

## Long lived hydroxyl radical generation in water by ultrafast laser irradiation

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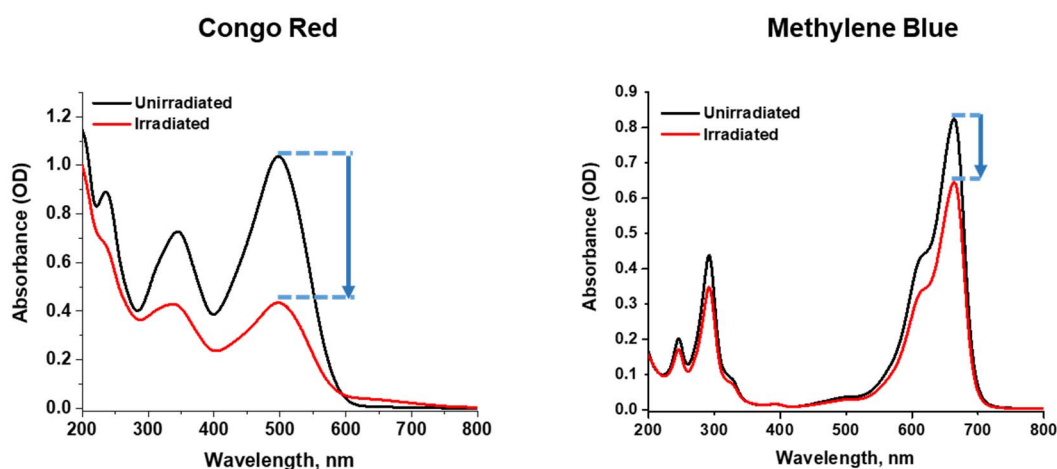
### 1. Introduction

Water, which is the most common liquid on earth, is also one most enigmatic. The fundamental understanding of its interaction with radiation and matter has been of vital interest for many disciplines. The ultrashort laser pulses have attracted a lot of research attention owing to its plausible applications in life sciences and surgery [1, 2]. The interaction of ultrashort laser pulses with water is known to generate free electrons, radical and reactive oxygen species by multiphoton absorption or tunnel induced ionization [2, 3]. Consequence of this, radiation-induced damage to DNA maintained under physiological conditions [4-7] have been observed using intense ultrashort laser pulses. This idea can be used to target specific diseased cells during laser based surgery or therapy. It has been proposed that due to better focusing of ultrashort laser pulses than attainable with standard radiation sources, damage to healthy tissue might be minimized [6]. Moreover, it has been observed that intense femtosecond laser pulses at 800 nm might yield similar radiation dosage in water as obtained using gamma-radiation [8].

The generation of radicals in water is known to progress through an ultrafast cation radical generation followed by proton abstraction by the surrounding solvent cluster. Many groups have attempted to characterize the cation radical of water by using pulse radiolysis or photolysis measurements [9] through indirect means of oxidizing a metal or other anions since the lifetime is too short to be directly observed. The subsequent step involving the proton abstraction and formation of the hydroxyl radical is relatively slower but produces a biologically relevant reactive intermediate. Thus, understanding the dynamics and quantification of these radicals becomes very crucial. Here, we use two common dyes, Congo red and Methylene blue to probe the changes in optical density subsequent to generation of radicals in water by ultrafast laser centered at 800 nm of 40 fs pulse duration. We observed that the generated radicals are long lived for duration of ten seconds and estimate the concentration.

### 2. Experimental Section

The output of a Ti:sapphire laser which generated 40 fs pulses of 800 nm with 1 kHz repetition rate was used for irradiation of water [4]. For all experiments the volume in the cuvette (path length of 10 mm) was 600  $\mu$ L. Deionized (Millipore) water was used in all our experiments and making the dye solutions.

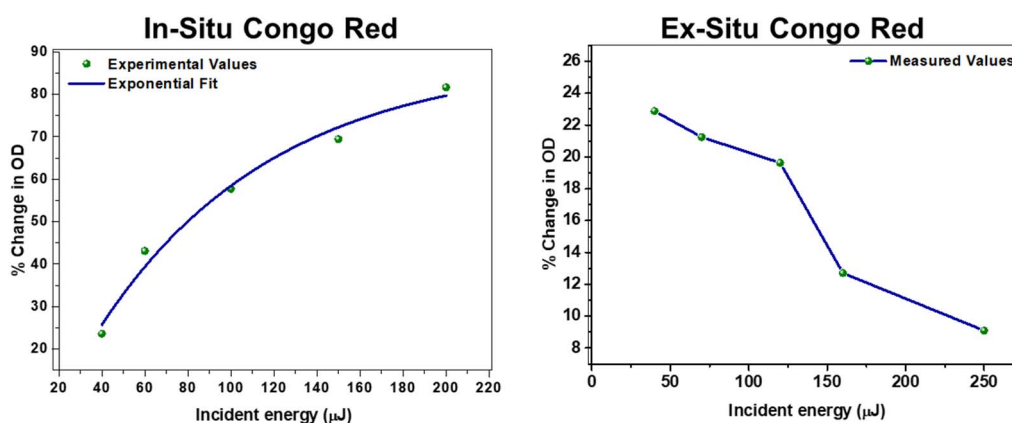


**Figure 1.** Change in OD monitored for the visible bands of Congo Red (CR) for *in-situ* and Methylene Blue (MB) for *ex-situ* irradiation.

Congo Red Dye (CR) and Methylene Blue dye (MB) were obtained from Sigma-Aldrich (Both certified biological stain standard). AR (>99%) grade Sodium Acetate, obtained from SD Fine- Chemicals Ltd, was used to make a 0.4M solution in water. The experiments performed can be broadly classified into two sets: *In-situ* and *Ex-situ*. *In-situ* experiments are when the dye is present in water during irradiation and *ex-situ* is when the dye is added 10 seconds after irradiation. In all *in-situ* experiments, the initial concentration of the dye was 50 ppm (CR: 9  $\mu$ M and MB: 18  $\mu$ M) and in all *ex-situ* experiments 10  $\mu$ L of 1 mM of the dye solution was added to 600  $\mu$ L of water after irradiation (reduced concentration was 16.4  $\mu$ M). The samples were irradiated for 3 minutes (180 sec) unless mentioned otherwise. For all experiments, the change in absorbance (OD) in the visible region (498 nm for CR and 664 nm for MB, figure 1) was monitored using a JASCO V-670 UV/VIS spectrophotometer using a quartz cuvette with a path length of 10 mm. Mass spectral analyses were done with an Electrospray Ionization (ESI) probe having both positive and negative ion modes in a liquid chromatography mass spectrometer (LCMS-2020, Shimadzu Corp.).

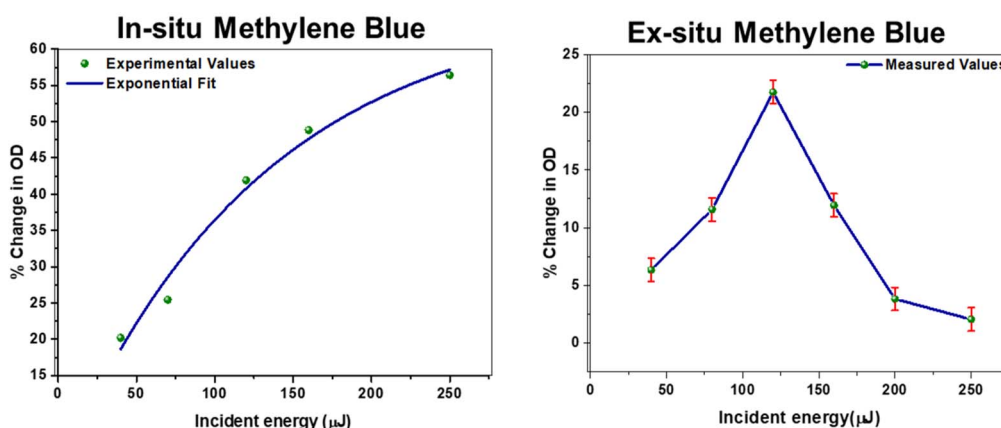
### 3. Results and Discussion

Figure 2 shows the change in OD upon irradiation using pulses of different incident energies. *In-situ* irradiation of CR shows an exponential dependence with pulse energy. It is worth noting that this change in OD can be attributed to the interaction of excited dye with water (multiphoton absorption in the dye) and the interaction of unexcited dye with hydroxyl radicals evolved as a result of ionization of water or both. To separate the effects of the multiphoton absorption process of water and multiphoton absorption process of the dye, *ex-situ* probing was performed. Although CR shows an overall decreasing trend, there is clear indication of two different regimes. CR degradation is slow up to an incident energy of 120  $\mu$ J following which a steep change is observed. We can explain the presence of two distinct pathways by looking at the factors that affect the overall population of the solvated hydroxyl radicals. With increase in energy there is an increase in the number of hydroxyl radicals generated by the plasma. Previously, it has also been observed that the formation of bubbles that sets up a flow pattern in water [4], which causes increased radical-radical annihilation. This can result in lesser number of effective radicals surviving for longer timescales.



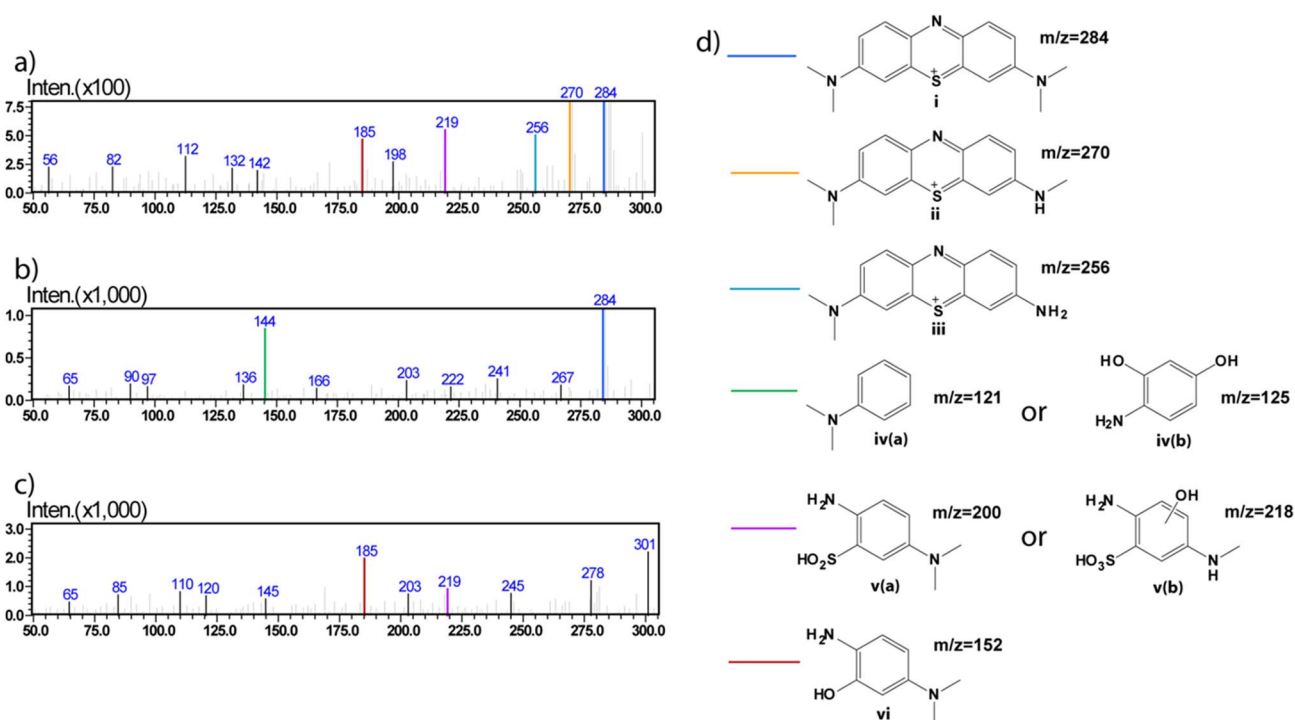
**Figure 2.** Energy dependence of the change in OD for Congo Red

The mechanism of dye degradation by the radicals occurs due to two major pathways; chromophore degradation and auxochrome degradation. Auxochromes are secondary functional groups for example the dimethylamino group for methylene blue (Figure 4d). Chromophore degradation is the main reason for the decrease in OD during dye degradation. In previously studied degradation pathways of CR, auxochrome degradation is followed by the fragmentation of the chromophore [10]. Due to the formation of closely resembling products initially in the case of CR, there is very little change in the OD values. This, along with the sheer number and complexity in the identification of products formed, lead us to the conclusion that CR is not giving an accurate quantitative response to the number of radicals generated. This could be because of the large size of the molecule opens up multiple points of attack even for auxochrome and chromophore degradation. Since methylene blue is known to undergo a chromophore and auxochrome degradation in an equally facile manner and it is a smaller molecule, we carried out similar experiments with it. For *in-situ* irradiation we see the trend is similar to CR (Figure 3). *Ex-situ* experiments showed an increasing trend followed by a decrease. The agreement between the hypothesis of different dominating factors determining amount of radicals present above and below 120  $\mu$ J irradiation energy and the experimental results, demonstrated the quantitative response of methylene blue dye.



**Figure 3.** Energy dependence of the change in OD for Methylene Blue

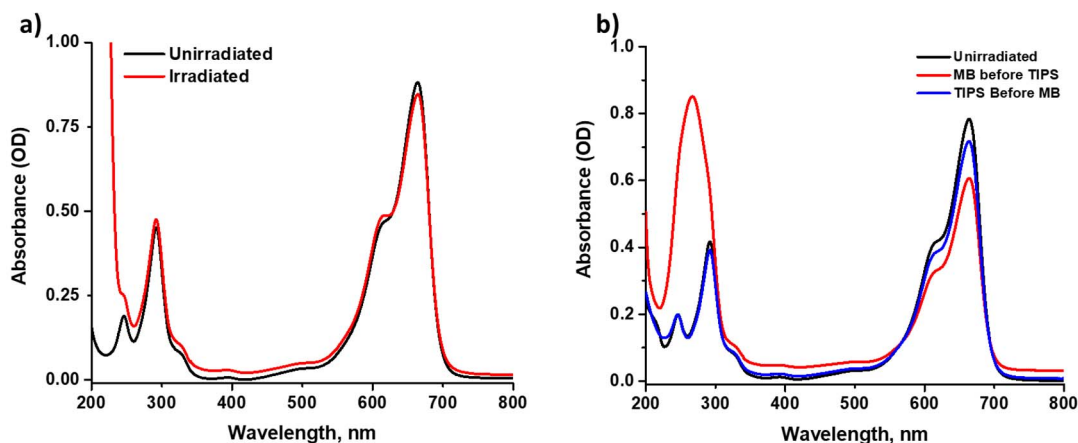
Figure 4 shows products of degradation for *ex-situ* experiment with methylene blue for different energies that were identified using ESI-MS. Most of the products were similar to the ones identified by generating  $\text{OH}$  using  $\text{TiO}_2$  nanocatalyst in water and UV light [11]. Incidentally, at lower energies we can see a large distribution of products that are attributed to both pathways of degradation. In figure 4d (i is the non-degraded dye, ii and iii result from auxochrome degradation while the others result from chromophore degradation (iv(b) and v(b) result from a combination of both)), compounds labelled i, ii, iii are observed with their own masses since they are already charged. The peak corresponding to 144 could arise from common adducts of two compounds, i.e.  $[\text{iv(a)}+\text{Na}^+]$  or  $[\text{iv(b)}+\text{H}_2\text{O}+\text{H}^+]$ . The peak corresponding to 219 can also arise from the adducts of  $[\text{v(a)}+\text{H}_2\text{O}+\text{H}^+]$  or  $[\text{v(b)}+\text{H}^+]$ . The mass 185 is obtained from the adduct of  $[\text{vi}+\text{MeOH}+\text{H}^+]$ .



**Figure 4.** Mass Spectra of *ex-situ* irradiation of Methylene Blue. Incident energies are (a) 40  $\mu\text{J}$ ; (b) 120  $\mu\text{J}$ ; (c) 200  $\mu\text{J}$ ; (d) Color key for identified products

The existence of long lived radicals has already been shown by Koyama et. al. [12] with respect to biological systems, however these radicals were produced by X-ray irradiation in cells and trapped within DNA complexes but these were not identified as hydroxyl radicals. To verify the nature of these long lived species as radicals and not ions, we introduced well known radical scavengers such as sodium acetate ( $\text{NaOAc}$ ) and Tri-Isopropyl Silane (TIPS) in water. Sodium acetate was used as an *in-situ* probe for CR and it showed 22-35% less degradation for varying proportions  $\text{NaOAc}$  solution and water when compared with irradiating a solution without sodium acetate. A test for *ex-situ* experiments was also carried out by irradiating water with

NaOAc solution (1:1 by volume) and then adding MB dye. The solution which was irradiated at 120 $\mu$ J showed almost no degradation as all the radicals were scavenged. In a similar method, water was irradiated and then TIPS and methylene blue we added successively within 5 seconds of time span (Figure 5). It is seen that when TIPS is added before MB, there is almost no degradation (<10%) whereas if MB is added before TIPS we see a similar degradation to what is seen for *ex-situ* MB experiments (~25% as seen in Figure 2). This result along with the presence of hydroxylated fragments of MB dye identified in the mass spectra, shows that the reactive species formed are hydroxyl radicals.



**Figure 5.** *Ex-situ* control experiments for Methylene Blue. (a) Irradiation of NaOAc and H<sub>2</sub>O followed by MB addition; (b) *Ex-situ* addition of TIPS and MB

The amount of radicals that are present in the solution for *ex-situ* experiments with MB can be estimated using Beer-Lambert's law, since the absorption is still in the linear regime. This can be given by:

$$N = \frac{\Delta OD}{\epsilon_{664}} \times 0.0006 \times N_A \times k$$

Where  $N$  is the calculated amount of radicals in solution,  $\Delta OD$  is the change in absorbance at 664 nm,  $\epsilon$  is the absorption coefficient, and  $N_A$  is the Avogadro number. Different numbers of hydroxyl radicals are involved in the degradation of the dye at different powers. To account for this, a weightage factor, ' $k$ ', is applied, which is a weighted average of the number of radicals involved in the formation of those products. Although a rough estimate, it shows a presence  $10^{14}$ - $10^{15}$  ( $\mu$ M concentration range) hydroxyl radicals present in water at the 10s mark. Although efforts are being made towards more accurate quantification of the amount of radicals present, there is agreement between our result and Chin et. al. [3], when they estimated the concentration of H<sub>2</sub>O<sub>2</sub> which was produced by the annihilation of  $\dot{O}H$  radicals after irradiation of water with femtosecond lasers.

## 4. Conclusions

We have shown the generation of long lived radicals (up to 10s) can be achieved by irradiation of water using intense ultrashort laser pulses. *Ex-situ* detection of these radicals showed that the amount of radicals generated are higher for lower incident energy of the laser. We have also shown the *ex-situ* degradation of these dyes is reversible to an extent by the use of known radical scavengers like sodium acetate and TIPS. Although the generation of these radicals is on ultrafast time scales, their stabilization within the solvent seems to be different from the way we think about reactive intermediate stabilization in water conventionally. We believe that our results will be of utility for ultrafast laser based applications in life sciences.

## 5. Acknowledgements

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