such an interface with a light-reaction flow cell, individual components of a dye mixture may be separately introduced to a reactor. The reaction products per original component can then be separated and identified.

Acknowledgments

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.culher.2019.01.003.

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