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Investigating the in-solution photodegradation pathway of Diamond Green G by chromatography and mass spectrometry

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Abstract

Diamond Green G (CI 42040, Basic Green 1) is a triarylmethane dye mostly employed in the industrial dyeing of miscellaneous natural and artificial textile fibres, in the production of printing inks and as an anti-fungal agent for aquaculture products. Diamond Green (DG) tends to fade under light irradiation, similar to several other dyes and pigments belonging to the triarylmethane family. In this paper, an in-solution degradation study was carried out to shed light on DG photostability. The experiments were performed by artificially irradiating DG in water and dimethyl sulphoxide, and analysing aliquots sampled at different time intervals by ultra-high performance liquid chromatography coupled with diode array detector quadrupole and time of flight tandem mass spectrometry. The degradation products formed were characterised and the product-ion spectra discussed and interpreted. On the basis of the structure of the identified compounds and their kinetic trend in relation to the accelerated ageing time, degradation mechanisms were proposed for DG, taking into account the role played by the solvent. Finally, the dyeing formulation of an historical textile sample, collected from a stage costume worn in a Zeffirelli lyric opera, was determined utilising the information collected from the analysis of irradiated DG solutions.

1 | INTRODUCTION

Diamond Green G (CI 42 040, Basic Green 1), also known as Brilliant Green, is a synthetic organic dye belonging to the class of triarylmethanes. The similar dye Diamond Green B (CI 42000, Basic Green 4), alternatively named Malachite Green, differs from the G form in the nature of the *N,N*'- alkyl substituents (ethyl groups for G and methyl groups for B).

Diamond Green was independently produced by Otto Fischer and Adolf von Baeyer in 1877.[1] The original and still used synthetic strategy for both forms is based on the condensation of benzaldehyde with *N,N*-diethylaniline/*N,N*-dimethylaniline in sulphuric acid or hydrochloric acid. The following step entails the oxidation of the leuco compound in hydrochloric acid solution with lead dioxide or manganese dioxide.[2] Diamond Green B is commercially sold as its sulphate salt. Alternatively, acetic acid can be used and the resulting leuco base oxidised catalytically with atmospheric oxygen in the presence of (dihydrodibenzotetraaza [14]-annulene)iron and chloranil.[3] The introduction of Diamond Green on the market determined an important change in the dyeing world. In the past, green textiles were obtained by a double dyebath: one for a blue base (vat dyeing with indigo or woad) and the other for yellow (mordant dyeing with weld, old fustic or other vegetal sources).[4] With the advent of synthetic colourants, true green dyes, such as Diamond Green, became available, enabling one-bath dyeing of textiles.[5] Where dyeing with natural dyes can take days or even weeks,[6,7] Diamond Green dyeing can be performed in just a matter of hours,

which was obviously a big advantage. It was thus defined "the first direct dyeing green of real value".[8] Even if the same green shade as Diamond Green may also be obtained combining synthetic pigments such as blue copper phthalocyanine and yellow monoazo, the resulting hue has a lower tinctorial strength.[9]

The dyeing of a wide variety of industrial materials, including cotton, leather, paper, silk, wool and polyacrylonitrile fibres, is the main application of Diamond Green.[10] Moreover, it is used with oil binders in paint tubes and printing inks[9] as a biological stain for bacterial endospores,[11] or even added to lipid-rich structures prior to embedding in resin and observation by transmission electron microscopy.[12] Being a low-cost, widely available and efficient fungicide, Diamond Green is also used for controlling fungal and protozoan infections in fish cultures,[13] even though it is potentially harmful to human health.[14] Due to the toxicity of Diamond Green, several ultra-sensitive methods based on liquid chromatography coupled with mass spectrometry (LC-MS) were developed for the qualitative confirmation and quantification of Diamond Green and its metabolites within aquaculture products.[14-17] Moreover, considering the issues related to the presence of hazardous synthetic dyes in industrial wastewater, many studies concerning Diamond Green are dedicated to fine-tuning approaches for its removal, as absorption on activated carbon prepared from biomasses[18-20] or degradation induced by microwave irradiation,[21] glow discharge,[22] electro-oxidation[23] or sonolysis.[24]

Investigating the photostability of triarylmethane dyes is fundamental for testing their performance on different kinds of textile fibres, but also if we are interested in dating ballpoint inks in documents or in defining the original composition and state of conservation of a formulation employed in artworks. Thus, the photo-fading processes of triarylmethane dyes such as Methyl Violet, Ethyl Violet, Crystal Violet and Fuchsine have been investigated in water and ethanol solution to simulate the natural ageing process occurring in different matrices.[25-29] Several degradation products have been characterised and plausible degradation processes proposed thanks to the use of surface-enhanced Raman spectroscopy,[30] matrix-assisted laser desorption/ionisation coupled with MS,[28,29] and LC coupled with diode array detector and tandem MS (LC-DAD and LC-MS/MS).[25-27] The evidence was consistent with the results collected from mock-ups,[25,27-29] and from historical samples such as van Gogh's drawings[25] and Japanese woodblock prints.[30]

To the best of our knowledge, this paper is the first tackling in-solution degradation of Diamond Green G (hereinafter called DG). The degradation phenomenon was studied in aqueous and dimethyl sulphoxide (DMSO) solutions to avoid possible interactions with complex and heterogeneous matrices (such as paintings or textiles) and to simplify the evaluation of the process. DG aqueous and DMSO solutions were artificially irradiated, sampled at different time intervals and analysed by ultra-high performance LC coupled with DAD and electrospray ionisation-quadrupole-time of flight (UHPLC-DAD-ESI-Q-ToF) to identify the several degradation products formed. A plausible degradation pathway was formulated taking into consideration the kinetic trend of the ageing products formed in relation to the accelerated ageing times. The differences in the DG degradation process occurring in the two solvents are discussed and compared with evidence collected during our previous study, in which a similar irradiation in-solution approach was used for another synthetic dye.[27] Finally, a textile sample collected from a bluish-greenish degraded area of a stage costume from a Zeffirelli lyric opera (1961), designed by Peter Hall,[31] was analysed to validate the reliability of the ageing pathway proposed for DG.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Diamond Green G powder was provided from the reference collection of the Cultural Heritage Agency of the Netherlands (RCE; reference number 3231, originally obtained from CIBA).

The solvents and reagents used for preparing the standard solutions and for sample treatment were DMSO (JT Baker) and water (LC-MS grade; Sigma-Aldrich). The eluents for the chromatographic systems were water and acetonitrile, both LC-MS grade (Sigma-Aldrich). For the UHPLC-DAD-ESI-Q-ToF set-up, triethylamine (TEA; ≥98% purity; Sigma-Aldrich) was deployed as a mobile phase modifier, while for the HPLC-ESI-Q-ToF system, formic acid 0.1% v/v (98% purity; JT Baker) was used. For the historical sample pretreatment, the following solvents were used: acetone (Sigma-Aldrich), methanol (Sigma-Aldrich) and oxalic acid dehydrate (99.8% purity; Carlo Erba).

2.2 | Photo-ageing in-solution experiment

The study was performed on 60 ppm DG solutions in water and in DMSO, which were artificially aged using a Spectrolinker XL-1500 ultraviolet (UV) crosslinker equipped with an Hg lamp (254 nm, *ca.* 1-3 μW/cm^2, Spectronics Corporation). Selecting a more energetic UV lamp enabled us to potentially induce more severe degradation of DG in aqueous and DMSO solutions.

Although the vials (Waters Corporation) only transmitted a very limited amount of light at 254 nm, this experimental set-up has already proven its potential for appropriately studying the degradation process of other organic dyes.[27]

An aliquot of 100 μL was sampled from each solution (total volume of 2 mL) at different time intervals (5, 10, 20, 30, 40, 60, 80, 90, 110, 240, 407 and 500 minutes) for a total exposure time of 1000 minutes. Before each sampling the vials were vortexed for 5 seconds.

2.3 | Historical sample

A sample, collected from a bluish-greenish stage costume made of viscose and belonging to the main female character of the lyric opera *I Puritani*, was analysed. This opera was directed by Franco Zeffirelli, a renowned Italian director, in 1961. The costume was designed by Peter Hall and manufactured by the Teatro Massimo tailoring shop in Palermo (Sartoria del Teatro Massimo di Palermo) and today it belongs to the Foundation Teatro Massimo of Palermo (Fondazione Teatro Massimo di Palermo). The sample was kindly provided by the restorer Claudia Cirrincione, who collected it during her thesis project in Conservation and Restoration of Cultural Heritage (University of Palermo).[31]

The sample pretreatment chosen was particularly suitable for extracting triarylmethane dyes from the fibres, and it was based on oxalic acid extraction[32]; the viscose yarns were added with 150 μL of an oxalic acid 0.5 mol/L oxalic acid/methanol/acetone/water solution (1:30:30:40 v/v/v/v) then sonicated in an ultrasonic Sonorex Supra 10P bath (Bandelin Electronics) for 30 minutes at 60°C. The yarns were then removed from the extraction solvent and subjected to extraction with 150 μL of DMSO by sonication for 10 minutes at 60°C. The oxalic extraction mixture was dried under nitrogen steam and added to 150 μL of DMSO. The two DMSO extracts were filtered with polytetrafluoroethylene filters (0.45 μm diameter), mixed and injected in the HPLC-ESI-Q-ToF system.

2.4 | Apparatus

The aliquots collected during the irradiation in-solution were analysed by UHPLC-DAD-ESI-Q-ToF. Both the chromatographic and mass spectrometric methods have previously been optimised, as reported in the literature.[33] The apparatus comprised an Agilent 1100 Quaternary pump (G1311A), a 1260 Infinity degasser (G1322A), a 1100 autosampler (G1313A), and a 1290 Infinity DAD (G4212A) containing a max-light cartridge cell (G4212-6008, V0 = 1.0 μL) coupled with a Bruker Micro Q-TOF by an ESI interface. The DAD operated with spectra acquisition in the range of 160-640 nm with 2 nm resolution. Nitrogen was the drying and sheath gas used for mass spectrometric detection. The ESI conditions were: drying gas temperature of 250°C, a flow rate of 10 L/min, a capillary voltage of 4.4 kV, nebuliser gas pressure of 200 kPa, and a sheath gas temperature of 250°C. The MS and MS/MS acquisition range was set from 50 to 1200 *m/z* in positive mode and the MS/MS experiments were performed in AutoMS/MS acquisition mode, using 50 V in the collision cell. The UHPLC conditions consisted of an Agilent ZORBAX Eclipse Plus C18 rapid resolution high-throughput column (50 × 4.6 mm; 1.8 μm particle size), an injection volume of 5 μL and a flow rate of 1.85 mL/min. The separation was achieved using a gradient of 95% water, 5% acetonitrile and a TEA buffer adjusted at pH = 3 (eluent A), and 5% water and 95% acetonitrile and a TEA buffer adjusted at pH = 3 (eluent B). The elution programme consisted of 100% A for 2.5 minutes, followed by a linear gradient to 0% A in 6 minutes, which was then maintained for 2 minutes. The re-equilibration time for each analysis was 1.5 minutes. The UHPLC-DAD data were elaborated using Agilent OpenLAB CDS Chemstation edition (REV. C.01.04) software while MS data were elaborated using Bruker Daltonics Data Analysis software (version 4.0 SP4 build 281).

The historical textile sample was analysed by HPLC-ESI-Q-ToF. The apparatus consisted of a 1200 Infinity HPLC coupled with a Q-ToF tandem mass spectrometer 6530 Infinity detector by a Jet Stream ESI interface (Agilent Technologies). The ESI conditions were: drying gas temperature of 350°C, flow rate of 10 L/min, capillary voltage of 4.5 kV, nebuliser gas pressure of 241 kPa, sheath gas temperature of 375°C and flow rate of 11 L/min. The high-resolution MS and MS/MS acquisition range was set from 100 to 1000 *m/z* in positive mode. The acquisition rate was 1.04 spectra/second for both MS and MS/MS. MS/MS experiments were performed in AutoMS/MS acquisition mode, using 50 V in the collision cell. The full width half maximum of quadrupole mass bandpass used during MS/MS precursor isolation was 4 *m/z*. The mass spectrometric and chromatographic parameters have already been optimised for the analysis of other triarylmethane dyes, as presented in our previous publication.[34] The chromatographic conditions used for the analysis of the historical costume are reported in the literature.[34,35]

3 | RESULTS AND DISCUSSION

3.1 | In-solution study: qualitative analysis

The irradiation in-solution of DG was carried out both in water and in DMSO to evaluate possible differences in the degradation process. In particular, because an extraction procedure based on the use of DMSO revealed its potential both in terms of its efficiency and speed for the analysis of synthetic dyes and pigments,[35-37] the photochemical behaviour of DG in this solvent was of great interest. The UHPLC-DAD chromatograms (corresponding to absorption at 600 and 254 nm) of the aliquots sampled from both solutions are presented in Figures 1A,B and 2A,B. The

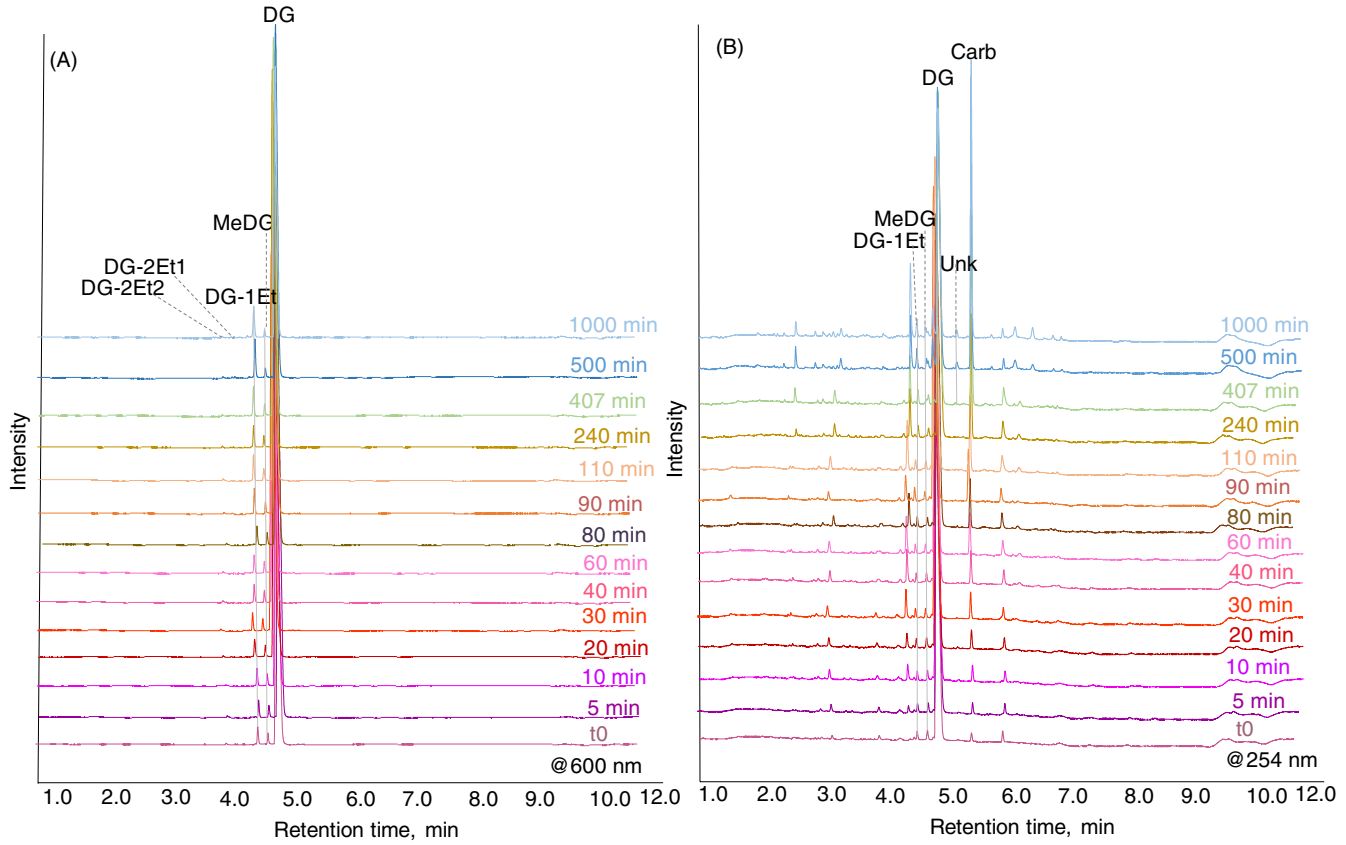


FIGURE 1 Ultra-high performance liquid chromatography coupled with diode array detector chromatograms of Diamond Green G (DG) aqueous solution irradiated at 254 nm, sampled at different times (from t0 to 1000 min). Detection wavelengths: (A) 600 nm and (B) 254 nm. All chromatograms are presented in the same scale, and are stacked for purposes of clarity

chromatograms are reported at 600 nm to highlight the trends of DG and of other triarylmethine compounds, and at 254 nm to evidence the presence of non-coloured ageing products, characterised by strong absorbance in the UV range. The identification of several labelled peaks was carried out on the basis of the exact mass values and interpretation of the product-ion spectra collected, as reported in Table 1.

The reference material used for preparing the solution already contained two synthesis by-products at time zero (t0): DG with one methyl group instead of an ethyl group as *N*- substituent (MeDG) (m/z = 371.248) and DG without an ethyl group as *N*- substituent (DG-1Et) (m/z = 357.233). This prevented the absolute quantitation of DG at different ageing times. A further impurity, 2MeDG, was detected. The position of the substituents cannot be easily determined based on the mass spectrum, and co-elution cannot be excluded.

The UHPLC-DAD chromatograms of the aliquots of DG in water and DMSO collected over time show new peaks ascribable to degradation compounds, although the intensity of DG only slightly decreases, and the change in colour of the aliquots is not visible to the naked eye. The products identified due to HPLC-ESI-Q-ToF analysis were two isomers of DG without two ethyl groups (DG-2Et2 and DG-2Et1) (m/z = 329.200), DG without three ethyl groups (DG-3Et) (m/z = 301.109) and DG without four ethyl groups (DG-4Et) (m/z = 273.137). DG-3Et and DG-4Et are missing in the water extracts.

The UV-visible (vis) spectra of the de-ethylated compounds (Figure 3A) exhibit a hypsochromic or blue shift of the absorbance maximum from DG to DG-4Et,[5] in accordance to what was already observed in the literature for triarylmethanes and xanthenes incurring in the *N*-dealkylation process.[25,26,28,35] The loss of electron-donating groups reduces the π electron density delocalised on the triarylmethane structure, thus increasing the energy for the electronic transition responsible for the hypsochromic shift.[38] The *N*-methyl forms show a less regular trend in the variation of the absorbance maxima, but the typical shape of triarylmethane spectra is unequivocally present (Figure 3B). The solution irradiated for 1000 min is still coloured green thanks to the relatively high amount of the original dye still present in the irradiated solution, and also because the main degradation products identified are absorbed in the same wavelength range.

The product-ion spectra of all the products identified are reported in Table 1 and in Figures S1A-F and S2A,B, while the fragmentation pattern is summarised in Table S1.

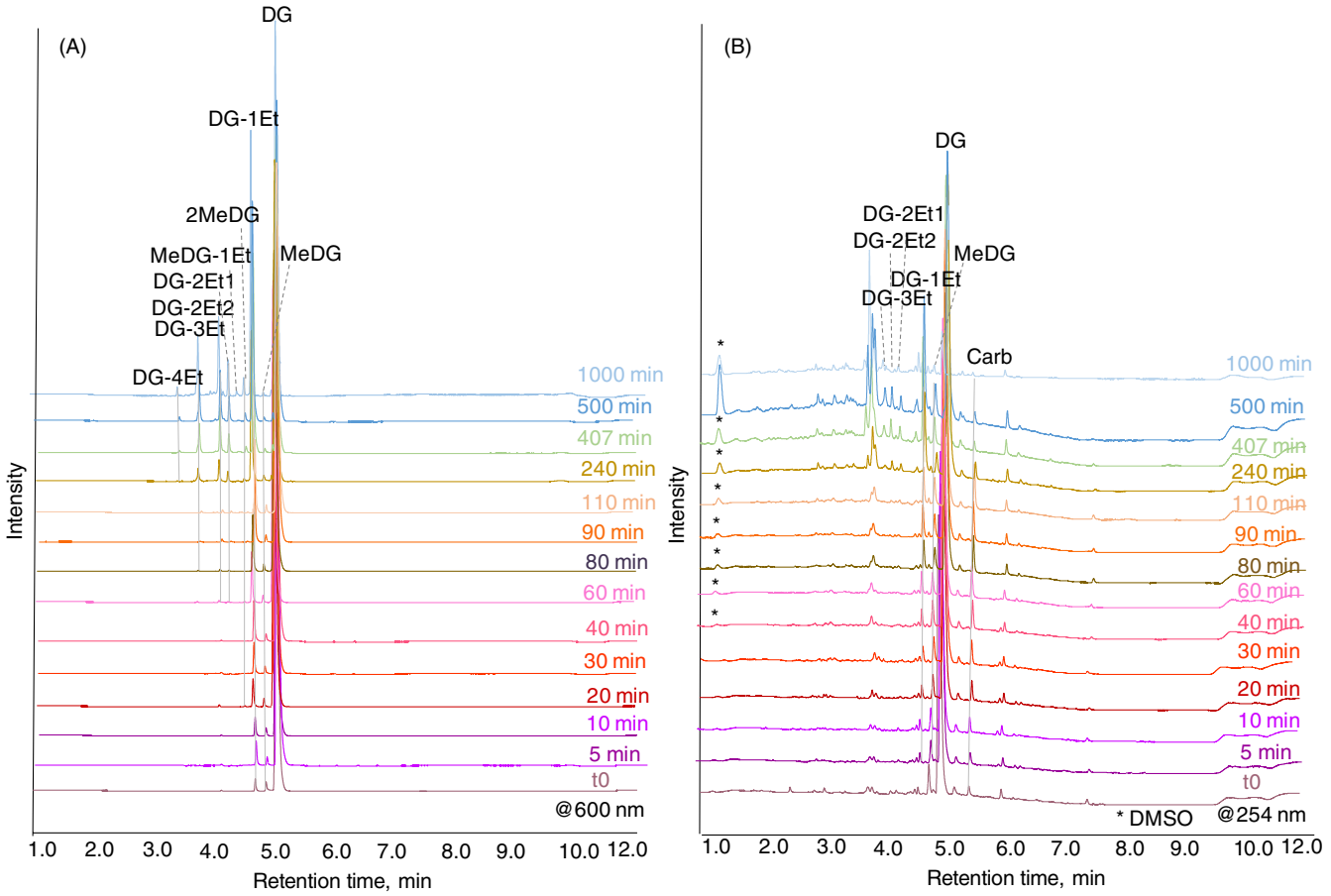


FIGURE 2 Ultra-high performance liquid chromatography coupled with diode array detector chromatograms of Diamond Green G (DG) solution in dimethyl sulphoxide (DMSO) irradiated at 254 nm, sampled at different times (from t0 to 1000 min). Detection wavelengths: (A) 600 nm and (B) 254 nm. All chromatograms are presented in the same scale, and are stacked for purposes of clarity. Peaks labelled with * are due to solvent

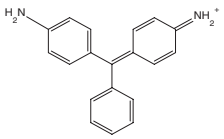
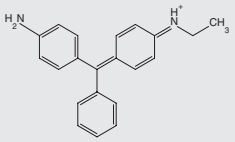
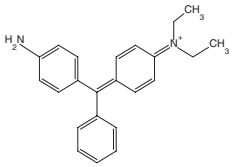
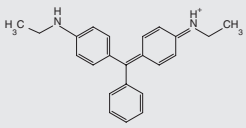
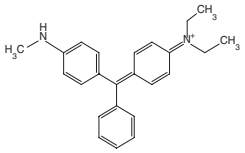
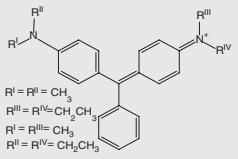
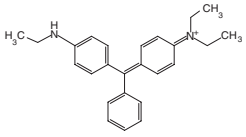
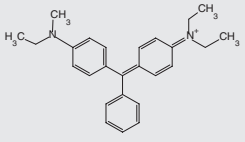
DG, DG impurities and degradation products all show a similar fragmentation pattern, entailing the consecutive homolytic cleavage of methyl and ethyl groups (as radical or as ethylene neutral loss, depending on the structure of the produced ion), in accordance with the literature on triarylmethine compounds.[39] The loss of a diethylamino group also occurs. DG-4Et, due to its chemical structure, cannot undergo the described pattern. Several *m/z*, corresponding to the base peak or to other intense fragments (Table S1), can generally be considered as triarylmethane markers; fragments with *m/z* values of 152.062, possibly corresponding to the biphenylene cation; fragments with *m/z* values of of 165.107, assigned to the benzotropylium cation; and fragments with *m/z* values of 180.081, assigned to the amino-benzotropylium ion, were identified.

During ageing, the new products prove that DG incurs in the so-called oxidative dealkylation of amines on the dye backbone (N-dealkylation): the ethyl groups of the dye are subsequently replaced by hydrogen upon exposure to light. This phenomenon has already been observed with the photo-irradiation of triarylmethanes such as Crystal Violet formulations[25,28,29] and xanthenes such as Rhodamine B.[35] The process is depicted in Figure 4. Note that some of the N-dealkylated products are barely visible in the UHPLC-DAD chromatograms in water and DMSO (Figures 1 and 2) although their presence was confirmed by LC-MS/MS.

The UHPLC-DAD chromatograms of the water extracts acquired at 254 nm (Figure 1) highlight several minor UV-absorbing peaks, some already present at t0 whose intensities do not readily change in time, while others start increasing as ageing progresses. More interesting results were obtained for two coloured compounds whose amounts increased with time, labelled in the figures as Unk and Carb. While Unk is only detected in low amounts, Carb is the most intense peak in the aqueous solutions irradiated for 500 and 1000 minutes (Figure 1B). Unk has absorbance maxima at 220, 285 and 480 nm (Figure S3A) and is characterised by a base peak at $m/z = 297.168$, but it was not identified because the response was too low to perform MS/MS analysis. The detection of Unk in the aqueous solution only indicates that it is related to DG carbinol form.

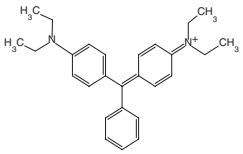
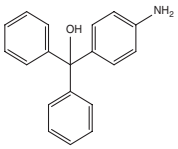
Carb, already present in small amounts in the starting water and DMSO solution, was identified as (4-aminophenyl)

TABLE 1 Compounds identified in the ultra-high performance liquid chromatography with electrospray ionisation-quadrupole-time of flight detector (UHPLC-ESI-Q-ToF) chromatograms of Diamond Green G (DG) in aqueous solution and dimethyl sulphoxide (DMSO) analysed in positive acquisition mode (the precursor and product ions of all the compounds are obtained as $[M]^+$, except for Carb, which is detected as $[M + H]^+$). The most intense product ions are highlighted in bold

	Molecules			MS[1]		MS/MS
Name	Assigned structure	Retention time [min]	λ abs max [nm]	Molecular formula	Precursor ion	Product ions
DG-4Et		3.3	300, 410, 582	$C_{19}H_{17}N_2^+$	273.137	269.121; 191.192; **180.077**; **152.061**
DG-3Et		3.7	315, 415, 596	$C_{21}H_{21}N_2^+$	301.169	269.101; 256.110; 240.071; 191.081; 180.081; **165.071**; **152.061**; 126.045
DG-2Et1		4.0	312, 410, 596	$C_{23}H_{25}N_2^+$	329.200	299.151; 285.139; 270.121; 256.111; 239.087; 207.094; 193.087; **180.081**; **165.069**; **152.060**
DG-2Et2		4.2	315, 415, 596	$C_{23}H_{25}N_2^+$	329.200	299.151; 285.139; 270.121; 256.111; 243.103; 228.092; 215.084; 202.078; 193.087; **180.081**; **165.069**; **152.060**
MeDG-1Et		4.3	320, 415, 612	$C_{24}H_{27}N_2^+$	343.216	299.137; 284.115; 269.115; 256.115; 241.108; 221.102; 207.095; **194.096**; 179.102; **165.073**; 152.065
2MeDG		4.5	280, 315, 422, 608	$C_{25}H_{29}N_2^+$	357.233	327.185; 313.171; 299.163; **284.138**; **270.127**; 256.112; 241.099; 229.101; **208.111**; **193.088**; 180.080; **165.070**; 152.062
DG-1Et		4.6	220, 315, 420 612	$C_{25}H_{29}N_2^+$	357.233	313.165; 299.161; 284.138; 270.115; 256.107; 241.106; 208.117; **193.091**; 180.080; **165.069**; 152.062
MeDG		4.7	245, 320, 430, 624	$C_{26}H_{31}N_2^+$	371.248	341.201; 327.187; 311.153; **297.140**; 284.142; 269.119; **241.102**; 222.127; 207.104; **192.082**; 165.070

(Continues)

TABLE 1 (Continued)

Name	Molecules: Assigned structure	Retention time [min]	λ abs max [nm]	MS[1]: Molecular formula	Precursor ion	MS/MS: Product ions
DG		4.9	320, 425, 627	$C_{27}H_{33}N_2^+$	385.264	355.219; **341.204**; 327.198; 311.156; **297.142**; 283.137; 269.121; **241.103**; 221.121; 192.083; 165.071
Unk	?	5.2	220, 285, 480		297.168	n.d.
Carb		5.4	248, 362	$C_{19}H_{17}NO$	276.136	n.d.

n.d., the intensity of the precursor ion was too low for the MS/MS experiment to be performed.

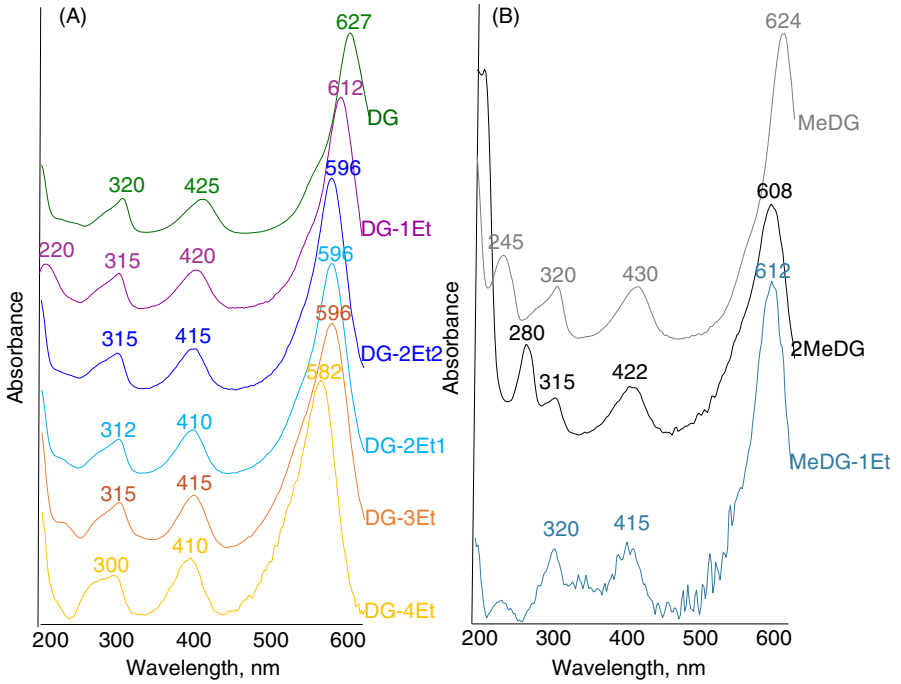


FIGURE 3 Ultraviolet-visible spectra of (A) DG and DG de-ethylated compounds and (B) DG demethylated compounds

diphenylmethanol (m/z = 276.137), based on the molecular formula, exact mass value and shape of the UV-vis spectrum reported in Figure S3B.

In order to justify the formation of this product, which is the main peak in the chromatogram of water extracts acquired at 254 nm (Figure 1B), the hydrolysis reaction of DG or generally that of triarylmethanes has to be taken into consideration (Figure 4). Carbinol is more sensitive to fading than the corresponding electrostatically stabilised cationic dye,[38,40] thus we expect this form to incur in the dealkylation process relatively faster than the corresponding triarylmethine, leading to Carb as the main degradation product in water. The maximum absorbance of Carb is at 360 nm in the near UV; the absorption band stretches into the blue region of the visible spectrum (ie, 380 nm and longer wavelengths), giving rise to yellow coloration, thus accounting for the colour shift of the aged solution towards green shades (Figure S3B). No other carbinol-type species were detected in the solutions analysed.

The UHPLC-DAD chromatograms of DG in DMSO solution (Figure 1A,B), collected after 240 minutes of irradiation,

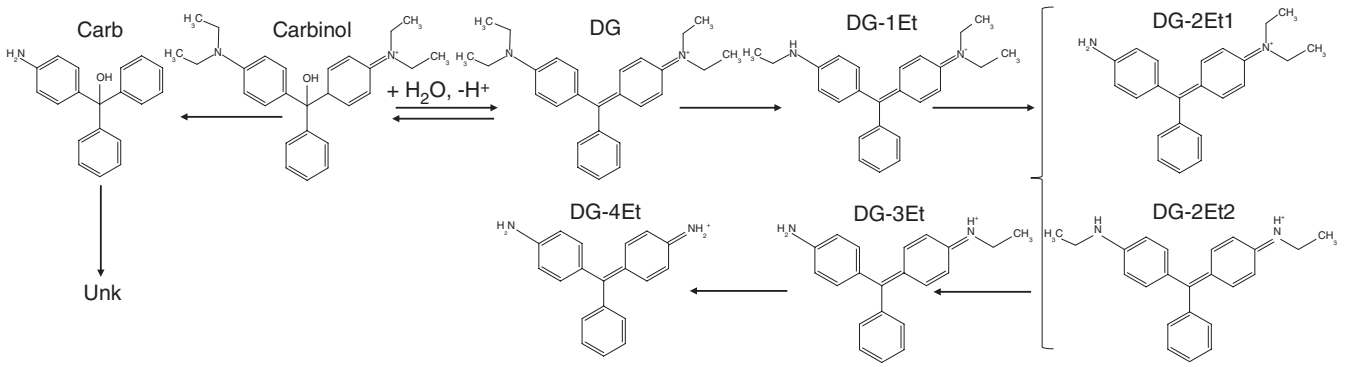


FIGURE 4 Hypothesised degradation pathways of Diamond Green G (DG): N-dealkylation on the right and hydrolysis reaction leading to carbinol formation[38] on the left

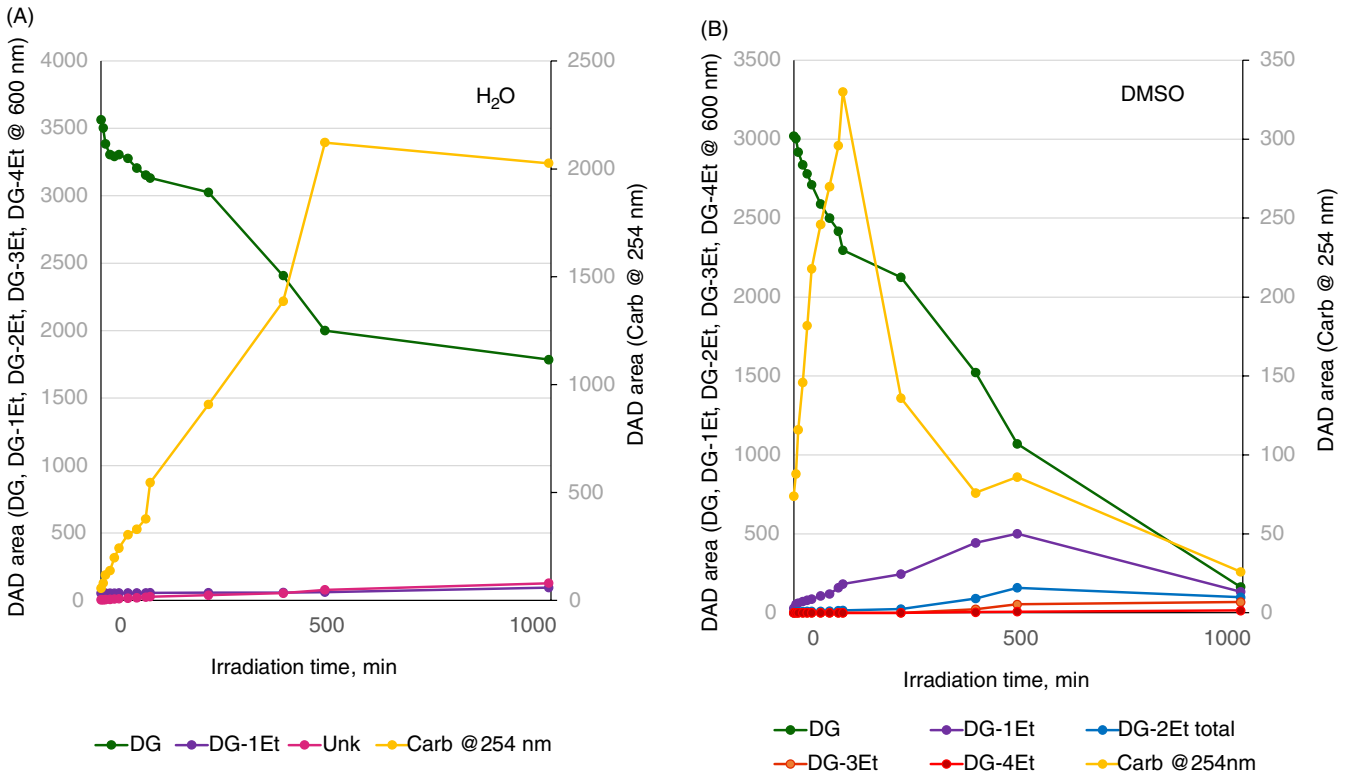


FIGURE 5 Peak areas (integrated in the ultra-high performance liquid chromatography coupled with diode array detector [UHPLC-DAD] chromatograms at 600 or 254 nm; Figures 1 and 2) vs accelerated ageing time of Diamond Green G (DG) and its impurities and degradation products formed in (A) water (H_2O) and (B) dimethyl sulphoxide (DMSO). The area of the peak due to Carb, integrated at 254 nm, is reported on the secondary axis in each graph

show formation of the same products as described for the water solution, but with different relative intensities, discussed thoroughly below in section 3.2.

The presence of Carb can be explained by the presence of trace water in DMSO, allowing equilibrium between the triarylmethine form and the respective carbinol to occur, even if at lower rates.

Two further compounds, characterised by the triarylmethine structure and not present in aqueous solution, were detected: DG with two methyl groups instead of two ethyl groups as *N*- substituents (2MeDG) (*m/z* = 357.233) and DG without two ethyl *N*-substituents and with one methyl group instead of an ethyl group as *N*- substituent (MeDG-1Et) (*m/z* = 343.216). The formation of the demethylated compounds can be explained by considering that DMSO is capable of interacting with a hydroxyl radical ($OH^{\bullet}$), generated by the irradiation of trace water present in DMSO, to generate a methyl radical ($CH_3^{\bullet}$).[41] These radicals may react with DG-2Et, leading to the formation of 2MeDG or MeDG-1Et. The latter was detected in the solution aged for 1000 minutes, thus it can be considered a final product under the selected ageing conditions and analysis time. No

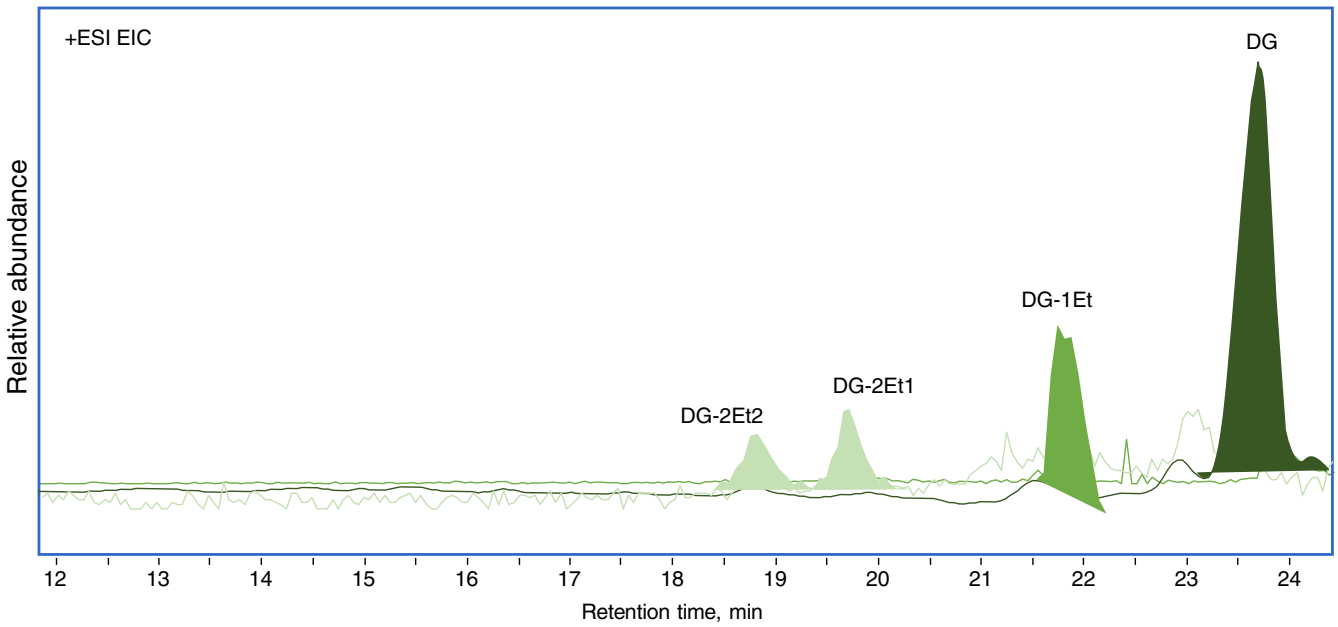


FIGURE 6 Ultra-high performance liquid chromatography and electrospray ionisation-quadrupole-time of flight (UHPLC-ESI-Q-ToF) extracted ion chromatograms (EICs) of $C_{27}H_{33}N_2^+$ (DG), $C_{25}H_{29}N_2^+$ (DG-1Et) and $C_{23}H_{25}N_2^+$ (DG-2Et) for the textile sample extract in positive acquisition mode

compounds containing sulphur (from DMSO) were produced, contrary to what was observed for the in-solution DMSO:ACN (1:1) study of eosin.[27] This was expected due to the lack of an electrophilic group, such as carboxyl or carbonyl, on DG.

3.2 | In-solution study: semi-quantitative analysis

The degradation processes occurring in water and DMSO were compared by plotting the areas of the peaks detected in the DAD chromatograms of the main compounds depicted in Figure 4 (integrated at 600 nm; except for at 254 nm for Carb) versus the irradiation time (Figure 5A,B). The data for the demethylated compounds (discussed in the text above) are not displayed, to ease the readability of the graphs.

In both solvents, DG decreases relatively slowly, but while in DMSO its peak area approaches zero at the end of the experiment (the final amount is 5% of the original dye amount), in water after 1000 hours of irradiation the amount of DG is only halved. The number of degradation products is also much higher in DMSO than in water. In DMSO, the following degradation products increase abruptly and then decrease: Carb (maximum at $t = 120$ minutes), DG-1Et/DG-2Et1/DG-2Et2 (maximum at $t = 500$ minutes) and DG-3Et (maximum at $t = 667$ minutes). The two isomers DG-2Et1 and DG-2Et2 display the same trend, decreasing more gradually than DG-1Et (their summed areas are reported in the graph for the ease of the reader). Based on their kinetics, these species can thus be considered intermediates in the ageing process. We expect the Carb form because of the amount of water dissolved in DMSO, and then it degrades completely to non-coloured species. DG-4Et and MeDG-1Et (not included in Figure 5B but included in Figure 2A) are present at $t = 1000$ minutes only, thus they can be considered final products in DMSO under the settled conditions and monitoring time. In water, the degradation processes are clearly still ongoing and it is possible that further final products will be produced. Notably, Unk is already produced in small amounts at very early irradiation times and it slowly increases (maximum at $t = 667$ minutes). Carb is produced very quickly and reaches a possible steady state around 500 minutes. Based on these results, not only were different kinetics of degradation in water or DMSO observed, but the two solvents also revealed a different degradation mechanism. The lack of appropriate analytical standards does not allow for an absolute quantitation of the different compounds.

3.3 | Analysis of an historical textile sample

A microsample was collected from a bluish-greenish viscose dress worn by the main female character of a Zeffirelli lyric opera and was analysed with HPLC-ESI-Q-ToF. Because of the characterisation of the composition of the reference DG formulation by tandem MS, the peaks evidenced in the extract ion chromatograms (Figure 6) were ascribed to DG and DG-1Et and to the two isomers DG-2Et1 and DG-2Et2. The

use of a DG formulation is consistent with the dating of this costume, as the lyric opera was performed in 1961.

No traces of Carb or any other carbinol species were detected in the sample extract. Thus, neither the environmental humidity that the sample was subjected to during its lifetime, nor the water present in the extraction solvents, allowed the formation of relevant amounts of carbinol forms.

4 | CONCLUSIONS

The current in-solution study enabled us to identify and unambiguously characterise DG synthesis by-products and degradation compounds, providing the UV-vis and production spectra. The main degradation pathways are depicted in Figure 4. On one hand, DG mainly incurs in the *N*-dealkylation process, consistently with observations of other triarylmethanes in different matrices. On the other hand, the degradation processes were different depending on the solvent in which the irradiation experiment was performed. Different final products can be identified for the two solvents, mainly due to the presence of carbinol forms in water, leading to the production of Carb as one of the main degradation compounds (and possibly of an unknown species, whose UV-vis spectrum is reported in Figure S3). In DMSO, radical dealkylation reactions entailing the loss of methyl groups are the main process competing with the main N-de-ethylation process.

The comparison between the kinetic trends of the components in the different environments allowed us to conclude that DG degrades faster in DMSO than in water, although for short times of irradiation the dye concentration is not affected by the chosen solvent (after 10 minutes, DG depletion is only 3% in DMSO and 2% in water). This finding proves that extracting unknown samples in DMSO at room temperature would not significantly affect the dye recovery, or the profile of its degradation products. The effect of temperature or ultrasound (even if applied for a short time) has still to be investigated, as do the use of oxalic acid and other sample treatment chemicals. Interestingly, analysis of a sample from an historical object showed that in addition to the main component, a few degradation products were found due to de-ethylation. In the solvent degradation experiments, many more components were detected, which could be due to the higher concentration of the starting material. From an ethical perspective, taking larger samples from an historical object is unacceptable, and future work should be devoted to ageing experiments on model samples (ie, dyed textiles for comparison). However, within this paper we showed that in-solution degradation is a rapid method with which to obtain fundamental knowledge about degradation mechanisms (as depicted in Figure 4), and relative behaviour in different solvents.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

FS and MVB designed the experiment. FS performed the analyses, ID and MVB contributed by interpreting the chromatographic and mass spectrometric data and provided explanations regarding the mechanisms. All the authors contributed equally to drafting the manuscript. All the authors read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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