Introduction

Industrially, with regard to the bleaching of cellulose based materials, the high annual volume of paper and raw cotton production makes the later process of substantial economic and environmental importance. Contemporary bleaching processes for raw cotton employ chlorine based oxidants such as sodium hypochlorite (NaOCl) to bleach undesired pigments by either increasing their solubility in water or reducing their ability to absorb (visible) light. However, since the early 1990s efforts have been directed towards “chlorine free” bleaching processes in particular with respect to avoiding the formation of dioxins. Atom efficient terminal oxidants such as dioxygen, hydrogen peroxide and ozone, whose primary degradation products are O₂ and H₂O, are ideal in this regard. A drawback of these oxidants is that they require activation due to their low intrinsic reactivity. H₂O₂, in particular, is used increasingly as a bleaching agent and while it is environmentally friendly, it requires temperatures in excess of 70-90 °C to achieve sufficient bleaching performance. Currently, industrial raw cotton bleaching makes use of aqueous alkaline media primarily, with the “bleach solution / wash mix” typically at pH 11 or higher, with hydrogen peroxide as oxidant and temperatures in the range of 70-90 °C. The bleaching process is also carried out with surfactants, to remove hydrophobic components such as waxes and long chain fatty acids, present in the cotton fibres.

Transition metal complexes, containing manganese, iron or cobalt (ions), activate H₂O₂ and O₂ and enhance stain removal at lower temperatures and at a reduced chemical cost. In the early 1990s, several detergent companies patented a range of manganese, iron and cobalt complexes for this purpose. The complex [Mn³⁺Mn⁴⁺(μ-O)(μ-CH₃COO)(Me₄dtne)][PF₆]₂ (1) (where Me₄dtne = 1,2-bis(4,7-dimethyl-1,4,7-triazacyclonon-1-yl)ethane), reported first by Wieghardt et al., was patented by Unilever together with analogous catalysts for laundry bleaching in the early 1990s (Figure 2). Since then, manganese triazacyclononane based catalysts have been employed in the bleaching of raw cotton and wood pulp with H₂O₂. The effectiveness of 1 has, however, been shown to be highly dependent on the conditions used (e.g., pH, temperature, H₂O₂ and catalyst concentration), with optimum activity observed for 1 at pH > 10 and at >40 °C. Over the last decade, mechanistic studies on manganese catalysed oxidations in organic solvents for fine chemical syntheses has provided a considerable body of data on the mode of action of these catalysts and a broad understanding of the role of additives in such catalytic systems. Furthermore, these Mn tmtacn catalysts have been used for oxidation of organic substrates, such as the epoxidation of alkenes. However, extending these mechanistic insights to understanding the behaviour of these catalysts in aqueous media under complex heterogeneous conditions, for example, cotton and pulp bleaching, is limited due to the lack of studies under such conditions. A key challenge is to understand how these catalysts achieve such high activity and how other reaction components interact with the catalysts to enhance or inhibit activity. However, the complex mixture of substrates and also their heterogeneity make direct study of bleaching reactions highly challenging. The use of model substrates which are stable in the reaction medium, thus providing a homogenous system, is therefore essential in such studies, to enable the elucidation of the mode of action of these catalysts.
The pigments responsible for the brown coloration of natural cotton include polyphenols, flavones and tannins, with flavonoid compounds being the primary source of colouration. Therefore the development of new methods for bleaching has targeted these substrate classes primarily. It is interesting to note that the pigments in other substrates which require bleaching, i.e. paper pulp and domestic laundry, and dishwashing applications, contain chemically similar compounds, lignin in wood pulp and tea and wine stains. In these processes peracids (e.g., peracetic acid) are often employed in conjunction with H₂O₂ and provide for increased bleaching activity at 50-60 °C, however, at temperatures lower than 40 °C sufficient stain bleaching cannot be achieved and the cost and waste generated means that peracids are less preferred.

The chemical structure of flavonoids includes a C6-C3-C6 flavone skeleton where the three-carbon bridge is cyclised with oxygen and the aromatic rings bear hydroxyl substituents (Figure 1). Morin, which is used frequently as a model dye for first stage screening of potential laundry bleach catalysts, is a flavon-3-ol, bearing a OH group at the 3 position. It is also generally the most reactive of the model substrates and hence a question arises as to its actual suitability to model the reactivity of the more stable naturally occurring dyes.

**Figure 1** General structure and numbering scheme of flavonoids under investigation in this chapter.

However, morin introduces specific complications since it has been shown to react directly with O₂ in the presence of manganese catalysts of Me₃tacn. Such an effect has not been observed in actual raw cotton bleaching. Furthermore, the dependence of the absorption spectrum of morin in the pH range 8-11 increases the complexity in studying the effect of pH on catalyst activity due to both changes in spectral shape and its susceptibility to oxidation.
Topalovic et al. studied the oxidation of the flavonoid morin catalysed by the catalyst [MnIV\textsuperscript{IV}(μ-O\textsubscript{3}(tmtacn))\textsubscript{2}(PF\textsubscript{6})\textsubscript{2}]\textsubscript{2} (where tmtacn is 1,4,7-trimethyl-1,4,7-triazacyclononane) in an effort to understand the action of this catalyst in the bleaching of raw cotton.\textsuperscript{24,25} Morin was shown to undergo oxidation in bicarbonate solution with O\textsubscript{2} as the terminal oxidant, which was catalysed, following a lag phase, by 2. The lag phase was proposed to be due to slow activation of the catalyst through direct electron transfer between the substrate morin and 2, followed by reaction with O\textsubscript{2} either with the reduced form of the catalyst or with the oxidised morin.\textsuperscript{25} It was postulated that the presence of a hydroxyl group at the C-3 position is a key factor in the activity of the catalysts in the presence of oxygen. It was noted that flavonoids without the C-3 hydroxyl group (e.g. chrysin) were unreactive towards oxidation with 2 under the same reaction conditions with O\textsubscript{2} as terminal oxidant.\textsuperscript{24}

Chrysin (Figure 1) is structurally similar to morin, differing by the absence of a hydroxyl group at the 3 position of the C ring and has been the subject of studies related to biological processes and physical chemistry.\textsuperscript{26,27} It bears a double bond between C-2 and C-3 and ring B is coplanar with rings A and C, facilitating conjugation.

In this chapter, morin is shown to be unstable in bicarbonate solution when exposed to UV light even from a spectrophotometer, undergoing metal catalysed oxidative photodegradation, which complicates kinetic analyses in bleaching studies. Morin and its limitations as a model are discussed first followed by a discussion of chrysin as a more suitable model substrate to study the activity of catalysts used in bleaching reactions. It is shown that chrysin is a more suitable model for mechanistic studies in oxidations with catalysts, e.g., manganese complexes, with H\textsubscript{2}O\textsubscript{2} and circumvents complications encountered due to side reactions such as photochemistry and reactions with oxygen and reduces the complexity of analysis since it shows little pH sensitivity in the pH range of interest. In addition, the activity of catalyst 1 in the oxidation of chrysin at pH 10 and 11 and various temperature 23, 40 and 60 was studied under conditions relevant to bleaching.

**Results**

The UV/vis absorption spectra of flavonoids are characterised by two resolved absorption bands. The transition in the (near) visible region (300 to 550 nm) is attributed to the B ring and the shorter wavelength transition (240 to 285 nm) attributed to the A ring (Figure 3).\textsuperscript{28}

The Raman λ\textsubscript{exc} 785 nm and resonance Raman spectra λ\textsubscript{exc} 266 nm of chrysin in the solid state and in solution, respectively, are shown in Figure 3. Although similar, the spectra show expected differences in intensities of the bands but also certain bands are shifted indicating that the compound has a slightly different structure in solution compared to that in the solid state.
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**Figure 3** (left) UV/vis absorption spectra of morin (40 μM) (purple) and chrysin (blue) (40 μM) in NaHCO$_3$ (10 mM) at pH 11, pH was adjusted with NaOH(aq). (right) Chrysin (black), solid state spectrum, $\lambda_{exc}$ 785 nm, (red) 1 mM solution in H$_2$O, at $\lambda_{exc}$ 266 nm.

**pH dependence of the absorption spectra of morin and chrysin**

The UV/vis absorption spectra of phenols and flavonoids often show pronounced pH dependencies, manifested in changes in band shape, position and intensity. Morin bears five hydroxyl groups, three of which are relatively acidic ($pK_a$ 5.2, 8.2 and 9.9). A bathochromic shift of the longest wavelength absorption band of morin is observed as the pH is increased ($\lambda_{max}$ = 394 nm at pH 8.2, 404 nm at pH 9.4, 412 nm at pH 10.2 and 417 nm at pH 11.4). By contrast, chrysin, in which the hydroxyl groups at C5 and C7 of the A-ring, have $pK_a$ values of 8.0 and 11.9, respectively, does not exhibit significant changes in its UV/vis absorption spectrum over the pH range of interest in the present study (i.e. pH 8.2 to 11.4, Figure 4).

**Figure 4** pH dependence of the absorption spectra of a) Morin and b) chrysin [40 μM] in NaHCO$_3$ [10 mM] at pH 8.2 (black), 9.4 (red), 10.2 (blue) and 11.4 (green).

**Photochemical stability of morin in the present of O$_2$ and metal ions**

An additional, and often overlooked complication in the use of flavones as model compounds in spectroscopic studies is their well-known photochemistry, together with the ability of hydroxyaromatic compounds in general and flavonoids in particular to react rapidly with $^1$O$_2$. Indeed, Garcia et al. have demonstrated recently the
photodecomposition of the flavonoids, quercetin, morin and rutin (Figure 1), under aerobic conditions.\textsuperscript{32} The oxidation of morin can be and is often monitored by UV/vis absorption spectroscopy. However, the propensity for morin to undergo photochemically driven degradation is such that the light used during monitoring can itself induce substantial oxidation. Indeed the difference in conversion when monitored continuously by UV/vis absorption spectroscopy (45 \%) and when spectra were recorded only at 0 and 300 min (< 11 \%) is apparent (Figure 5).

![Figure 5](image1.png)

\textbf{Figure 5} (left) UV/vis absorption spectra of Morin [400 $\mu$M] in NaHCO$_3$ [10 mM], pH 10.2, (1 mm path length), I) Initial spectrum, $t = 0$, II) spectrum after 5 h (sample held in the dark), III) After continuous online monitoring (spectra was obtained every 30 s for 5 h) (right) change in absorption over time under continuous spectral acquisition and after 5 h for a sample held in dark (shown as a red square). The dashed line indicates the initial absorbance.

The 45\% decrease in visible absorbance is consistent with the concentration of dioxygen in solution (200-350 $\mu$M) limiting conversion under these conditions (Figure 6).

![Figure 6](image2.png)

\textbf{Figure 6} Change in concentration of morin (a) [400 $\mu$M], and b) [40 $\mu$M], in NaHCO$_3$ [10 mM], pH 10.2. Spectra recorded every 30 s for 5 h. Note that the initial absorbance is the same in each case due to the use of 1 mm and 10 mm path length cuvettes, respectively.

Importantly solutions of morin, that had been purged with argon prior to monitoring by UV/vis absorption spectroscopy, showed no changes even with extended irradiation.
The underlying mechanism/s involved in the photoinduced oxidation of morin was examined further. Even without a deliberately added catalyst, morin underwent slow photochemical degradation, which is suppressed substantially by the addition of the sequestrant DTPA (diethylene-triamine-pentaacetic acid) (5 μM), indicating that even trace metal ions present are directly involved in the observed photochemistry under aqueous conditions (Figure 7 and Figure 8), and that the combination of metal ions and oxygen together with irradiation are necessary to observe oxidation of morin.

**Figure 7** Morin [40 μM] in NaHCO₃ [10 mM], pH 10.2, without the sequestrant DTPA (red), and with sequestrant DTPA (5 μM), (black). Spectra obtained at 30 s intervals over 5 h.

**Figure 8** Change in absorption (at maximum absorbance) of morin with continuous irradiation in the presence of DTPA at pH 8.2 (blue), 9.4 (red), 10.2 (green) and 11.4 (purple). Conditions: Morin [40 μM], 1 [1 μM], DTPA [5 μM], NaHCO₃ [10 mM], oxygen as terminal oxidant. Right: full spectra shown for pH 10.

Addition of 1 (1 μM) to an air equilibrated solution of morin at several pHs between 8.0 and 11.5 resulted in a decrease in absorbance at 412 nm with a concomitant increase in absorbance at 321 nm. The reaction rate showed a pronounced dependence on pH with the rate increasing with increasing pH (Figure 9 and Figure 10). The initial spectra are different at each pH examined. The absorption bands a, b and c are indicated in Figure 9. Two isosbestic points, at 287 and 370 nm, were maintained for most of the reaction, suggesting the initial formation of a single oxidation product in which the chromophoric system is disrupted, e.g. oxidation at the
flavone double bond. At later times the isosbestic points were lost and the band at 321 nm underwent a red shift to 331 nm together with a decrease in absorbance. These data indicate that the primary product had reacted further albeit not-necessarily undergoing further oxidation but rather hydrolysis.

**Figure 9** UV/vis spectra of morin (40 μM), 1 [1 μM], over time (spectra taken at 50s intervals for 30 min, for clarity only the spectra at 100s intervals are shown) at pH 11.4, in NaHCO₃ [10 mM], 23 °C.

**Figure 10** Change in absorbance with time at a (275 nm), b (316 nm), and c (406 nm), at pH 8.2 (blue), 9.4 (red), 10.2 (green) and 11.4 (purple). Morin [40 μM], 1 [1 μM], NaHCO₃ [10 mM], 23 °C, oxygen [200-350 μM] as terminal oxidant.

In comparison, the oxidation of morin in the presence of 1 at pH 10 and 11 upon addition of H₂O₂ is faster than with O₂ is the terminal oxidant. At pH 11 conversion was complete within ~ 10 min, compared to pH 10 for which it was only complete after ~30 min (Figure 11).
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**Figure 11** Absorbance at $\lambda_{\text{max}}$ 412 and 417 nm vs time in the oxidation of morin with H$_2$O$_2$ as terminal oxidant at pH = 10 (blue) and at pH = 11 (red). Conditions: morin [40 µM], 1 [1 µM], H$_2$O$_2$ [200 µM], NaHCO$_3$ [10 mM], 23 °C.

Hence, under conditions relevant to bleaching, morin is shown, even in the absence of H$_2$O$_2$ and 1, to undergo oxidation provided that oxygen, traces of metal ions and UV/vis irradiation are available. The absence of any one of these components, as exemplified by the lack of oxidation when irradiated under argon or in the presence of a sequestrant.

**Photochemical stability of Chrysin**

In contrast, little or no change to the absorption of resonance Raman spectrum of chrysin was observed even when under continuous visible and UV (266 nm) irradiation in the presence of O$_2$ (Figure 12).

**Figure 12** (left) UV/vis absorption spectrum of chrysin [400 µM] in NaHCO$_3$ [10 mM], pH 10.2. Initial spectrum t = 0 (solid line), spectra were obtained at 30 s intervals over 5 h (dotted line) and taken at t = 5 h (dashed line) e). (right) Resonance Raman ($\lambda$,exc 266 nm) spectrum of chrysin [1 mM] in H$_2$O at pH 10.2, under continuous monitoring for 3 h, Spectra shown at t = 0 (blue) and t = 3 h (red).

Furthermore chrysin does not show significant changes in its UV/vis absorption spectrum under air, neither at pH 10 nor 11, in the presence of 1, in contrast to morin which undergoes oxidation under the same conditions (Figure 13).
Figure 13 Change in absorbance at 359 nm of chrysin [40 μM] (red at pH 10.2 and blue at pH 11.0) and morin (purple at pH 10.2 and green at pH 11.0) over time in air equilibrated solution with 1 [1 μM], in aqueous NaHCO₃ [10 mM] at 23 °C.

Continuous monitoring of a mixture of chrysin and morin (10:1) by UV/vis absorption spectroscopy under air with 1 (Figure 14) shows changes (Figure 15) which are consistent with oxidation of the morin present only.

Figure 14 Change in the UV/vis absorption spectra of chrysin [40 μM], (left) in the presence and (right) absence of 0.1 equiv of morin at pH 11.4 and 1 [1 μM], in air equilibrated aqueous NaHCO₃ [10 mM], at 23 °C.

Figure 15 (Left) UV/vis absorption difference spectra corresponding to Figure 14. (right) Absorption at 450 nm over time.
Oxidation of chrysin with H$_2$O$_2$ catalysed by 1

A peculiar feature of catalyst 1 is that it is stable and retains its catalytic activity in oxidations with H$_2$O$_2$ even at high pH (pH > 11). In chapter 3, it was shown that at pH > 10, the acetato ligand of 1 dissociates partly and/or fully to form 1a and/or 1b, respectively (Scheme 1). The structural changes provide exchangeable coordination sites for coordination of H$_2$O$_2$. It was also noted that addition of carboxylates, such as acetate or carbonate (e.g., dissolved CO$_2$ from atmosphere) resulted in reversion to a carboxylate bridged complex (e.g., 1d) and a decrease in activity (Scheme 1).

Scheme 1 Equilibrium between 1 and the acetate dissociated forms (1a and 1b) present at high pH and possible structure of hydroperoxy intermediate (1c) formed at high pH.

Raw cotton bleaching with H$_2$O$_2$ is carried out at elevated temperatures (~70 to 95 °C), and hence catalytic oxidation of chrysin was investigated in water between 23 and 60 °C. The stability of chrysin towards oxidation with oxygen even under UV irradiation and in the presence of 1, allows oxidation with H$_2$O$_2$ to be studied spectroscopically at pH 10 and 11 (Figure 16 and Figure 17).

Figure 16 Changes in the UV/vis absorption spectrum of chrysin (40 μM) at pH 11, with 1 [1 μM], in aqueous NaHCO$_3$ [10 mM], with H$_2$O$_2$ [200 μM], 23 °C. Spectra are at 50 s intervals.
Figure 17 Absorbance at 359 nm vs time showing the extent of oxidation of chrysin with O$_2$ or H$_2$O$_2$: at pH = 10 (left), at pH = 11 (right), (I) under air, (II) with H$_2$O$_2$. Conditions: chrysin [40 µM], catalyst [1 µM], H$_2$O$_2$ [200 µM], in aqueous NaHCO$_3$ [10 mM], at 23 °C.

The rate of oxidation of chrysin increased with increase in temperature (Figure 18). In all cases the highest activity was observed at pH 11. In the absence of catalyst, chrysin was stable at room temperature and degraded relatively slowly at higher temperatures (Figure 18 and Figure 19). These data correspond to data obtained under industrial bleaching conditions with 1 and H$_2$O$_2$.$^6$

Figure 18 Change in absorption at 358 nm for chrysin [40 µM] with H$_2$O$_2$ [10 mM] in H$_2$O at (left) pH 10, right) pH 11, without catalyst, at 23°C (black), 40°C (red), 60°C (blue), with 1 [1 µM], 23°C (green), 40°C (pink), 60 °C (orange).

Figure 19 (right) Maximum rate of the reaction (ΔAbs s$^{-1}$) and (left) conversion of Chrysins [40 µM] with H$_2$O$_2$ [10 mM], 1 [1 µM] in H$_2$O at 23, 40 and 60 °C. At pH = 10 (blue) pH =11 (red).
Discussion

The photo degradation of morin in water and aqueous bicarbonate solution under irradiation with UV light from a spectrometer together with its oxidation with $O_2$ and the pH dependence of its UV/vis absorption spectrum limits its applicability as a model compound in the study of bleaching in basic solutions. In the present study, for morin $O_2$ is shown to be the terminal oxidant in the presence of metal ions (whether manganese catalysts, Mn$^{II}$ salts or trace metal ions) and under UV/vis irradiation. This reactivity is in stark contrast to raw cotton which does not undergo oxidation with oxygen alone and requires high temperatures and $H_2O_2$ to undergo bleaching \textit{vide infra}. The background reaction of morin in bicarbonate buffer resulted in 11 % conversion (in the dark) whereas monitoring resulted in a 45 % conversion. This increase in conversion is due to photo-bleaching, in the presence of metal ions present in the reaction mixture, as a consequence of reaction monitoring. The data indicate that morin is susceptible to photo-degradation and reaction with $O_2$, which complicates kinetic analyses. This photo-bleaching was not observed for chrysin where the online vs. offline spectra are identical. The proposed mechanism based on data obtained in the current study is summarized in scheme 2.

Scheme 2 Proposed mechanism for light induced degradation of morin catalysed by metal ions with $O_2$. A central question is as to the mechanism by which oxygen is activated in the oxidation of morin. When held in the dark some degradation of morin is still observed suggesting that light is accelerating the process. The complete absence of activity observed when oxygen was excluded confirms that oxygen is the terminal oxidant. Furthermore, metal ions must be available for activity to be observed with the sequestrant DTPA inhibiting the reaction. Given that transition metal ions can react with $O_2$ albeit more slowly than $^1O_2$, the effect of irradiation can be viewed as an accelerating factor rather than an essential component. However, the mechanism by which the reaction proceeds requires that electrons are available to reduce oxygen, at least to superoxide.

Topalovic \textit{et al.}, investigated the potential intermediacy of superoxide $O_2^-$ and/or $H_2O_2$ in the oxidation of morin by $O_2$ catalyzed by 2. The reaction rate was reduced by the presence of superoxide dismutase and catalase enzymes. It was suggested that formation of superoxide by electron transfer from morin to $O_2$ occurred, following by dismutation of superoxide to hydrogen peroxide.

Montana \textit{et al.}, have described the antioxidant properties of several flavonoids, including quercetin, morin and rutin, and their activity against reactive oxygen species (ROS) that were generated by visible light irradiation of riboflavin (which can generate...
singlet oxygen) in methanol. Morin was found to be the most reactive with around 80% of the $^{1}$O$_{2}$-morin collisions resulting in oxidation. The authors reasoned that the high activity of morin and O$_{2}(^{1}A_g)$ was due to the presence of a phenolate structure under the conditions employed, which is absent in the case of the other flavonoids studied, and differences in the B-ring, especially the presence of an OH group in the 7-position in morin. Hydroxy-substituents increase the nucleophilicity of the aromatic structure towards the electrophilic $^{1}$O$_{2}$.

Agrawal et al. noted that the hydroxyl groups of chrysin were deprotonated in the order 7-OH > 5-OH. The hydroxyl groups of the structurally related quercetin were also shown to be deprotonated in the order 7-OH > 4'-OH > 5-OH. Musialik et al. reported that, with the exception of morin, all compounds in their study with a 7-OH group have a similar acidity and this group is the most acidic, regardless of the hydroxylation pattern. Matsuura et al. noted the photosensitized oxygenation of 3-hydroxyflavones previously and that methylation at the 3-hydroxy group resulted in a loss of photosensitivity. Furthermore, no reaction took place with visible irradiation in the absence of rose bengal.

In a study by Colombini et al. two degradation pathways for morin were proposed: one involving metal ions and the other involving generation of singlet oxygen.

![Degradation pathways for morin with O$_{2}$ proposed by Colombini et al.](image)

In the case of chrysin, which does not bear a hydroxyl group at the 3 position of the C ring nor in the B ring, oxidation by O$_{2}$ was not observed. Morin exhibits two activating, i.e. electron donating, OH substituents. Indeed at high pH, the hydroxyl group is deprotonated making it even more susceptible to oxidation. As a consequence of having fewer hydroxyl moieties, chrysin is less nucleophilic and a less favourable substrate for an oxidising species such as $^{1}$O$_{2}$. So, in contrast to morin, bleaching of chrysin was only observed when H$_{2}$O$_{2}$ and Mn-tmtacn were used and occurred faster at higher pH and elevated temperatures. As mentioned above, the UV/vis absorption
The spectrum of morin is highly dependent on pH, in contrast to chrysin which shows negligible changes in its absorption over the pH range of interest to this study. It was reported by Hage et al. that 1 shows higher activity at high pH in the bleaching of cotton. However, at higher pH the rate of decomposition of \( \text{H}_2\text{O}_2 \) increases, which reduces overall bleaching activity. In raw cotton bleaching, 1 provides best result at low concentrations at high pH and at elevated temperatures. The rate of the reaction of Morin and chrysin with 1 were compared at high pH (10-11). The behaviour of chrysin (i.e. only bleaching observed in presence of \( \text{H}_2\text{O}_2 \) and a catalyst and not by \( \text{O}_2 \) highlights it as a more relevant model compound in regard to bleaching process rather than morin under same conditions.

**Conclusions**

In this chapter the suitability of chrysin as a model compound for homogenous reactions used in mechanistic studies relating to raw cotton bleaching catalysed by 1 is demonstrated. The use of morin as a model compound is fundamentally flawed due to its photochemistry and sensitivity to oxygen not observed for the colourants present in natural fibres. Even exposure of morin to UV/vis irradiation during monitoring of the progress of morin oxidation is enough to induce photochemical degradation when at least traces of metal ions are present. Furthermore, the complexity of the interactions between morin with oxygen and metal ions was not recognized previously when used as model substrate for raw cotton bleaching. Oxidation with metal ions and \( \text{O}_2 \) introduces complexity as oxidation with \( \text{O}_2 \) proceeds at a similar rate as with \( \text{H}_2\text{O}_2 \). Obtaining mechanistic information regarding oxidative processes is therefore difficult due to the inherent instability of morin in aqueous media and its photochemical bleaching. The data presented here indicates that chrysin is sufficiently stable and requires \( \text{H}_2\text{O}_2 \) and elevated temperatures for oxidation to occur. In addition, chrysin is amenable to reaction monitoring by UV resonance Raman as well as UV/vis absorption spectroscopy.

In conclusion, we have shown that morin is an inappropriate compound used as a model in cotton bleaching as it is subject to oxidation by reactive oxygen species and metal ion under online monitoring. In contrast the reactivity of chrysin is consistent with that observed with cotton.

**Experimental**

**Materials**

\[ \text{[Mn}_{\text{II,IV}}(\text{O})_2(\text{CH}_3\text{COO})(\text{Me}_4\text{dtne})](\text{PF}_6)_2 \] (1) Elemental analysis (calc. for \( \text{Mn}_2\text{C}_{20}\text{H}_{43}\text{N}_6\text{O}_4\text{P}_2\text{F}_{12} \): C 28.89 % (28.89 %), H 5.26 % (5.21 %), N 10.11 % (10.11 %). was available from earlier studies and provided by Catexel BV (Leiden, The Netherlands). MnSO\(_4\) was obtained from Fluka. Morin, chrysin, sodium hydroxide and sodium bicarbonate were purchased from Sigma Aldrich. Hydrogen peroxide (50% w/w in H\(_2\)O) was obtained from Acros Organics. All chemicals were used as obtained without further purification. Doubly distilled water was used unless stated otherwise.
UV/vis absorption spectra were recorded with a HP8453 spectrophotometer or a Specord600 (AnalytikJena) in stoppered 1 cm pathlength quartz cuvettes unless stated otherwise. The pH was determined using a Hanna Instruments pH 211 microprocessor pH meter previously calibrated with standard buffer solutions at 4.01 and 7.01.

Oxidation reactions were performed in 1 cm path length quartz cuvettes (Hellma) unless stated otherwise. Bicarbonate buffered solutions of morin and chrysin were prepared freshly and the pH was adjusted with NaOH or H$_2$SO$_4$ (1 M) before and, where necessary, after addition of H$_2$O$_2$. All reactions were performed, at least, in triplicate at room temperature (23 ± 2 °C) and under air unless stated otherwise. pH was measured at the end of the reactions also and in all cases there were no significant changes. Initial rates of reaction were obtained by fitting the linear portion of the plot of absorbance vs time and the slope of this best fit line is reported as the maximum rate.

**General method for oxidation of morin and chrysin catalysed by 1 with air.** The pH of a freshly prepared solution of morin (40 µM) in NaHCO$_3$(aq) was adjusted as necessary using NaOH (1M) or H$_2$SO$_4$ (10 %). 25 µL of freshly prepared solution of the catalyst (100 µM) was added directly to a cuvette containing 2.5 mL of morin (40 µM).

**General method for oxidation of morin and chrysin catalysed by 1 with H$_2$O$_2$.** The pH of a freshly prepared solution of morin (40 µM) in NaHCO$_3$(aq) was adjusted as necessary. Hydrogen peroxide was added to the cuvette containing morin, (200 µM, 5 eq. with respect to substrate), followed immediately by the addition of catalyst (1 µM).

**General method for oxidation of morin catalysed by 1 with O$_2$ or H$_2$O$_2$ in presence of Na$_5$DTPA (Pentasodium diethylene triamine pentaacetate).** The pH of a freshly prepared solution of morin (40 µM) in NaHCO$_3$(aq) was adjusted as necessary. Na$_5$DTPA (5 µM) was added to a cuvette containing morin. H$_2$O$_2$ (1 eq. with respect to substrate) was added to a cuvette containing morin (40 µM) and Na$_5$DTPA followed immediately by the addition of catalyst (1 µM).

**References**

5. O$_3$ is more reactive but is itself an environmental pollutant and is expensive to produce.
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It is clear that, in terms of colour, from the spectra that chrysin is also not an ideal model for the colourants in raw cotton, as at > 400 nm there is hardly any absorption, whilst raw cotton has a yellow-brown colour.
