

A Perspective on the Photofading of Organic Colorants

 Anthony Harriman 

Molecular Photonics Laboratory, School of Natural & Environmental Sciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK; anthony.harriman@ncl.ac.uk

Abstract: This perspective presents an account of the underlying features associated with the photofading of organic colorants. Photofading is commonly known to the scientific community as photodegradation or photooxidation, while in earlier times the more grandiose term “light fastness” was commonplace. This is a subject of immense diversity and significance, but there are many challenges to be faced when attempting mechanistic reasoning. The text is illustrated by descriptions of several systems taken from the scientific literature, together with anecdotes related to the principal researchers. The chemical challenges to be overcome in order to design photostable materials are outlined and reference is made to the natural world. It is stressed that the journal *Colorants* would welcome submissions in this field.

Keywords: photochemistry; bleaching; autooxidation

1. Introduction

This is the first of several Editorial Perspectives introducing research topics of direct relevance to the journal *Colorants*. The style allows for personal observations in addition to hard science. The term “photofading” has many alternatives, such as “photobleaching” or “photodegradation”, but these are rather negative and fail to register the fact that photofading can be both useful and desirable. An acceptable alternative is “light fastness”, but this has fallen out of favour in recent years. It was with light fastness that I started my own endeavours in the subject when, immediately upon leaving secondary school, I obtained a job as a junior researcher in the Industrial Research Laboratory of a local paint manufacturer. One of my duties was to monitor the state of painted panels exposed to the atmosphere on the roof of the building. This responsibility was taken very seriously, and I designed a numerical grading scheme that simplified life for the senior researchers in the laboratory. Not bad for a sixteen-year-old with no knowledge of organic chemistry. Even so, I felt the seniors were greatly relieved when, a few years later, I left the Company to undertake a PhD; the topic being the design of photodegradable materials.

There are very few photofading reactions where the bleaching mechanism is known unequivocally, mainly due to the fact that the reactions are slow and the steady-state concentration of reactive intermediates is correspondingly close to zero. This was not the case with certain anthraquinone dyes that were found to fade quickly in sunlight and to damage the underlying fabric. The reaction mechanism was exposed by Bridge and Porter in 1958, and published in the Proceedings of the Royal Society, using the recently introduced flash photolysis technique. It was observed that many common quinones formed the triplet-excited state via intersystem crossing and that the triplet abstracted a hydrogen atom from any suitable medium, including cotton. The resultant free radicals could initiate a chain reaction, resulting in loss of colouration and destruction of the surroundings. The intermediate radicals were detected and identified by transient absorption spectroscopy. A spin-off from this research was the recognition that certain 1-hydroxyanthraquinone derivations underwent extremely fast intramolecular hydrogen abstraction, by-passing the intermolecular reaction, and were highly photostable. Some years later, when I was fortunate enough to join George Porter’s research group at the Royal Institution of Great



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Britain, I used the same flash photolysis instrument. I joined the group on a two-year contract with the understanding I would stay for one year before looking for a permanent placement. I stayed for fourteen years.

One of the many visitors to the Royal Institution was Jan Verhoeven from the University of Amsterdam. Jan was a pioneer of through-bond charge transfer at a time when the approved mechanism involved a through-space process. Convincing a sceptical audience about the merits of a through-bond mechanism was a hard sell at the time, but this is now universally accepted. Jan was also instrumental in solving a major problem associated with the photobleaching of colorants under the microscope. It is well known that many dyes fade quickly under high power microscopes and it was soon recognised that the triplet-excited state was an important intermediate. The mechanism, however, was unknown, and singlet molecular oxygen was frequently blamed for degradation of the dye. Detailed examination of the reaction led to the realisation that the critical reaction involved electron transfer between triplet-excited and ground-state molecules of the dye, work published in the *Biophysics Journal* in 1996. Reverse electron transfer is intercepted by reaction of the radical anion with molecular oxygen, leading to peroxy-radical formation and a chain reaction. This reaction is ubiquitous and is involved in many diverse photobleaching processes. In contrast, singlet molecular oxygen, although highly damaging in certain cases such as cyanine dyes, is relatively innocent.

A third example of a breakthrough discovery was reported by Karlsson et al. who described the photobleaching of Erythrosine, a popular food dye, in air-saturated water [1]. The rate of fading was shown to be highly sensitive to the experimental conditions, such as O₂ concentration and light intensity. The critical reaction step involved geminate attack on a ground-state molecule of Erythrosine by singlet molecular oxygen, for which the reaction followed first-order kinetics. In turn, this realisation allowed determination of the activation energy for the bleaching step. This is one of the simplest photofading reactions, with the rate doubling in D₂O relative to H₂O, which makes an ideal undergraduate laboratory practical. Amazingly, it proved possible to measure the formal quantum yield for photofading—a rare occurrence—using an automated LED-based setup. Even so, at higher dye concentrations, auto-catalysis could be observed [1]. Incidentally, Joshua Karlsson, who is now an independent researcher at Tampere University, is in the process of establishing a research group to further explore photofading mechanisms.

2. Lessons from Nature

Photosynthesis provides much of the inspiration for photochemical research and continues to afford new mechanisms and unexpected reaction intermediates. Nature takes great pains to eliminate triplet-excited states, usually by incorporating suitable quenchers, and to circumvent the consequences of singlet-singlet annihilation. The latter could provide access to unstable intermediates via electron transfer reactions. It is also clear that the architecture of the photosynthetic reaction centre complex has been designed to ensure rapid electronic energy transfer over relatively long distances. The importance of this latter point is that no single pigment molecule exists in an excited state for longer than a picosecond or so. Since photofading tends to be quite slow, it simply cannot compete with electronic energy transfer. This is an important lesson and has been used by Alamiry et al. to design an elaborate multi-component molecular array wherein rapid electronic energy transfer occurs along a thermodynamic gradient [2]. The lowest-energy acceptor is the only chromophore that is susceptible to bleaching and, under continuous illumination, this component slowly degrades. In turn, this shortens the energy transfer pathway and increases the excited-state lifetime of the new terminal chromophore. The latter now starts to fade under illumination. This cycle is continued until much of the array has been destroyed [2].

A common problem inherent to many artificial colorants involves auto-catalysis, whereby a photochemical product catalyses further degradation of the chromophore. Such processes have been seen in numerous cases and can be quite spectacular. For example,

Woodford et al. observed that certain symmetrical pyrrole-BF₂ (BOPHY) derivatives were quite stable over more than 100 h of continuous illumination before suddenly beginning to fade [3]. Further illumination caused rapid loss of colour due to the onset of auto-catalysis. A related process occurs during the photocatalysed bleaching of indigo in solution [4]. Clearly, such behaviour makes it impossible to determine a quantum yield for photofading and renders prediction of longevity a hazardous process. Interestingly, autocatalysis might play an important role in natural photosynthesis by promoting the well-known colour changes seen at autumn time. Nature, it would appear, has found out how to make good use of undesirable reactions but has successfully hidden the secret code.

Certain natural processes have evolved in such a manner as to incorporate self-repair mechanisms that stabilise the organism against photodegradation. This realisation has led to the introduction of self-healing dyes, which are crude mimics of the sophisticated natural machinery but did not need 2.3 billion years to perfect. These self-healing chromophores have been developed primarily for use in super-resolution microscopy and comprise a fluorescent dye covalently linked to a photostabiliser moiety, such as cyclooctatetraene. The latter quenches any accidentally formed triplet state before damage can occur. Similar reaction cycles can be invoked to intercept oxidized or reduced fluorophore before the onset of secondary reactions can bleach the dye. There are now many such molecular dyads and triads that can operate in this manner and ensure improved performance for super-resolution microscopy. This is a vibrant research topic and many novel systems are likely to hit the market in the near future.

Photosynthesis faces a great challenge from climate change, which is faster than evolution. Already, we are seeing the need for farmers to reconsider which crops can be grown while there is an ever-growing need to eliminate wastage of water. We might expect similar changes in photostability for artificial materials and colorants that demand adaptations in procedure. It will be interesting to see how Mother Nature responds to this upheaval but evolution might be too slow for us to learn new tricks. We have to anticipate what will be the effects of increased exposure to sunlight on local surroundings.

3. Inhibition of Photofading

Various additives can reduce the effects of photofading and retain the inherent colour for significantly longer periods. Such additives can be generic, as in the form of anti-oxidants employed to prevent photodegradation of sunscreens, or specific for a particular class of colorants. A common feature for this latter type of inhibitor is complexation between the two reagents, which shortens the excited-state lifetime and/or curtails intermolecular reactions with adventitious quenchers. A nice example of such behaviour concerns the effects of urea on the photobleaching of Methylene Blue in aqueous solution [5]. In the absence of urea, Methylene Blue undergoes photobleaching via intermediate formation of singlet molecular oxygen, followed by geminate attack to form the leuco-dye. Urea blocks the site where geminate attack occurs and thereby stabilises the dye [5]. A similar situation arises during the photofading of pyrrole-BF₂ complexes in solution [3]. Here, for example, the rate of photobleaching is decreased by a factor of 2000-fold in toluene relative to cyclohexane. The key stabilising step involves formation of a weak complex between the dye and toluene. William McFarlane, who sadly died earlier this year, was able to deduce the geometry of the complex using high resolution NMR spectroscopy. Incidentally, this work provides clear evidence that the rate of photobleaching in solution depends on the fractional contribution of polar resonance forms of the colorant [3]. Covalent attachment of the inhibitor directly onto the scaffold of the colorant allows far greater control over the reaction profile and minimises the amount of inhibitor needed to stabilise the dye. This strategy does not always work as expected and, on occasion, the inhibitor can be converted into an activator during photolysis. In the extreme case, rather than form an intermolecular complex, the chromophore can be encapsulated in a three-dimensional cage, thereby seriously hindering attack by radical species.

In the field of opto-electronics, it is essential that the materials are highly photostable. This requires careful engineering of the entire product to optimise performance and minimise degradation. As in natural photosynthesis, there is a need to eliminate any long-lived singlet excitons and triplet states, to avoid oxygen and UV light, and to minimise losses through exciton annihilation. In other areas of everyday life, photobleaching cannot be eradicated completely. This is the case with cosmetic products, such as hair dyes and sunscreens, which are exposed to high levels of sunshine for prolonged periods. This is also the case with art works and, in particular, paintings. Preventing the discoloration of oil paintings is a massive problem that needs innovative solutions [6].

4. Applications of Photofading

It is a tribute to the resourcefulness of the research community that applications have been found for photofading, although the most popular application relates to the fading of new indigo-dyed jeans to make them appear old. A more scientific use has been the development of Fluorescence Recovery After Photobleaching (FRAP) which allows determination of the diffusion coefficient of fluorescent reagents in solution. The FRAP process, which is now installed on commercial instruments, was developed primarily by Webb in the 1970s. The basic operating principle is that a strong laser pulse is used to bleach a small volume of solution observed under a fluorescence microscope, and subsequently, the kinetics are measured for the partial recover of emission as fresh fluorophore enters the reaction volume. A major success for this protocol has been the detailed investigation of protein binding within the membrane. A spin-off has been the development of Fluorescence Loss in Photobleaching (FLIP), which uses confocal fluorescence microscopy to monitor movement of fluorescent-labelled proteins inside cells and membranes. A related application lies in the arena of super-resolution microscopy where transient photobleaching of a strong fluorophore allows detection beyond the diffraction limit. Indeed, direct stochastic optical reconstruction microscopy (*d*STORM) makes use of fluorescent-labelled antibodies or chemical tags for subdiffraction resolution fluorescence imaging with a lateral resolution of ~20 nm. Before the experiment starts, the sample is strongly fluorescent, but the fluorophore is transformed into a dark state by in situ illumination with a strong laser pulse of visible light. The dark state remains for around one second or so before a small subset is reconverted to the original state with a second excitation pulse, such that their location can be resolved with high precision. There are now numerous alternatives to the *d*STORM technique, with several relying on the bleaching of fluorescent labels.

Away from advanced microscopy, photofading of chromophores has been used for such diverse applications as contrast enhanced photolithography, diagnosis, and imaging guided surgery, and the in situ preparation of photo-therapeutic agents. The latter protocol works via the injection of a coloured but benign reagent that can be converted into a cytotoxic material under far-red illumination [7]. This type of photoactivation strategy was initiated by Kirpal Gulliya at the Baylor Medical Center in Dallas using Merocyanine 540. At the other extreme of the application range, Woodford has introduced an elaborate chemical actinometer for measuring exposure to sunlight [8]. Here, the target compound is a member of the symmetric pyrrole-BF₂ family suitably modified for absorption at long wavelength. Under illumination, the compound bleaches to form a stable material that absorbs at higher energy. Both the original compound and its photoproduct exhibit strong fluorescence when dispersed in a plastic film. Radiometric measurements using fluorescence and absorption allow accurate determination of the sunlight exposure history.

5. Research Challenges

The problems caused by photofading of colorants have been evident since the onset of history, although much has been learned and there is now widespread appreciation of which colorants should be avoided. The fundamental challenge that remains is to understand the bleaching mechanism in sufficient detail to be able to predict how and when bleaching will occur. The difficulty associated with exposing the reaction mechanism

depends on the application for the colorant of interest. Thus, the type of photobleaching inherent to super-resolution fluorescence microscopy involves high quantum yields such that the reaction intermediates should be visible by transient spectroscopy. Usually, these intermediates are believed to be radical ions formed by sacrificial light-induced electron transfer between the fluorophore and materials present in the buffer. There is, however, a scarcity of spectroscopic information covering the properties and spectral features of these radical ions that can be used to identify intermediates in the bleaching event. This situation has led Lisovskaya et al. to characterise the radical ions formed from certain cyanine dyes using the technique of pulse radiolysis [9]. Karlsson et al. [1], on the other hand, have gone so far as to suggest that the bleaching intermediate is a photoisomer. It is only by characterising the reaction intermediates that these photosystems will be fully optimised for advanced microscopy.

The elimination of photobleaching is a bigger challenge, and progress in this field has resulted largely from engineering of the device rather than through chemistry. The problem here relates to the very slow reaction, which means the steady-state concentration of intermediates is too low for conventional analysis. It can take years, even decades, before the damage is visible; this is certainly the case with paintings and prints. It is also clear that auto-catalysis is far more common than previously thought, and this adds a further difficulty for establishing a reaction mechanism. Singlet molecular oxygen is often blamed but rarely with real justification. Examining very slow reactions is considerably more difficult than the study of very fast reactions and requires great insight. Hopefully, more researchers will be attracted to this topic in the very near future.

6. Conclusions

Editorial perspectives present personal but authoritative viewpoints on contemporary research topics of particular interest to the journal *Colorants*. They should present a brief overview of the subject, with key references, and provide a forward-looking commentary on where a particular research area is heading and where new insight is needed. The articles reflect the authors' own research interests and should highlight matters that fit nicely into the scope of the journal.

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