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The generation of superoxide and hydrogen peroxide by exposure of fluorescent whitening agents to UVA radiation and its relevance to the rapid photoyellowing of whitened wool

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Abstract

Various fluorescent whitening agents (FWAs), chosen from the three major classes used commercially on textiles (stilbenes, pyrazolines and coumarins), produced hydrogen peroxide and superoxide radical anions when irradiated in aqueous solution with UVA light at 366 nm, near their absorption maximum. In contrast, none of these FWAs produced singlet oxygen on irradiation under similar aqueous conditions. The formation of superoxide, rather than $^1O_2$, suggests a mechanism where the excited singlet state of the FWA may undergo ionization to produce either an FWA radical cation and a free electron which is accepted by molecular oxygen, or an electron transfer reaction via formation of semi-reduced and semi-oxidized FWA radicals. Aqueous tryptophan also generates hydrogen peroxide and superoxide when irradiated at 366 nm, but the rate of H_2O_2 production increases significantly in the presence of an FWA. When wet FWA-treated wool fabrics are irradiated with simulated sunlight, they produce significantly more H_2O_2 (by a factor of four) than peroxide-bleached wool. Photogeneration of H_2O_2 and $O_2^{-\bullet}$ by electron transfer reactions from the excited state of the FWA, rather than energy transfer to $^1O_2$, probably contribute significantly to the rapid photoyellowing of wet FWA-treated wool and silk fabrics which remains a serious commercial shortcoming of these fibres.

Keywords: Fluorescent whitening agents; Superoxide radical anion; Hydrogen peroxide; Singlet oxygen; Photooxidation; Wool

1. Introduction

Fluorescent whitening agents (FWAs) are important additives which improve the appearance of various commercial products, particularly in the paper, plastics, detergent and textile industries. In the absence of FWAs these products usually absorb in the blue spectral range of natural sunlight, causing them to appear off-white or yellow. FWAs absorb UVA radiation in the range 360–380 nm and reemit visible blue or violet light as fluorescence. Small quantities of FWAs dispersed into appropriate products can compensate for the unwanted yellow appearance, making them appear whiter and brighter.

There are three major chemical classes of commercial FWAs, based on the stilbene, coumarin and pyrazoline structures, of which the most widely used are the stilbenes, including distyryl biphenyls, (DSBP). The photochemistry of FWAs has been extensively studied, both in solution and in the solid phase, as stability to sunlight is important in maintaining a white product with good lightfastness. The presence of oxygen and moisture during irradiation eventually leads to oxidation of the FWA which is then rendered less effective, causing the substrate ultimately to revert to a yellow appearance. Photoisomerisation of the fluorescent *trans*-isomer to the non-fluorescent *cis*-isomer is also important for stilbene whiteners, especially in solution [1]. However this gradual loss of effectiveness of the whitener through life does not explain the very rapid photoyellowing observed when FWAs are applied to the proteinaceous fibres wool and silk, especially when wet [2,3]. The severe lack of photostability for whitened wool products is an ongoing commercial shortcoming, and a problem that would clearly benefit from a better understanding of the photochemistry. Of particular relevance to the photoyellowing of FWA-treated wool by sunlight is the fact that no yellowing occurs in the absence of atmospheric oxygen [4].

It is well known that irradiation of certain dyes in dilute solution can produce singlet oxygen ($^1O_2$) via a Type II or energy transfer process between the excited triplet state of the dye and molecular oxygen in its ground triplet state (Scheme 1, where D represents the dye molecule).

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$$D + h\nu \rightarrow {}^1D^* \quad (1)$$

$${}^1D^* \rightarrow {}^3D^* \quad (2)$$

$${}^3D^* + O_2 \rightarrow D + {}^1O_2 \quad (3)$$

Scheme 1. Singlet oxygen formation (energy transfer mechanism).

Common examples of singlet oxygen sensiters are Rose Bengal, Methylene Blue and Eosin Y.

In the presence of an electron donor (E) some dyes generate H_2O_2 and O_2^- via an electron transfer mechanism shown in Scheme 2 [5]. At dye concentrations $>10^{-5}$ M, the ground state of the dye itself can sometimes act as the electron donor, resulting in semi-reduced and semi-oxidised dye radicals (Equation (6)) [6].

Some dyes have the ability to act as both singlet oxygen sensitisers and $H_2O_2/O_2^{-\bullet}$ generators, depending upon the particular conditions [6]. It was of interest to consider which of these mechanisms is dominant when FWA-treated textiles are exposed to sunlight, particularly for wool. The theory that singlet oxygen is the primary reactive oxygen species involved in the photoyellowing of untreated wool was first proposed in 1976 by Nicholls and Pailthorpe [7], and extended to include bleached wool and wool treated with a fluorescent whitening agent (FWA) in a subsequent publication by Nicholls in 1980 [8]. Nicholls suggested that interaction of the triplet FWA molecule with oxygen to produce $^1O_2$ (Scheme 1) was the more likely mechanism for the photoyellowing of FWA-treated wool than an electron transfer process [8].

In recent years significant doubt has been cast on the singlet oxygen mechanism of wool photoyellowing. One major criticism is that the photoyellowing of wool is far more rapid when the wool is wet, but the lifetime of singlet oxygen in water is 4.2 μs, compared with 14 ms in the gas phase [9]. Recent work by Millington and Kirschenbaum [10] has shown that the rate of photoyellowing of wet wool by simulated sunlight in water and in D_2O is very similar. The lifetime of $^1O_2$ in D_2O (67.8 μs) is significantly higher than in water, and reactions which involve $^1O_2$ are usually greatly enhanced in D_2O [9]. Millington and Kirschenbaum also showed that hydroxyl radicals are produced when wet wool is irradiated with both UVA (366 nm) and blue (425 nm) light using a fluorescent probe [10]. The amounts of $^{\bullet}OH$ produced on irradiation of wool are the same in solutions of the probe in H_2O and D_2O, showing that the formation of $^{\bullet}OH$ does not occur via $^1O_2$. Although $^1O_2$ has been detected in irradiated wool by Smith [11], he suggested that singlet oxygen is involved in the photobleaching of wool. He found that when wool is irradiated at 265 and 350 nm, $^1O_2$ was detected at the higher wavelength only. He also postulated that photoyellowing of wool by sunlight is much faster in the wet state because any $^1O_2$ generated by visible wavelengths, which would lead to concurrent photobleaching in the dry state, is rapidly quenched by water [11].

$$D + h\nu \rightarrow {}^1D^* \quad (4)$$

$${}^1D^* \rightarrow {}^3D^* \quad (5)$$

$${}^3D^* + E \rightarrow E^{+\cdot} + D^{-\cdot} \quad (6)$$

$$D^{-\cdot} + O_2 \rightarrow D + O_2^{-\cdot} \quad (7)$$

$$O_2^{-\cdot} + H^+ \rightarrow {}^{\cdot}OOH \rightarrow H_2O_2 \quad (8)$$

Scheme 2. Formation of superoxide and hydrogen peroxide (electron transfer mechanism).

It is also relevant that in earlier work on FWA-treated wool fabrics exposed to simulated sunlight when wet, hydrogen peroxide was detected during the rapid photoyellowing of the wool [12]. When FWA-treated wool was doped with H_2O_2 before irradiation it yellows rapidly. However in the presence of reducing agents such as sodium bisulphite or thiourea dioxide, far less yellowing was observed. It was suggested that the generation of H_2O_2 during exposure to sunlight could be responsible for the increased rate of yellowing of FWA-treated wool, especially when wet [12].

It was not clear from earlier work whether the wool protein, the FWA or both were involved in the photochemical mechanism resulting in H_2O_2 generation, although only wet FWA-treated wool produced measurable amounts of H_2O_2 after irradiation with simulated sunlight [12]. Certain proteins and their photoproducts, particularly those found in eye lenses such as β-crystallin, have been shown to generate H_2O_2 and superoxide when exposed to UVA radiation [13–15]. Previous work also has shown that irradiation of pigment dispersions such as cadmium sulphide [16], zinc oxide [17] and metal-free phthalocyanine [18] can also generate superoxide and H_2O_2.

In this study aqueous solutions of FWAs chosen from the three major classes used commercially on textiles (stilbenes, pyrazolines and coumarins) were irradiated using a UVA source in the presence of atmospheric oxygen and analyzed for H_2O_2, superoxide radical anion and singlet oxygen. The assay methods used in this study have recently been applied to the study of reactive oxygen species produced in human eye lenses during cataract formation [13,15,19].

Some further studies were also carried out on FWA-treated wool fabrics and, together with data from previous published studies, the probable photochemical mechanisms involved in the yellowing of FWA-treated wool are discussed.

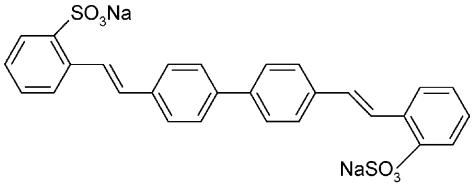
2. Experimental

2.1. Materials

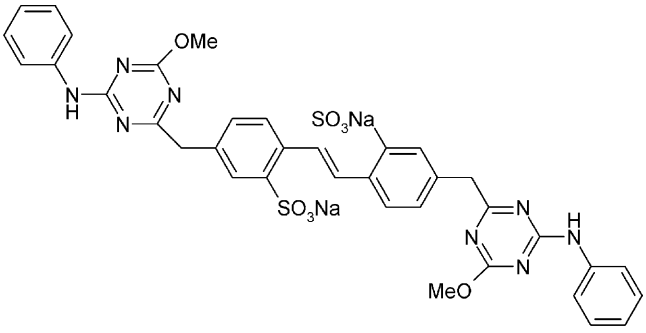
2.1.1. Materials

L-Tryptophan, imidazole, methylene blue (MB), xylenol orange (XO), sorbitol, iron (II) ammonium sulphate and *N*,*N*-dimethylnitrosoaniline (RNO) were obtained from

Sigma Aldrich. The enzymes catalase (ex bovine liver) and superoxide dismutase (SOD) were obtained from Bohringer Mannheim (Castle Hill, NSW). The fluorescent whitening agents Uvitex NFW, CF and WGS solids (Ciba) and Leucophor PAT, Hostalux PN and Hostalulx N2R liquids (Clariant) were kindly supplied by the manufacturers. Of these only the structures of Uvitex NFW (**I**) and CF (**II**) have been openly disclosed, and these two FWAs were recrystallised twice from aqueous ethanol. Other commercial FWAs were used as supplied.



(I) *Uvitex NFW (Ciba)*



(II) *Uvitex CF (Ciba)*

2.1.2. Fabric, FWA application and irradiation conditions

Lightweight Merino wool challis fabric was obtained from Armitage Ltd (UK) and was scoured thoroughly in warm water before use. A conventional alkaline bleaching procedure [20] using hydrogen peroxide (0.75% (w/v), with tetrasodium pyrophosphate {6 g/l} for 1 h at 60 °C) was given to the fabric before FWA treatment. All fluorescent whitening agents (2% on weight of fibre) were applied to the bleached wool fabrics at 60 °C for 60 min in the range of pH 3.5–4.0. An Ahiba Turbomat laboratory scale dyeing apparatus was utilized for all fabric treatments using a liquor to goods ratio of ∼40:1.

The preparation of wool fabrics for wet or dry photostability testing (in simulated sunlight) was achieved by placing fabric strips into separate compartments of UV-transparent polyethylene bags. Samples undergoing wet irradiation were sealed in these compartments. All samples were irradiated on a water-cooled stage inside the sample chamber of a Hereaus Suntest accelerated weathering machine (xenon arc) fitted with a combination of quartz and dichroic filters.

Reflectance spectra and CIE Ganz 82 whiteness indices of fabrics were measured using a Gretag Macbeth Color-Eye 7000 A spectrophotometer with Optiview software.

Photoirradiation of FWA solutions for H_2O_2 and $O_2^{-\bullet}$ analysis was carried out using a custom-built UVA apparatus containing up to twelve Pyrex tubes held in an annular carousel around a central blacklight UVA source (Eye H125BL, Iwasaki Electric Co, Tokyo). This apparatus produced peak UV output (typically 14 mW/cm^2 at the sample position) between 360 and 370 nm and no UV radiation below 300 nm [21]. The lamp was cooled by incorporating a small fan beneath the sample carousel, and the temperature of the solutions during irradiation was kept below 30 °C even during extended (up to 6 h) irradiation periods. Irradiation of samples for singlet oxygen assays was carried out using either a Philips TLD blacklight tube (for irradiation of the FWA-containing solutions at 366 nm) or a Philips TL15 red fluorescent tube (for 660 nm irradiation of MB).

2.1.3. Reactive oxygen species assays

2.1.3.1. Hydrogen peroxide. Hydrogen peroxide was assayed using the xylenol orange technique described by Jiang et al. [22]. H_2O_2 oxidises iron (II) to iron (III) in the presence of sorbitol, which acts as a catalyst. Iron (III) then forms a purple complex with xylenol orange. A 5 cm^3 aliquot of irradiated FWA solution was placed in a 25 cm^3 graduated flask, and mixed with 2.5 cm^3 each of sorbitol (0.1 M), sulphuric acid (0.25 M) and xylenol orange (1.0 mM). The reaction was initiated by the addition of 2.5 cm^3 of iron (II) ammonium sulphate (2.5 mM) prepared fresh daily and RO water (purified by reverse osmosis) was added to make up to 25 cm^3, and the flask was then shaken for 45 min. After shaking, the absorbance at 560 nm was determined and compared with a hydrogen peroxide standard curve. In order to obtain optimum accuracy, the assay was also performed in the absence of peroxide and this was used as a blank, as described by Gay et al. [23]. The concentration of H_2O_2 in stock solutions was calculated using its extinction coefficient of 43.6 M^{-1} cm^{-1} at 240 nm.

When the enzyme catalase was added to the FWA aliquot before carrying out the assay, any H_2O_2 was selectively converted to water and oxygen. As catalase is highly specific for H_2O_2, the presence of any other oxidants capable of producing iron (III), including organic hydroperoxides ROOH, could be discriminated [22].

2.1.4. Hydrogen peroxide formed on irradiated wool fabrics

Small squares of untreated, bleached and fluorescent whitened fabric (10 cm × 10 cm) were dried in a microwave oven for 6 min to remove moisture and weighed, then immersed in water containing ∼0.1% (v/v) Leophen M wetting agent for no less than 1 h. The fluorescent whitener used was Uvitex NFW (2% owf). Each sample was passed through a pad mangle set at a pick up of 100%, to ensure that the ratio of mass of wool to water was constant for each sample. The samples were then sealed in polyethylene

bags transparent above 250 nm and photoirradiated for 3 h using a Hereaus Suntest machine. After irradiation, the samples were placed into a centrifuge tube equipped with a PVDF membrane with pore size 0.45 μm (Millipore, Australia), and centrifuged to remove as much of the liquid in the wool as possible. Typically between 0.5 and 0.6 ml of solution was obtained from each sample. The xylenol orange peroxide assay was then performed on this liquor. An identical experiment was performed without irradiation, and the assay of these liquors was used as the appropriate blank. Blank values were comparable to the error of the measurement.

2.1.5. Superoxide radical anion

The superoxide radical anion ($O_2^{-\bullet}$) can be detected using a modification of the Jiang et al. assay described above for hydrogen peroxide, in which the enzyme superoxide dismutase (SOD) is added [13,15]. SOD is a highly specific probe for the presence of $O_2^{-\bullet}$ (and its protonated form •OOH) by enhancement of H_2O_2 production.

2.1.6. Singlet oxygen

The system imidazole plus *N*,*N*-dimethylnitrosoaniline (RNO) can be used as a sensitive and selective test for the presence of $^1O_2$ in aqueous solution, as described by Kraljic and El Mohsni [24]. Recently Linetsky et al. [19] compared the sensitivity of three techniques for detecting $^1O_2$ in aqueous dispersions of human eye lens (crystallin) proteins following UVA irradiation, one of which was a variant of Kraljic's method used here. They used the thermal decomposition of 3-(4-methyl-1-naphthy) propionic acid endoperoxide to make a quantitative estimation of the amount of $^1O_2$ generated in solution. All three methods gave similar estimates of the amount of $^1O_2$ produced by irradiated lens protein [19], demonstrating that the Kraljic method is a reliable one.

The assay was performed on irradiated solutions using a concentration of the "sensitiser" (MB or Uvitex NFW) of 20 μM, an RNO concentration of ~40 μM and various imidazole concentrations ranging from 0 to 7 mM, as previously described by Kraljic [24]. Hostalux N2R and other FWAs with undisclosed structures were diluted by a factor of 10,000. Solutions containing the "sensitiser", RNO, and various concentrations of imidazole, were prepared and placed in 1 × 1 cm polystyrene spectrophotometer cells. The cells were then held in direct contact with the appropriate light source (fluorescent tube) for 60 min using rubber bands, removed and the UV-Vis spectrum obtained using a Cary 300 instrument (Varian). The absorbance of the RNO peak at 440 nm was also measured on each sample before and after irradiation to monitor RNO bleaching, and the difference recorded as ΔA (440 nm). Kraljic [24] has shown that a plot of RNO bleaching {i.e., Δ*A* (440 nm)} against the logarithm of imidazole concentration should be sigmoidal for reactions involving singlet oxygen at low imidazole concentrations (<8 mmol/l).

3. Results

3.1. Detection of H_2O_2 and $O_2^{-\bullet}$ in FWA and tryptophan solutions

The three major classes of FWA that can be applied to textiles, stilbenes, pyrazolines and coumarins, have different structures and chemistries, but it has been shown previously that all three types of FWA vastly increase the rate of photoyellowing of wool [3]. Since a previous preliminary study [21] on two stilbene FWAs in solution also showed that both produced H_2O_2 when irradiated with UV light near their absorption maxima at 366 nm, it was of some interest to see if H_2O_2 production was independent of the class of FWA used.

The three FWAs chosen for this part of the study were Uvitex NFW (Ciba, stilbene), Leucophor PAT (Clariant, pyrazoline) and Uvitex WGS (Ciba, coumarin). Fig. 1 shows that all three FWAs produced similar amounts of H_2O_2 after irradiation with UVA light for 90 min. The linearity of the plot of [H_2O_2] with irradiation time, whilst good for Uvitex NFW, was poor for the coumarin FWA Uvitex WGS. Other FWAs used on wool and nylon textiles (Uvitex CF, Leucophor PAF, Hostalux PN) gave similar results and generally linear plots.

Fig. 2 compares the amounts of H_2O_2 generated using two different concentrations of Uvitex NFW (2 and 0.2 mM). As expected, the higher concentration of FWA produces a higher concentration of H_2O_2.

Fig. 3 shows that adding the enzyme superoxide dismutase (SOD, 250 units/ml) to Uvitex NFW solution before irradiation results in the generation of significantly higher levels of H_2O_2 for irradiation periods of 30 min or more. UVA irradiation of SOD in the absence of FWA produced no detectable H_2O_2. Since SOD dismutes the superoxide anion

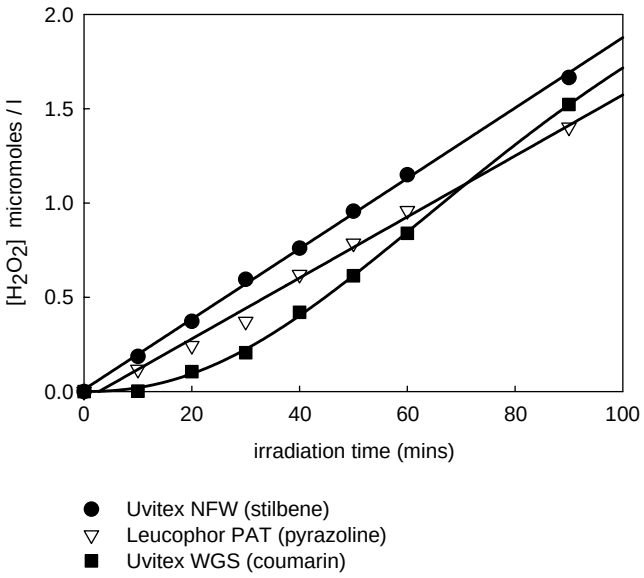


Fig. 1. Photogeneration of H_2O_2 by aqueous FWA solutions exposed to 366 nm UVA light.

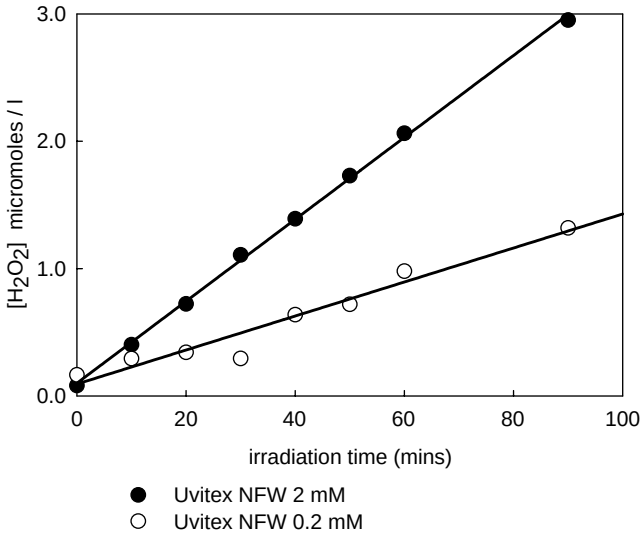


Fig. 2. Effect of FWA concentration on rate of photogeneration of H_2O_2.

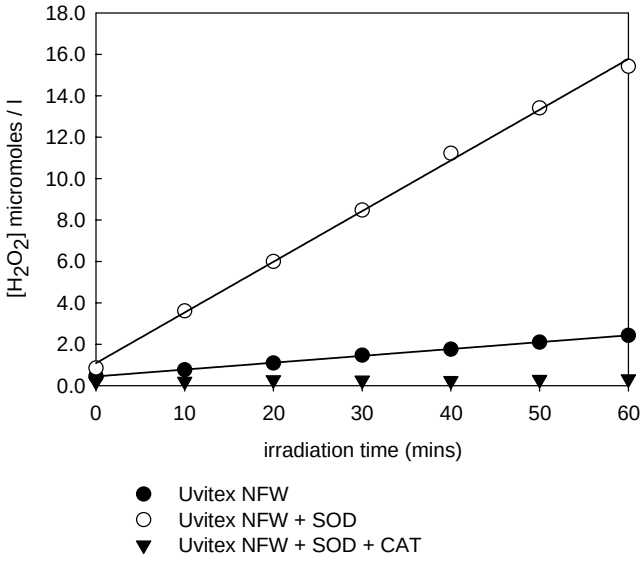


Fig. 3. The effect of superoxide dismutase and catalase on the photogeneration of H_2O_2 by aqueous Uvitex NFW (2 mM).

to hydrogen peroxide and molecular oxygen (Scheme 3), detection of higher levels of H_2O_2 in the presence of SOD shows that Uvitex NFW generates superoxide on exposure to UVA radiation. Similar results were found for other FWAs (Uvitex CF, Hostalux PN, data not shown).

The formation of both $O_2^{-\bullet}$ and H_2O_2 from irradiated FWAs also provides a route to the hydroxyl radical [25], which is the most reactive oxygen radical (Scheme 4).

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (9)$$

Scheme 3. Dismutation of superoxide radicals to hydrogen peroxide and oxygen by superoxide dismutase.

$$O_2^{\cdot-} + H_2O_2 \rightarrow {}^{\cdot}OH + {}^{-}OH + O_2 \quad (10)$$

$$^{\cdot}OOH + H_2O_2 \rightarrow {}^{\cdot}OH + H_2O + O_2 \quad (11)$$

Scheme 4. Formation of hydroxyl radicals from $O_2^{-\bullet}$ and H_2O_2.

Having shown that UVA irradiation of aqueous FWA solutions generates $O_2^{-\bullet}$ and H_2O_2, it was of interest to investigate the effects of the presence of aqueous tryptophan on H_2O_2 generation by irradiated FWAs. In previous work by McCormick and Thomason [26], $O_2^{-\bullet}$ and H_2O_2 were generated from tryptophan solutions irradiated using a source generating UV in the range 315–400 nm. A mixture of Uvitex NFW and tryptophan (both 2 mM) was irradiated and analyzed in a similar manner.

Fig. 4 shows that irradiation of tryptophan (2 mM) at 366 nm produces significantly more peroxide than the FWA (2 mM) alone. It is also clear that the rate of increase in $[H_2O_2]$ for tryptophan initially is quite slow, but the rate increases significantly after about 10–20 min irradiation, following a sigmoidal relationship. This is believed to be due to the photosensitisation of tryptophan oxidation by its oxidation products, in particular *N*-formylkynurenine (NFK) [26]. Fig. 4 also shows that a combination of tryptophan and FWA (both 2 mM) produces significantly higher concentrations of peroxide very rapidly. There is a clear synergistic effect between the FWA and tryptophan with regard to H_2O_2 production. The effect must be due, at least in part, to the far more effective light absorption in the near UV by the FWA.

This synergy between the FWA and tryptophan to produce H_2O_2 is an interesting and important observation. If the tryptophan residues in wool behave in the same way, this photosensitising effect of the FWA could explain the rapid photoyellowing of FWA-treated proteinaceous fibres containing Trp residues, such as wool and silk. It is interesting to

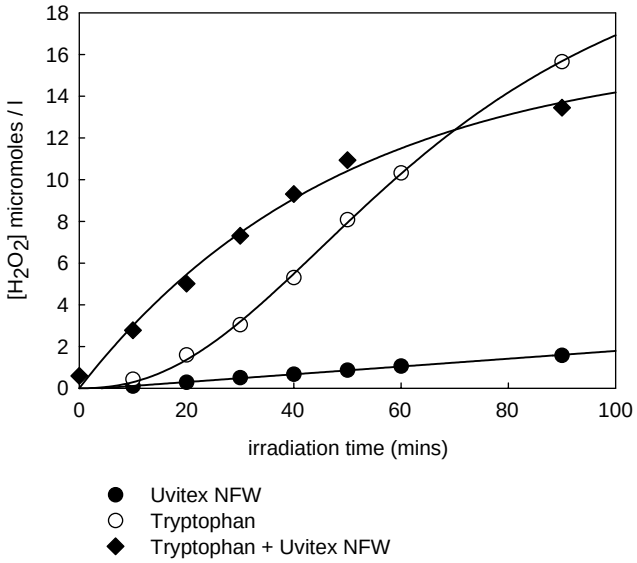


Fig. 4. Photogeneration of H_2O_2 by irradiated tryptophan and Uvitex NFW.

Table 1
Concentration of H_2O_2 in liquor centrifuged from photoirradiated wool fabric after 3 h wet exposure to simulated sunlight

Wool sample	H_2O_2 (μmol/l)
Untreated	8.0
Peroxide bleached	5.6
Bleached/FWA-treated	25.7
Bleached/FWA-treated (pre-yellowed dry for 24 h)	31.5

note that in previous work [12], H_2O_2 was clearly detected in irradiated FWA-treated wool, but not in untreated wool.

3.2. Detection of H_2O_2 in irradiated wool fabrics

Table 1 shows a comparison of the peroxide concentrations measured in various irradiated wool samples after 3 h exposure to simulated sunlight. The four wool samples compared are untreated wool, peroxide bleached wool, bleached/fluorescent whitened wool, and bleached/fluorescent whitened wool which had been pre-yellowed by simulated sunlight irradiation in the dry state for 24 h. From the results presented in Table 1 it is clearly demonstrated that when UV light is absorbed by untreated wool, a significant amount of peroxide is produced, resulting in a solution of 8.0 μM hydrogen peroxide. Even when many of the visible chromophores in wool are removed by bleaching, a significant amount of peroxide is still produced (ca. 5.6 μM). When a fluorescent whitener is present, the concentration of the peroxide in the liquor after irradiation increases by a factor of four from 5.6 μM (in bleached wool) to 25.7 μM (in bleached/fluorescent whitened wool). The largest amount of peroxide measured was obtained from the pre-yellowed fluorescent whitened wool, which indicates that the yellow chromophores generated in the wool contribute significantly to peroxide production. Note that the blank measurement on the pre-yellowed fluorescent whitened wool was zero, showing that there was no peroxide present on the wool prior to the wet irradiation.

3.3. Detection of singlet oxygen

The results of the singlet oxygen assay using the known singlet oxygen sensitiser methylene blue (MB) following irradiation near MB's visible absorption maximum with red light at 660 nm for 60 min in the presence of various amounts of imidazole are shown in Fig. 5. It is clear that the peak at 440 nm (due to the absorption of the RNO) is bleached as expected, and that the degree of bleaching increases with the concentration of imidazole present, as previously observed. These plots agree well with the earlier data reported by Kraljic for known singlet oxygen sensitisers [24]. Fig. 6 shows that the bleaching of RNO depends on the initial concentration of imidazole, as recommended in Kraljic's paper [24].

Having confirmed Kraljic's earlier work, the $^1O_2$ assay was then applied to the two FWA solutions. Figs. 7 and 8 show absorption curves for Uvitex NFW and Hostalux N2R, respectively, with RNO and various amounts of imidazole present, following irradiation with UVA light (366 nm) for 60 min. These plots are very different from Fig. 5, and the principal effects here are that absorption of the FWA is greatly decreased in all cases after irradiation, and little

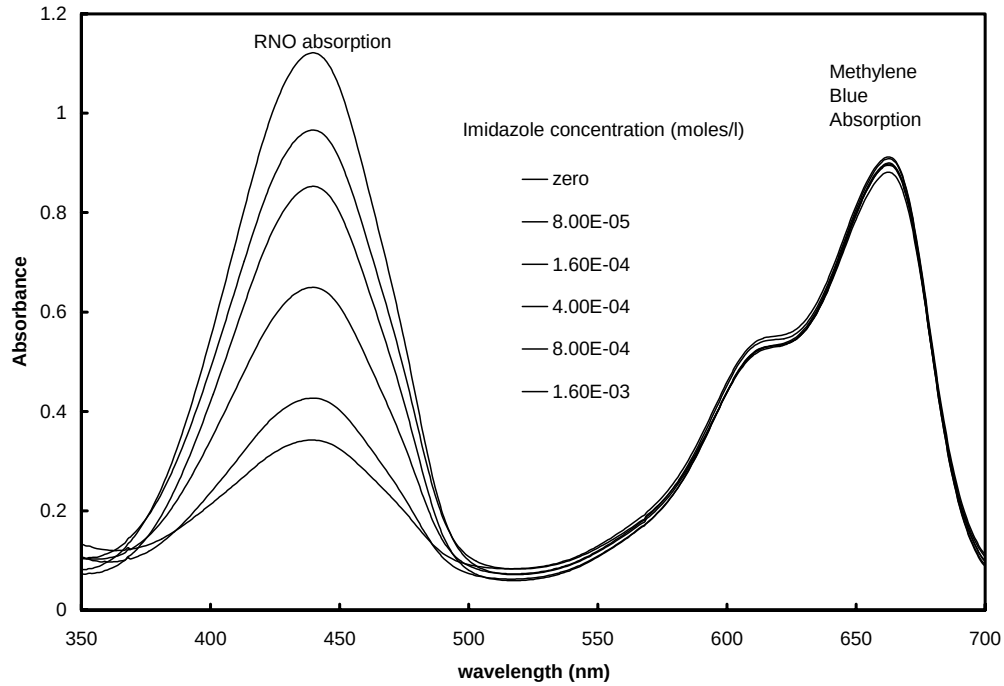


Fig. 5. Bleaching of RNO by $^1O_2$ generated by irradiation of aqueous methylene blue at 660 nm at various imidazole concentrations.

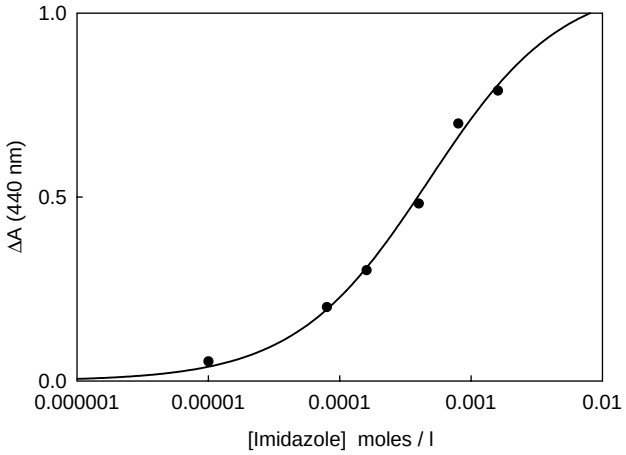


Fig. 6. Dependence of the degree of bleaching of RNO (ΔA 440) on imidazole concentration for photoirradiated MB showing the characteristic behavior of a singlet oxygen sensitiser.

or no bleaching of RNO occurs. This suggests that irradiation at 366 nm effectively destroys the FWA after 60 min exposure and there is no evidence for any $^1O_2$ being generated. The difference in RNO absorbance for the FWA studies (measured at 440 nm before and after exposure) varies only by ±0.05 absorbance units, which is more than an order of magnitude lower than the corresponding measurements for MB (cf. Fig. 5).

It is clear from Figs. 7 and 8 that the photostability of the FWAs in solution is significantly inferior to MB. One contributing factor is the difference in the energies of the radiation absorbed. MB absorbs red light at 660 nm, equivalent to an energy of 181 kJ/mol, and the FWAs absorb at 350 nm, equivalent to 342 kJ/mol. The bond energies of most organic compounds lie in the range 200–400 kJ/mol, so that the higher the energy of absorption the more likely photolysis is to occur. It is also interesting to note that most recognized singlet oxygen sensitisers are highly colored dyes, and that the energy difference between ground state triplet and the lowest energy state of singlet oxygen ($^1\Delta$) is relatively small, (92.4 kJ/mol) [27], allowing excitation by energy transfer from the triplet states of many dyes using visible light [6].

Another important factor which affects the stability of FWA solutions is the tendency for *trans–cis* photoisomerisation to occur. Previous work [28] has been carried out on the *trans–cis* photoisomerisation of Uvitex NFW, showing that in solution the FWA is far less stable than when it is either applied to a textile or dispersed in a solid PVA film. In the solid phase and in viscous media, the twisting of the molecules that is required for a transition from the fluorescent *trans*- form to the non-fluorescent *cis*-isomer is inhibited [28]. In previous studies on FWA-treated wool, a reduced level of *trans–cis* photoisomerisation occurs on wool than in solution, but an increased level of FWA photooxidation products is produced.

Although the low photostability of the two FWA solutions examined in this study is clear, there is no evidence that any $^1O_2$ is produced when these FWA solutions are irradiated, in contrast to the results for MB and for other recognized singlet oxygen sensitisers [24].

4. Discussion

The almost linear increase in H_2O_2 formation with time during UVA irradiation of stilbene and pyrazoline FWAs

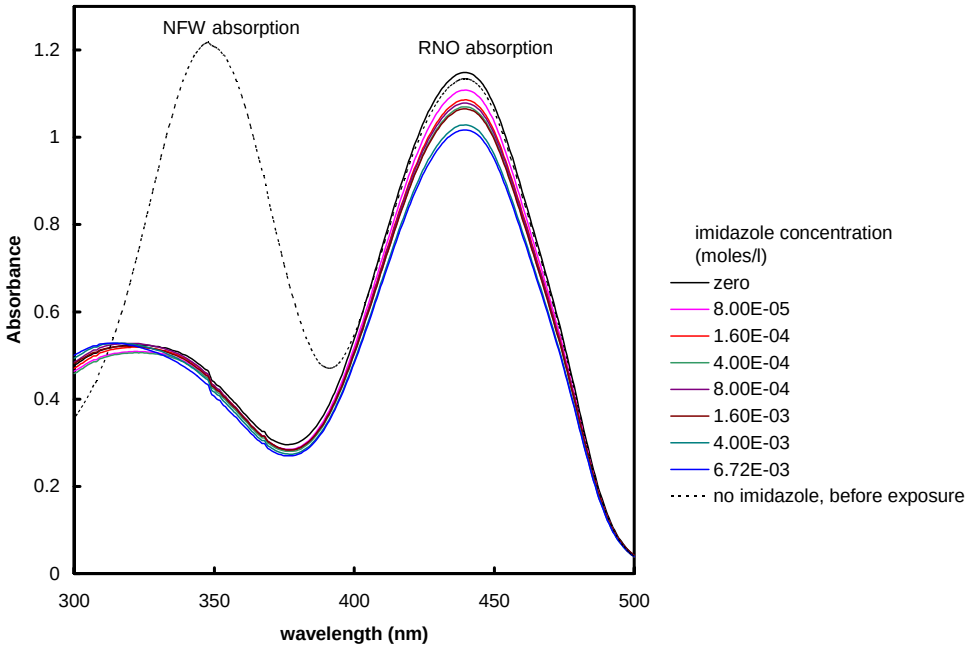


Fig. 7. UV-Vis spectrum of Uvitex NFW and RNO absorptions after irradiation at 366 nm at various imidazole concentrations.

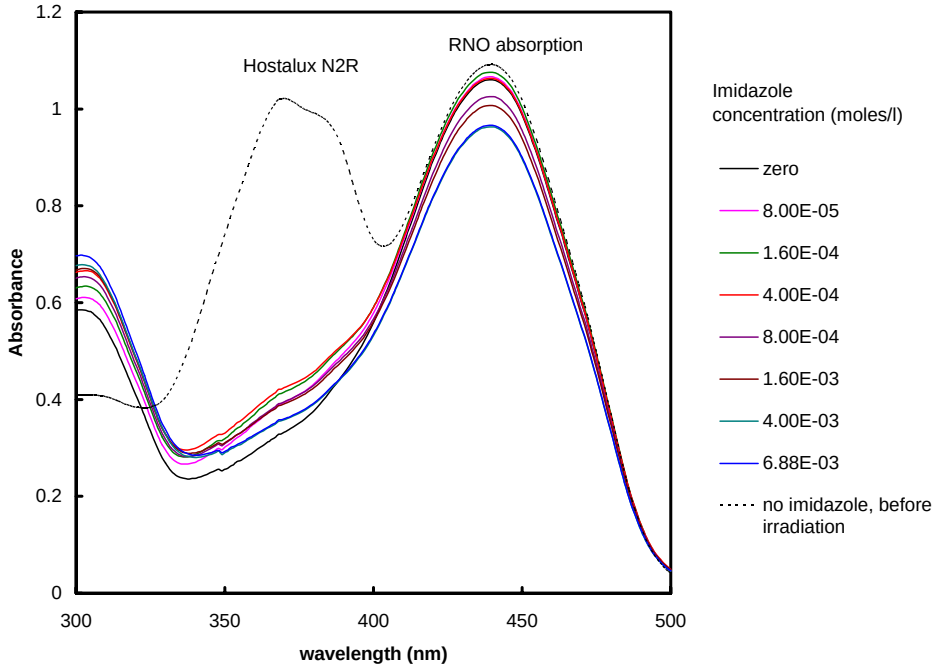


Fig. 8. UV-Vis spectra of Hostalux N2R and RNO absorptions after irradiation at 366 nm at various imidazole concentrations.

and the increased levels of H_2O_2 formed in the presence of SOD (Fig. 3) suggest that the H_2O_2 is produced by dismutation of the superoxide anion as shown in Scheme 3. The fact that no $^1O_2$ is produced by irradiated FWA solutions is further evidence for an electron transfer rather than an energy transfer mechanism. The reactions shown in Scheme 2 are certainly one possibility, but a direct Type I mechanism involving photoionisation of the excited singlet state of the FWA is also possible (Scheme 5).

This mechanism is consistent with previous laser flash photolysis work on Uvitex NFW by Smit and Ghiggino [29] which suggested the formation of semi-oxidized radical cations at 354 nm excitation.

The results presented here clearly demonstrate the formation of superoxide and hydrogen peroxide from irradiated FWAs, and not singlet oxygen. Therefore the energy transfer mechanism previously proposed by Nicholls [8] and more recently by Auer and Pailthorpe [30], suggesting that the FWA excited triplet reacts with ground state oxygen to produce singlet oxygen, (Scheme 1) is considered far less likely than an electron transfer mechanism (Schemes 2 and 4).

$$D + h\nu \rightarrow {}^1D^* \quad (12)$$

$$^1D^* \rightarrow D^{+\cdot} + e^- \quad (13)$$

$$e^- + O_2 \rightarrow O_2^{-\cdot} \quad (14)$$

$$O_2^{-\cdot} + H^+ \rightarrow {}^{\cdot}OOH \rightarrow H_2O_2 \quad (15)$$

Scheme 5. Electron transfer to oxygen via photoionisation of FWA (Type I) photooxidation.

According to a recent study by Santus et al. [31], the mechanism of superoxide production by irradiated aqueous tryptophan in direct or sensitized reactions involves photoionisation and release of a hydrated electron, followed by the loss of a proton from the Trp radical cation ($Trp^{+\bullet}$) to give a neutral radical (Scheme 6). The neutral Trp radicals react rapidly at the diffusion-controlled rate with superoxide to form oxidation products, but do not react with molecular oxygen on a millisecond timescale. Since Trp oxidation products have been shown to be major contributors to the yellow pigmentation produced in wool exposed to sunlight [3], the increased rate of H_2O_2 production by FWA/Trp mixtures observed in our study suggests that H_2O_2 and $O_2^{-\bullet}$ could be significant in the rapid photoyellowing of FWA-treated wool. If the rate of production of H_2O_2 and superoxide in wool textiles could somehow be reduced, this might offer a potential solution to this longstanding problem.

Table 1 shows that hydrogen peroxide is generated when wet wool fabrics are irradiated with simulated sunlight, which might well be expected from previous work showing

$$Trp + h\nu \rightarrow {}^1Trp^* \quad (16)$$

$$^1Trp^* \rightarrow Trp^{+\cdot} + e^- \quad (17)$$

$$e^- + O_2 \rightarrow O_2^{-\cdot} \quad (18)$$

$$Trp^{+\cdot} + H_2O \rightarrow Trp^{\cdot} + H_3O^+ \quad (19)$$

Scheme 6. Superoxide generation by photoionisation of tryptophan [31].

the generation of hydroxyl radicals by irradiated wool [10]. However earlier work using a less sensitive peroxide assay [12] had only been able to detect H_2O_2 in FWA-treated wool. For FWA-treated wool, the increased rate of H_2O_2 generation by a factor of four relative to bleached wool strongly suggests that that photogeneration of H_2O_2 and superoxide on wool is involved in the photoyellowing mechanism, at least under wet conditions. It is interesting to note that the pre-yellowed FWA-treated wool produced the highest H_2O_2 concentration, showing that not only the FWA, but also the yellow chromophores formed in irradiated wool, are capable of photogenerating peroxide in sunlight. This finding suggests that any successful treatment developed to limit the rate of photoyellowing of FWA-treated wool would not only need to reduce the rate of formation of H_2O_2 and superoxide by the FWA, but also prevent the formation of any yellow chromophores from the amino acid residues responsible in wool.

5. Conclusions

It has been demonstrated that aqueous solutions of several commercial FWAs from the three main classes (stilbenes, pyrazolines and coumarins) produce both the superoxide anion and H_2O_2 and not singlet oxygen on exposure to UVA radiation. The formation of superoxide suggests that an electron transfer process from the FWA to oxygen is the dominant mechanism. This probably occurs either via formation of semi-reduced and semi-oxidized dye radicals as shown in Scheme 2, or via a direct Type I mechanism involving photoionisation of the excited singlet state of the FWA (Scheme 5).

The increased rate of H_2O_2 photogeneration by a mixture of aqueous tryptophan and FWA exposed to UVA light suggests that this could be an important factor in understanding the rapid photoyellowing of FWA-treated wool and silk. The fact that peroxide is formed during irradiation of FWAs in solution and at an increased rate on wet FWA-treated wool fabric suggests that H_2O_2 and superoxide, rather than singlet oxygen, are involved in the mechanism of photoyellowing of FWA-treated wool and silk.

Acknowledgements

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