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Photodynamic Cancer Therapy
A Study of the Photolysis Reaction between Porphyrin and 9,10-Diphenylanthracene

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in
Chemistry

By
James Zachary Akins

Under the mentorship of Dr. Jim LoBue

Abstract

Photodynamic therapy (PDT) is a method of combating cancerous tumors. PDT works when certain molecules called photosensitizers absorb light and transfer that energy to dissolved oxygen in solution. Next, this singlet oxygen will interfere with nearby molecules that are necessary for cancer cells to function. This research focuses on the kinetics (rates) of the reaction with respect to the photosensitizer, light, and the target molecule. A laser set at 514nm was used in photolysis with the photosensitizer 5,10,15,20-Tetrakis(2,3,5,6-tetrafluorophenyl)porphyrin (TPP). To mimic cancer cells and monitor the effectiveness of the chosen photosensitizer, 9,10-Diphenylanthracene (DPA) was used as the target molecule. In knowing the kinetics of the reaction, practical things like the dosage of the photosensitizer can be better determined for PDT. In this research, consistent data was found that allows for the prediction of the decomposition of DPA when the concentrations of TPP and DPA and the intensity of the laser light are known. Also, using Carbon-13 NMR, the product of the photolysis was analyzed.

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I would also like to thank April Berlyoung for sharing chemical solutions, allowing me to mention her work in this paper, and having fun with me in the laboratory.

Introduction

Photodynamic Therapy (PDT) is a method that can be used to combat cancer using a light-sensitive drug to destroy tumors¹. This technique has been widely used for thirty years, but research is still necessary to improve the performance of the drugs. PDT only works when light and oxygen are present with the drug, and the treatment can be described in a few steps. First the drug is administered to a patient, and then that person is shielded from light before the therapy². Once the drug has accumulated on the cancer, a specific wavelength of light is directed onto the tumor. This activates the drug which then transfers its excitation energy to dissolved oxygen molecules to form excited singlet oxygen which will then kill tumor cells^{1,3}. Tumor specificity is highly important because if the drug becomes activated on non-cancerous tissue, unwanted side effects result⁴. Also, easily metabolized drugs are favored to reduce the longevity of sensitivity to light. Another important aspect of PDT is the penetration of the light waves into the body. Longer wavelengths are able to travel further into the skin; therefore, drugs that are activated at longer wavelengths are desirable⁵. PDT's drawbacks are that the therapy can only affect cancerous material on the external parts of the body, its current drugs are not as tumor specific as preferred, and it results in patients having easily damaged, light-sensitive skin.

Porphyrin is a photoactive molecule that is most studied for PDT⁶. In this research, porphyrin with substituted phenyl rings containing fluorine atoms is used as the light absorbing molecule. While other molecules were used, the molecule used most often was 5,10,15,20-Tetrakis(2,3,5,6-tetrafluorophenyl)porphyrin (TPP). Fluorine is substituted onto the phenyl groups mainly to test its influence. Since the fluorine atoms

seem to have no effect on rates of photolysis, it is useful to show that they can be attached to the porphyrin since fluorine improves metabolic stability and affects protein binding affinity⁷. Medicinal chemists in the future may find variation of the fluorine substitutions useful, but this research does not include any in vivo experiments. Porphyrin works as a catalyst to produce singlet oxygen.

It is known that cancer cells tend to be disrupted by singlet oxygen that is produced by photosensitizers, so in order to measure the effectiveness of a photosensitizer, any method of measuring the amount of dissolved singlet oxygen will do. In the following experiments, 9,10-diphenylanthracene (DPA) is used as the target molecule as it is sensitive to these excited oxygen molecules. Specifically, the absorption spectrum of DPA changes after it comes into contact with singlet oxygen. DPA normally has five absorbance peaks between 262nm and 394nm, but as soon as it interacts with singlet oxygen, these peaks disappear. This interaction forms 9,10-diphenylanthracene endoperoxide (DPA EPO) which has extremely low absorbance values, if any, since it has a significantly interrupted conjugated system⁸.

In this paper, multiple experiments were done. Early experiments were done to learn more about how concentrations affected the rate of photolysis and how much these solutions absorbed light so that desired concentrations could be found. After finding a suitable wavelength at which to photolyze, kinetics experiments were performed in order to determine the kinetic orders of the reactants. The orders were found with respect to TPP and DPA. How the power of the laser affects photolysis rate was studied, and lastly, evidence that an enderperoxide was formed was found using Carbon-13 NMR.

Experimental

Stock solutions were diluted to concentrations between 0.5mM and 100mM for most solutions. Most solutions used chloroform or 1,4-Dioxane as the solvent.

A Shimadzu 2401 PC Recording UV-Vis Spectrophotometer was the machine used to measure the absorbance of TPP and DPA. Data was collected between 200nm and 800nm. Clean quartz cuvettes were used for all experiments except for the NMR photolyses in which SOG (glass) cuvettes were used. Data from the UV-Vis was saved as a data print table and was later transferred to Excel.

To perform a photolysis experiment, a laser was used as the excitation source. The Coherent I90 Argon Ion laser was set at 514nm for all experiments in this paper, but other wavelengths like 488nm and 635nm were tested. The argon ion laser was typically set at 50 mWatts, but in the power study, this wattage was systematically varied. The photolysis experiments consisted of shining the laser through a cuvette containing a sample solution and recording UV-Vis spectra at regular intervals. C-13 NMR spectra were recorded for the DPA solution (approximately 1.09mM) and for the photolyzed solution. The solvent used during NMR was deuterated chloroform.

Data analysis was performed in Excel 2010. Using the solver function, coefficients of the spectra of pure samples of TPP and DPA were optimized by minimizing the sum of squares of the residual difference between linear combinations of these individual spectra and the spectra of the photolysis solution. In doing this, the percent contribution to peaks in the combined solution could be ascertained for TPP and DPA separately. This allowed for the quantification of the percent decrease of each molecule over the course of the photolysis.

Data and Discussion

Figure 1 through Figure 10 and Images 1 and 2 are included to explain this research. Understanding this data is important in understanding the rest of the study.

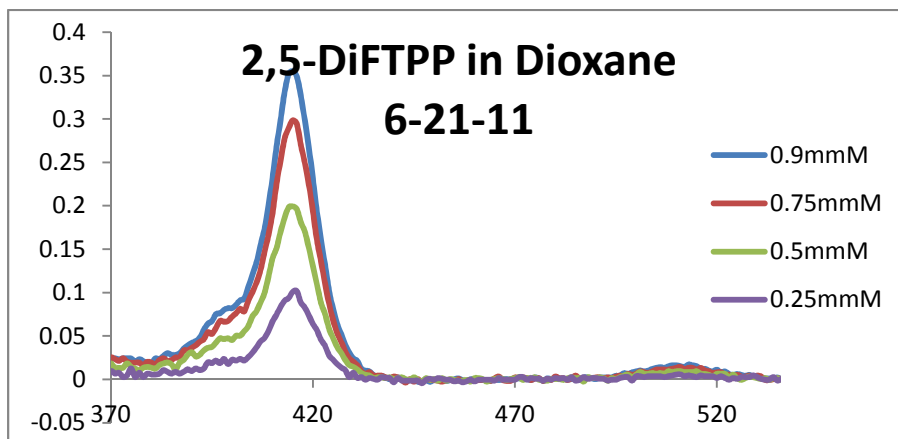


Figure 1. ABS vs. Wavelength for 2,5-DiFTPP in Dioxane at 4 concentrations

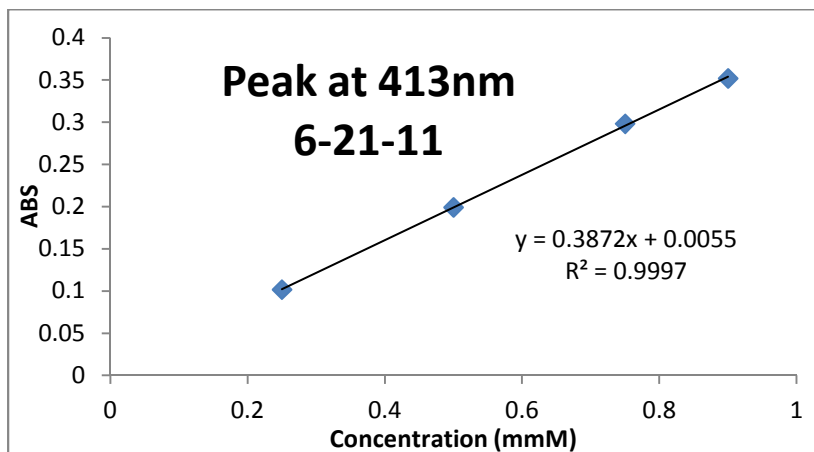


Figure 2. ABS vs. Conc - Beer's Law Plot of 2, 5 DiFTPP in Dioxane at 4 concentrations

Figure 1 is the spectrum of 2,5-DiFTPP at four different concentrations. These spectra show the peaks of porphyrin to be around 413nm and 507nm. The absorbance is known to be a result of the porphyrin ring and not of the groups substituted to it; although some substituents may change the wavelength absorbed, all substituents in the following

experiments did not. Another peak that is smaller than the other two is at 584nm but is not shown on the graph. Figure 2 is a graph of the absorbance value at the top of the peak at 413nm for the four concentrations. Since this graph is linear, it shows that Beer's Law holds true for the absorbance of this molecule. Beer's Law is used to determine the remaining percent of reactants in all following experiments.

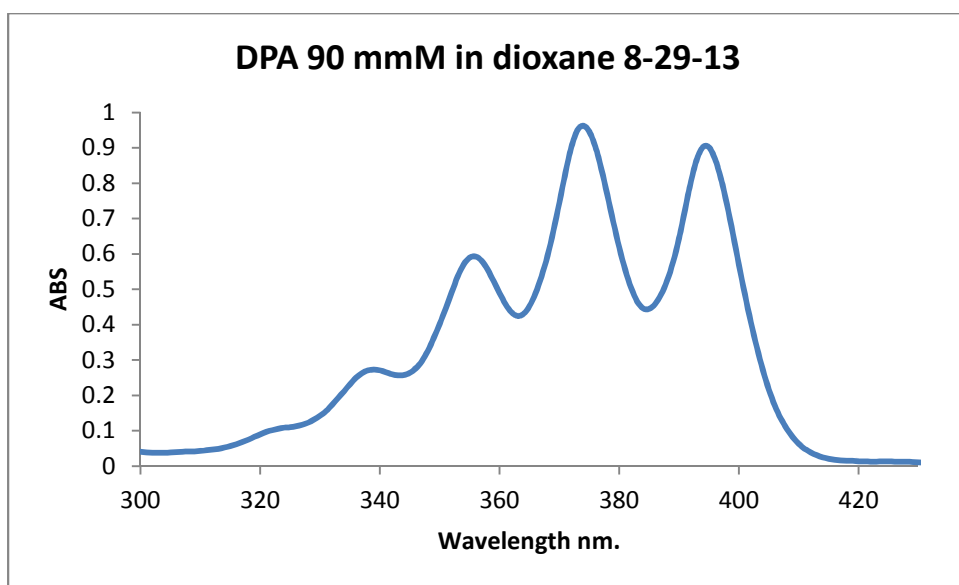


Figure 3. 9,10-Diphenylanthracene in Dioxane-high concentration

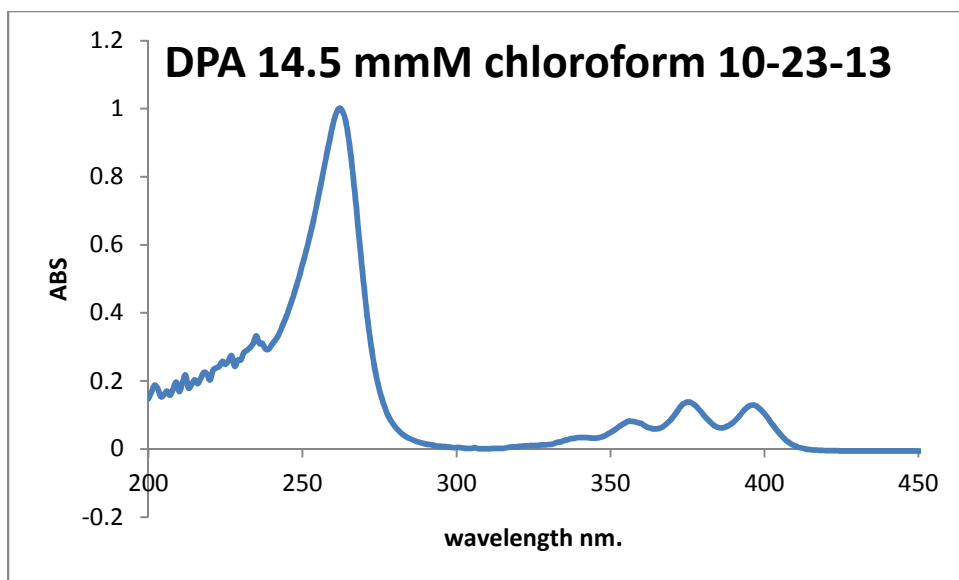


Figure 4. 9,10-Diphenylanthracene in Chloroform-low concentration

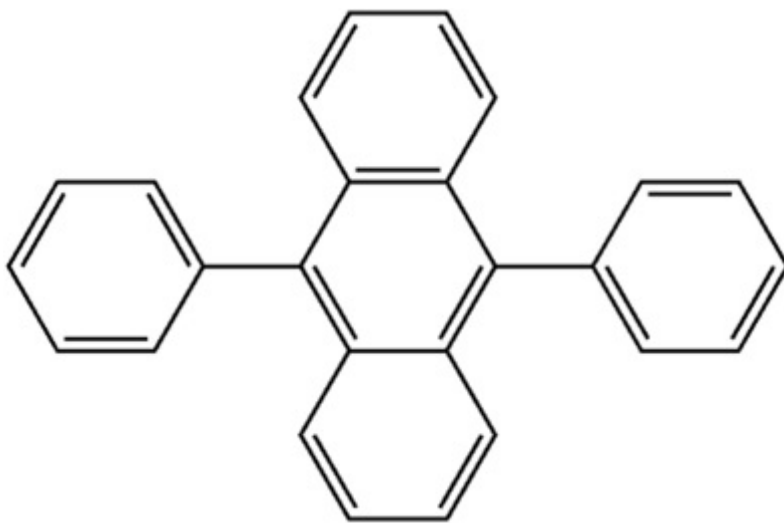


Image 1. 9,10-Diphenylanthracene (DPA) – Hydrogen atoms are left off for clarity

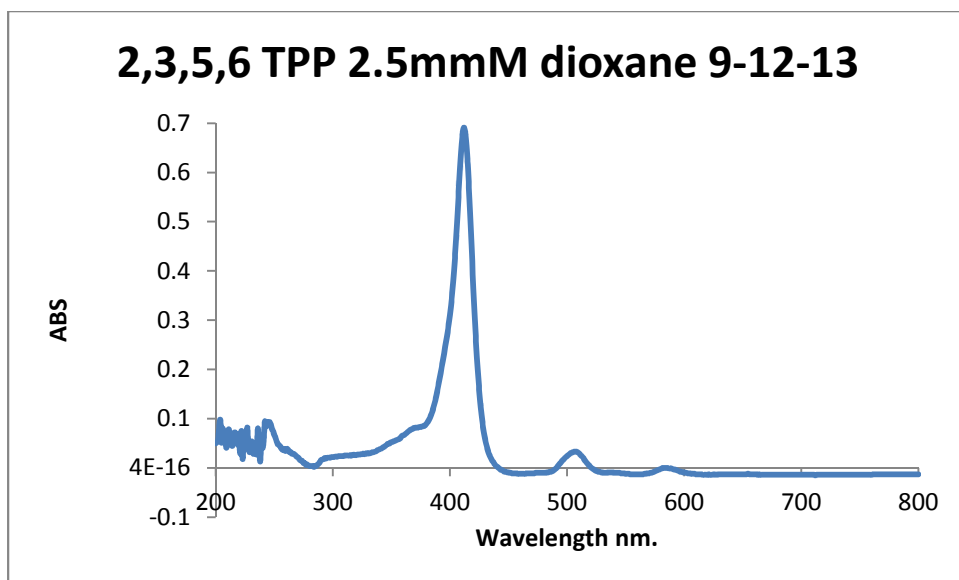


Figure 5. 5,10,15,20-Tetrakis(2,3,5,6-tetrafluorophenyl)porphyrin (TPP)

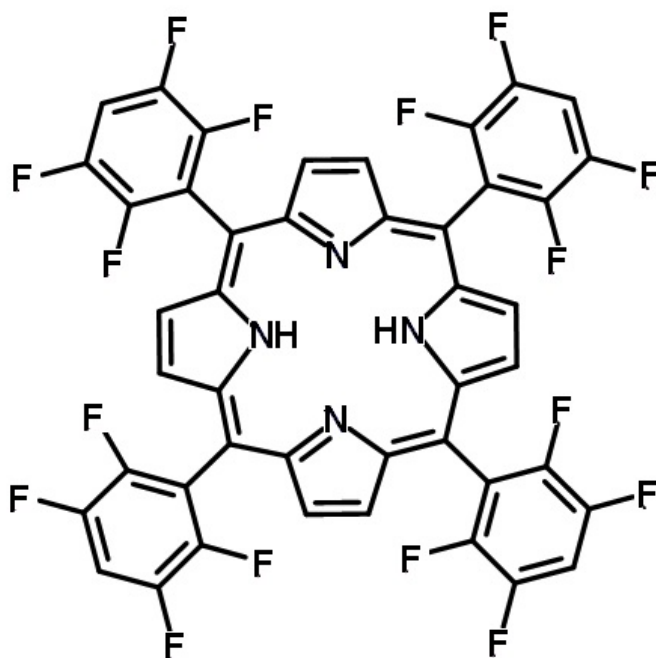


Image 2. 5,10,15,20-Tetrakis(2,3,5,6-tetrafluorophenyl)porphyrin (2,3,5,6 TPP)
Hydrogen atoms are left off for clarity

Figure 3 and Figure 4 are absorbance spectra of 9,10-Diphenylanthracene (DPA) in dioxane and chloroform respectively. The differing appearances of the spectra are not due to the solvent choice; instead, the difference stems from the change in concentration. The photolysis process involves radical electrons, and while chloroform could cause radicals in solution, it is not believed to be a part of the reaction. To rule out that the solvent is a part of the reaction, 1,4-Dioxane is used since it does not add radical electrons. Photolysis is successful in both of these solvents. Oxygen is a part of the photolysis reaction, and 1,4-Dioxane and chloroform have different oxygen solubilities that can affect the photolysis if the oxygen concentration depletes hours into a photolysis. In Figure 3, four peaks can be seen at 341nm, 357 nm, 375nm and 396nm. In Figure 4, which is at a much lower concentration, another peak can be accurately found at 262nm. Image 1 is of DPA. In Figure 5, although the baseline is slightly off, it can be seen that TPP has 3 peaks that occur at 413nm (the Soret band), 507nm and 584nm (the Q bands). This variation of porphyrin is 5,10,15,20-Tetrakis(2,3,5,6-tetrafluorophenyl)porphyrin (2,3,5,6 TPP) and is the main catalyst for photolysis used in the following experiments. 2,3,5,6 TPP can be seen in Image 2. DPA is the molecule used to indicate the effectiveness of the porphyrin in all of the following experiments.

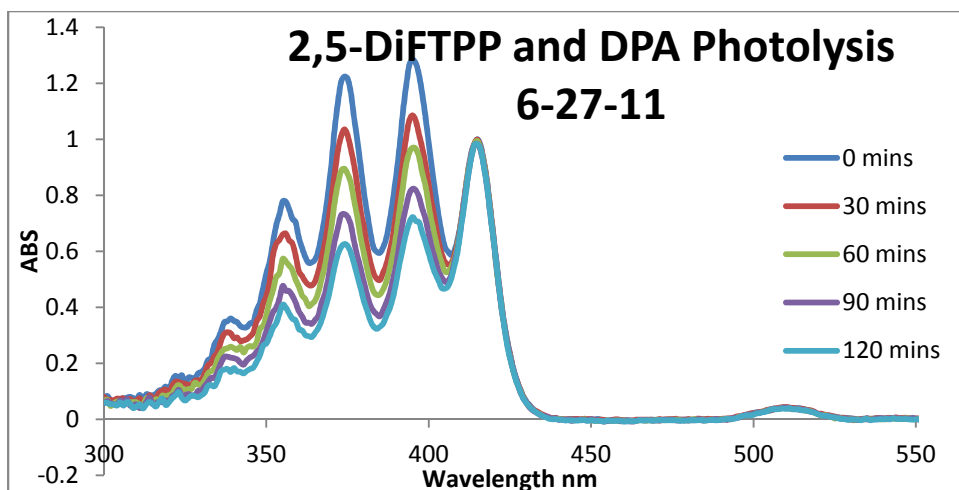


Figure 6. Photolysis of 2, 5 DiFTPP and DPA in Dioxane at 514nm

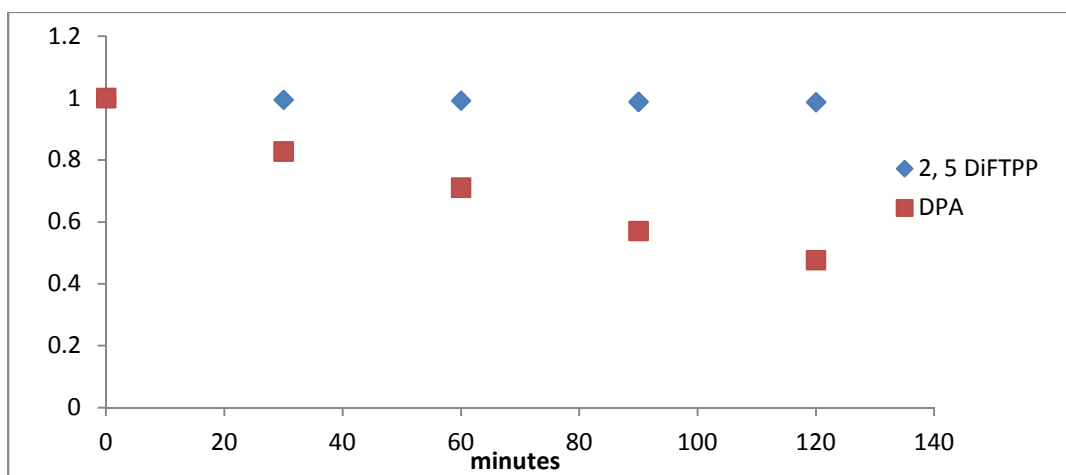


Figure 7. Percent vs. Minutes - Percent 2,5-DiFTPP and DPA remaining at a given time

Figure 6 is a photolysis with DPA and 2,5-DiFTPP. Concentrations are not the focus of this graph; instead, what species decrease in photolysis is the focus. It can be seen that the peaks at 341nm, 357 nm, 375nm and 396nm decrease over time, and it can be seen that the peaks at 413nm and 507nm do not decrease. This indicates that the DPA is decreasing in solution and that the TPP is remaining the same concentration. Figure 7 shows how the DPA (red squares) and TPP (blue diamonds) vary over time. Even though

all photolyses in this paper were done at 514nm, a photolysis can be done at any wavelength at which TPP absorbs light, but as mentioned before, longer wavelengths are preferred because they can penetrate deeper into body tissue. That being said, not much photolysis occurred at 635nm because TPP does not absorb as well at that wavelength.

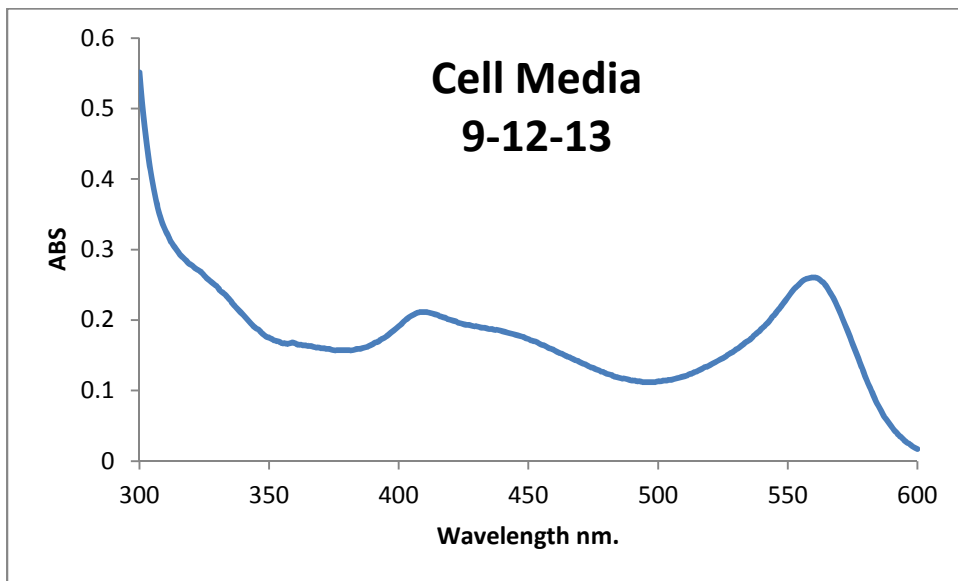


Figure 8. Cell media absorbance before 4 Amino TPP was added

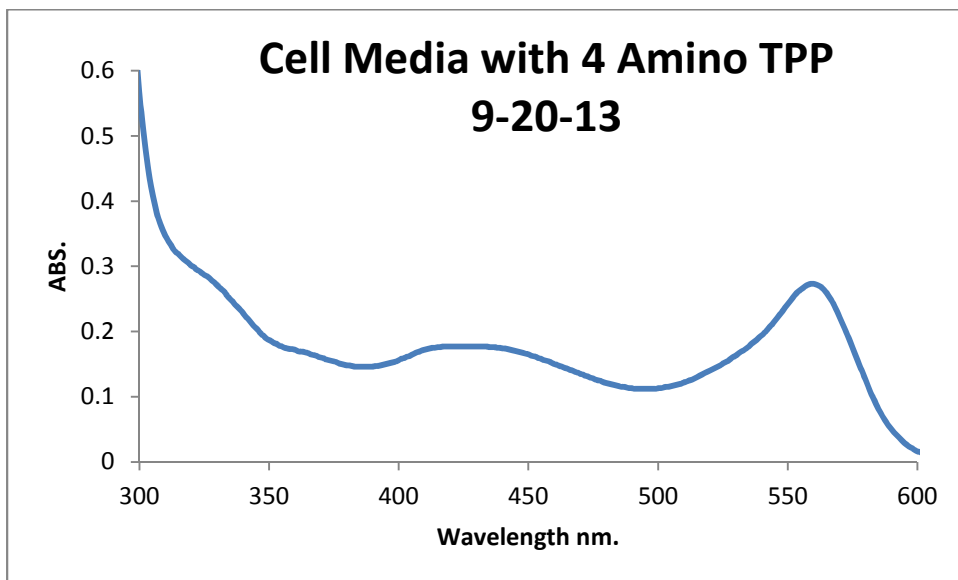


Figure 9. Cell media absorbance after 4 Amino TPP was added

One problem that was not solved concerns a solubility issue with the porphyrin. A variant of porphyrin called 4-amino TPP, which is more polar than other variants used in these experiments, could not be dissolved in cell media, which is a polar solvent. This can be seen in the lack of a peak at 413nm between Figure 8 and Figure 9. There was also no success in dissolving the porphyrin in water. Pharmacologists may be able to solve this polar solubility problem with encapsulation or chemical modification of the porphyrin itself.

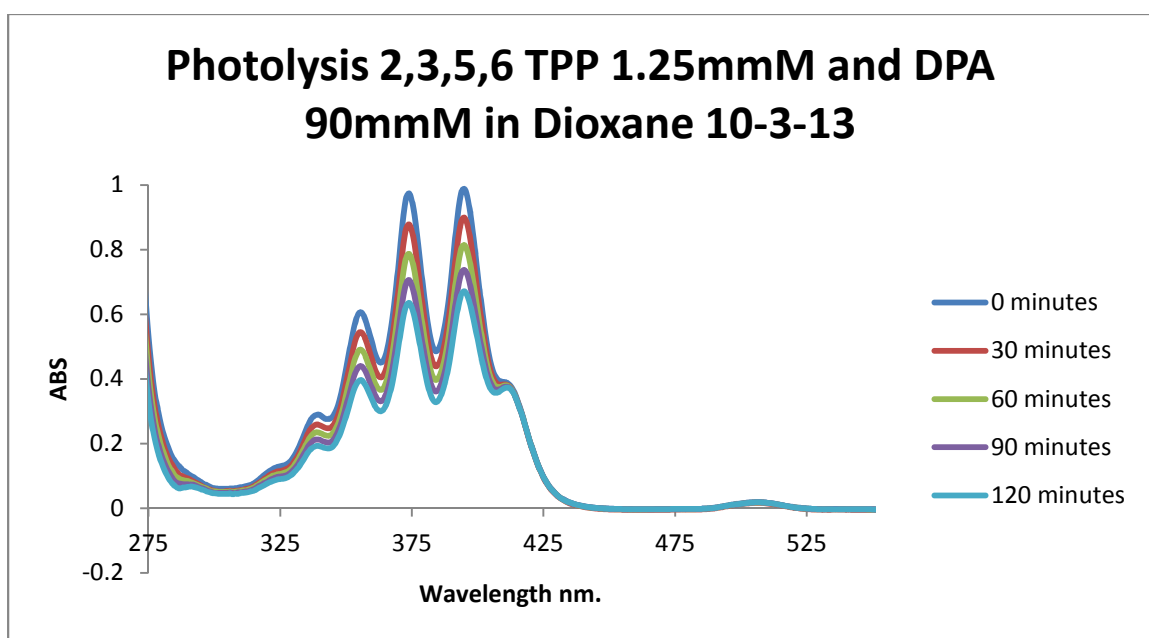


Figure 10. Photolysis with 2,3,5,6 TPP 1.25mM and DPA 90mM in Dioxane

Figure 10 emphasizes some of the same points in Figure 6. The area under the peaks (at 341nm, 357 nm, 375nm and 396nm) that is attributed to DPA was used to calculate the percent of DPA in the solution at that time. Photolyses with relatively high concentrations of DPA should use these peaks for quantification purposes while low concentration DPA should be quantified in a different way that is described later.

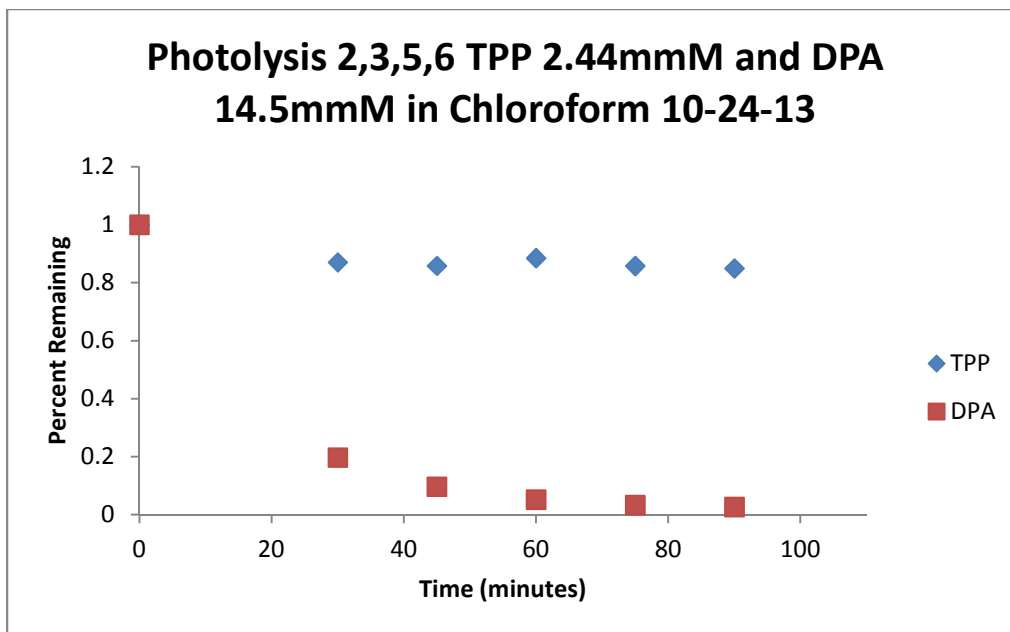


Figure 11. Photolysis Kinetics Experiment-TPP 2.44mmM-Percent Remaining

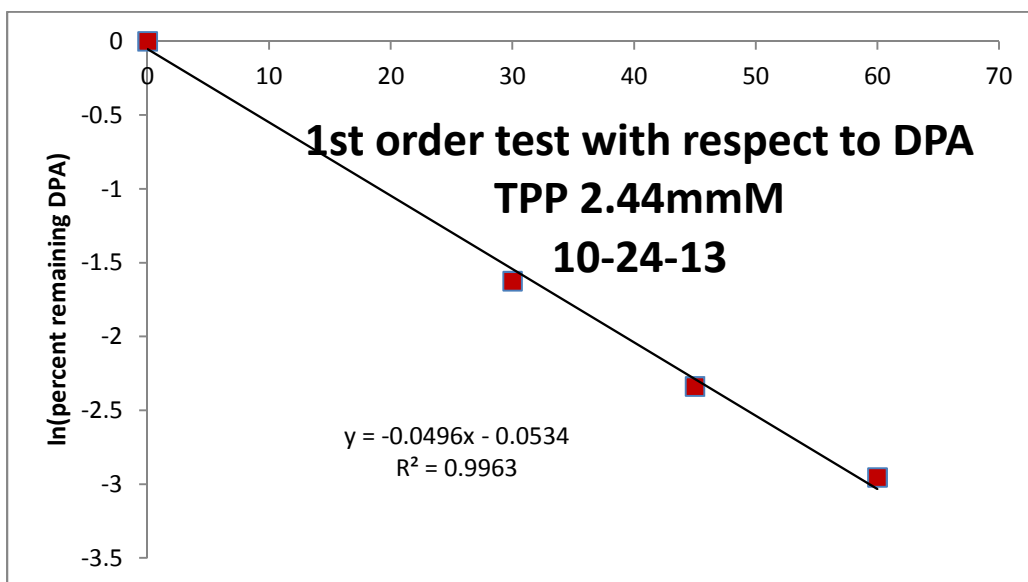


Figure 12. Photolysis Kinetics Experiment-TPP 2.44mmM-Natural Log of initial rate

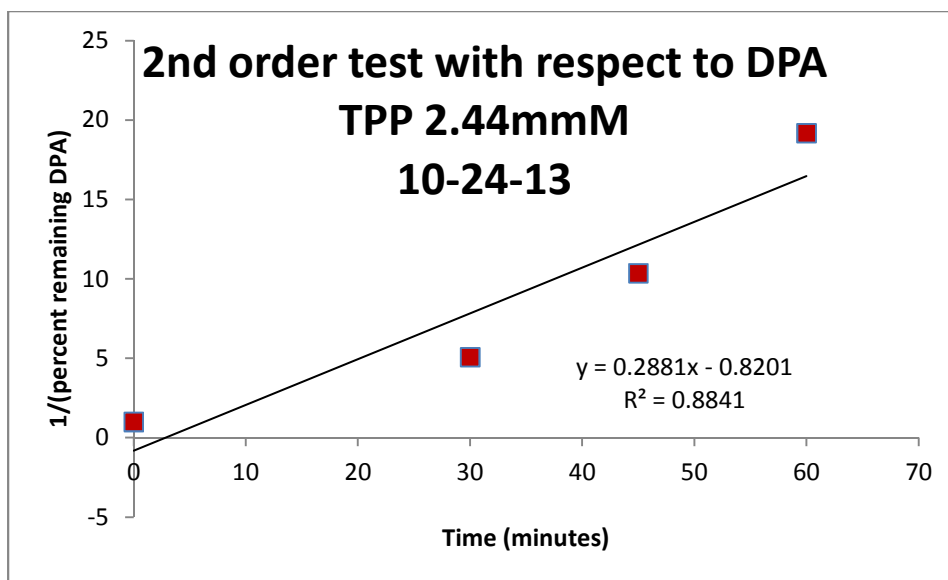


Figure 13. Photolysis Kinetics Experiment-TPP 2.44mmM-Inverse Function

Using spectra like those shown in Figure 10, graphs like Figure 11 were constructed. Except for a missing data point at fifteen minutes, Figure 11 shows the fraction of each component of the solution that remains at regular intervals in the photolysis. Using knowledge of kinetics, the initial decline of DPA was tested, and it was found that the data fit a first order dependence better than other orders because the natural log of the concentration of DPA was linear with the natural log test. This can be seen in Figure 12. When comparing Figure 12 with Figure 13, it is clear that the reaction is first order with respect to DPA and not second order. It should be noted that the initial rate was not accurately measured in the photolysis of Figure 11. The concentration of TPP was so high that the absorbance of the photolyzed solution should have been measured at shorter intervals. In the future, similar photolyses should have many data points early in the process to accurately measure initial rate.

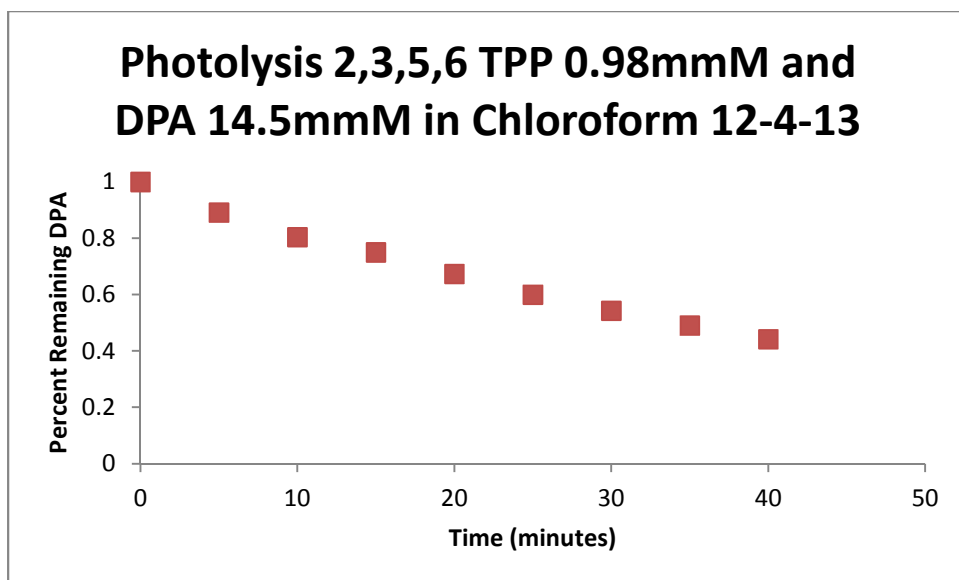


Figure 14. Photolysis Kinetics Experiment-TPP 0.98mmM-Percent Remaining

Figure 14 demonstrates a photolysis in which the concentration of TPP was lowered and in which the interval of taking absorbance values was shortened. The slope in Figure 15 is a measurement of the initial rate from data described in Figure 14.

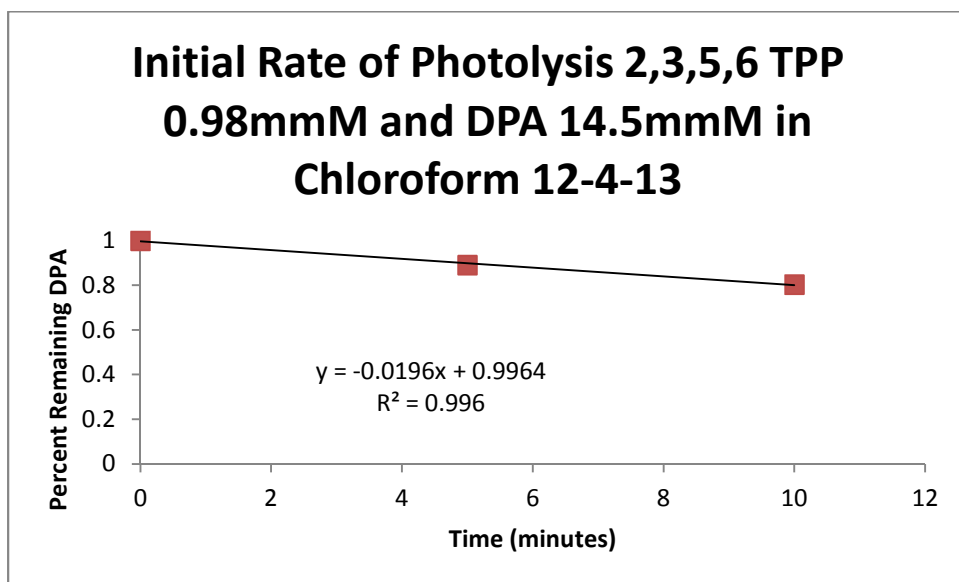


Figure 15. Initial Rate of the Photolysis in Figure 14

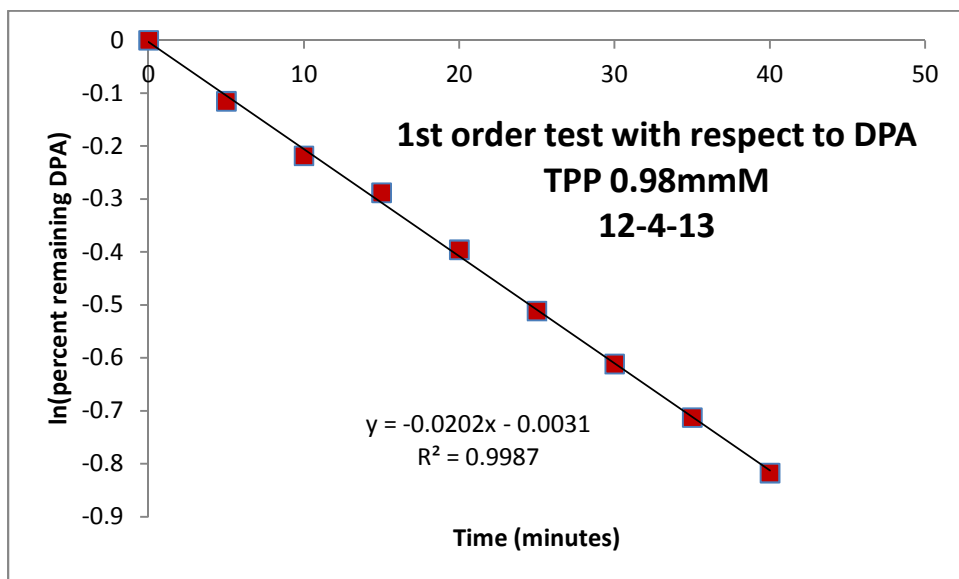


Figure 16. Photolysis Kinetics Experiment-TPP 0.98mmM-Natural Log

Figure 16 shows that this reaction rate follows first order kinetics extremely well with respect to DPA even over the entire photolysis since it was done over such a short period of time and at such a low TPP concentration. Figures 17, 18 and 19 are of a similar photolysis with half of the TPP concentration of Figures 14. In the future, all photolyses should be done at low concentrations and sampling intervals should be short in order for initial rates to be accurately measured.

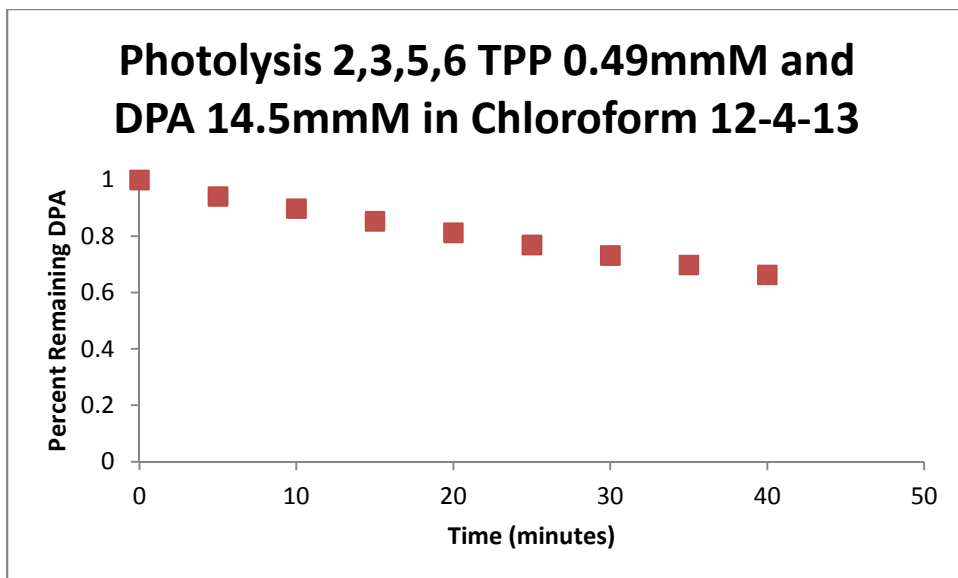


Figure 17. Photolysis Kinetics Experiment-TPP 0.49mmM-Percent Remaining

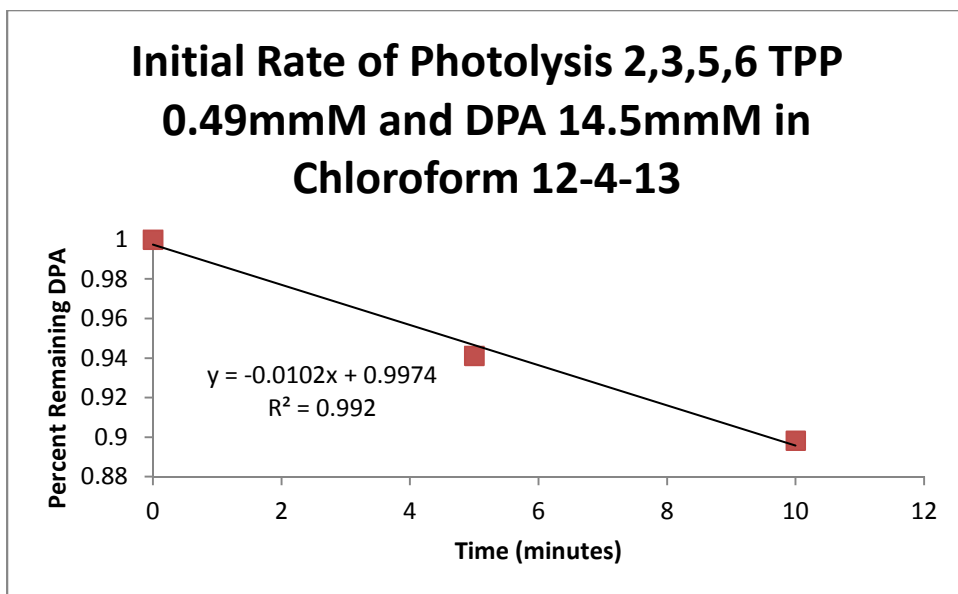


Figure 18. Initial Rate of Photolysis in Figure 17

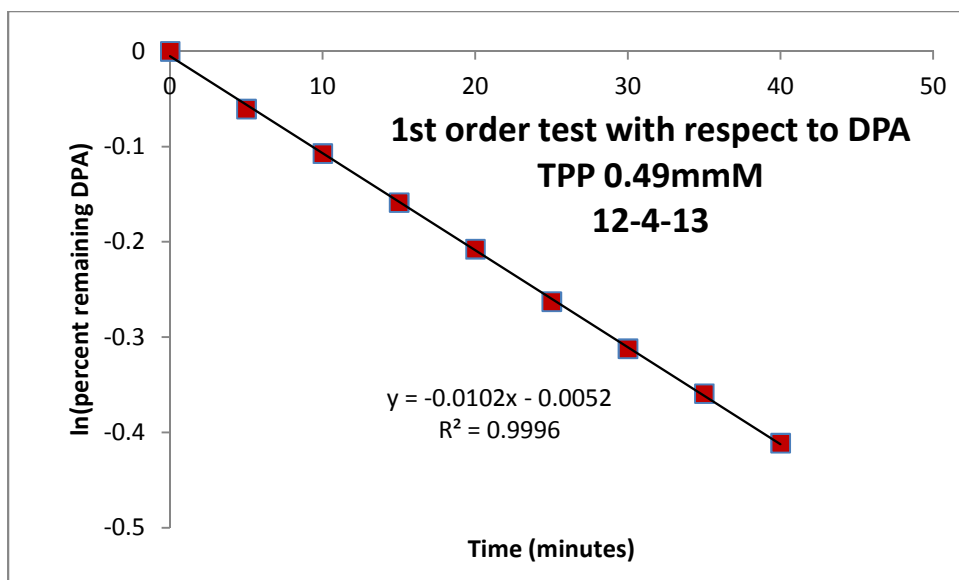


Figure 19. Photolysis Kinetics Experiment-TPP 0.49mmM-Natural Log

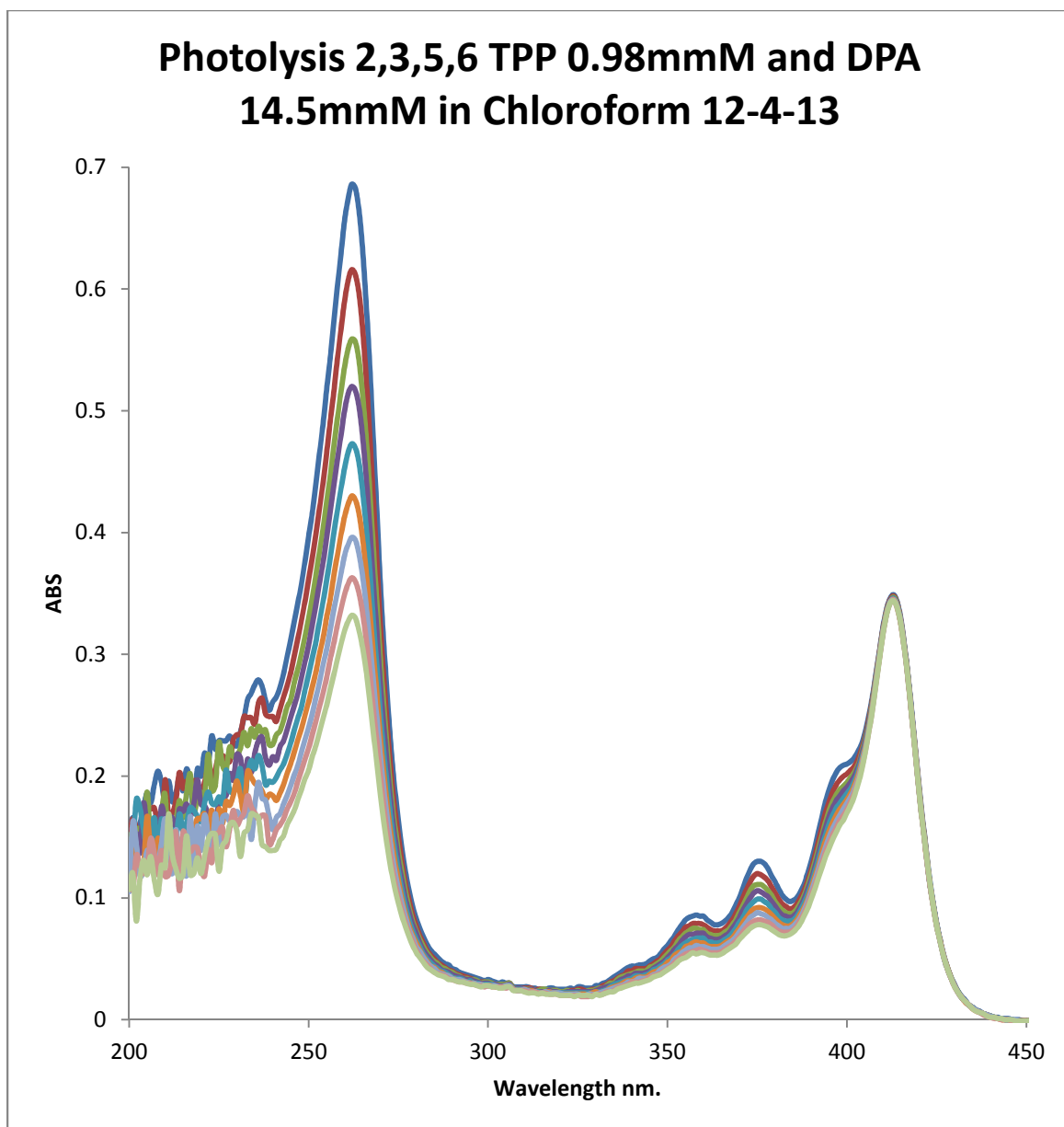


Figure 20. Photolysis 2,3,5,6 TPP 0.98mmM and DPA 14.5mmM in Chloroform 12-4-13

Figure 20 is simply a graph of the absorbance peaks in the photolysis that is in Figure 14. Here it can be seen that the area under the UV peak (at 262nm) was used at these low concentrations of DPA to quantify the DPA. Data intervals in this spectrum are every five minutes.

Further Kinetic Analysis

$$R = k[DPA]^x[TPP]^y$$

$$\frac{R_1}{R_2} = \frac{k [DPA]_1^{-1} [TPP]_1^y}{k [DPA]_1^{-1} [TPP]_2^y} = \frac{[TPP]_1^y}{[TPP]_2^y} = \left(\frac{[TPP]_1}{[TPP]_2} \right)^y$$

$$y = \frac{\ln\left(\frac{R_1}{R_2}\right)}{\ln\left(\frac{[TPP]_1}{[TPP]_2}\right)}$$

It was already shown that the photolysis is first order with respect to DPA. Using Table 1 and the equations above, it is possible to find the order with respect to TPP (y). Plugging in the values from Table 1 into the last equation gives y=0.9423. This value is reasonably close to 1, which would mean the photolysis reaction is first order with respect to TPP as well. With further experimentation, the order of TPP can be more accurately measured, but from this experiment, it can be seen that the reaction is at least close to first order with respect to TPP.

Table 1. Summary of Initial Rates from Figures 15 and 18

[TPP] mM	[DPA] mM	Initial Rate
0.98	14.5	0.0196
0.49	14.5	0.0102

In a photodynamic therapy, there are four components to the reaction that takes place. These components are the photosensitizer, target molecule, light and oxygen. After

finding that the reaction was first order with respect to TPP and DPA, the order of light intensity was chosen as the next project. As seen in Figures 21, 22, 23 and 24, four photolyses were conducted in which the only variation was the wattage of the laser.

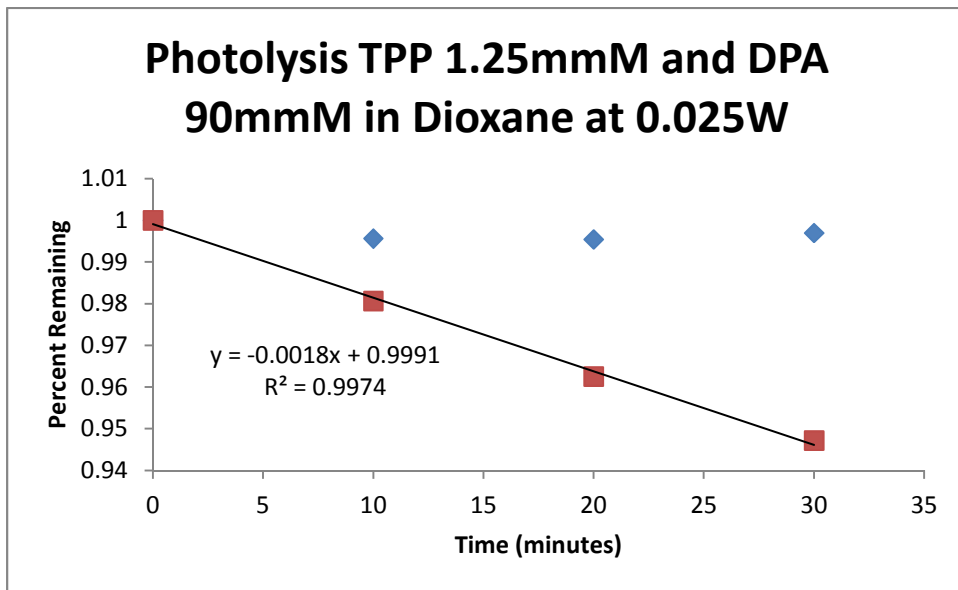


Figure 21. Light Intensity Rate Experiment – Laser set at 0.025W

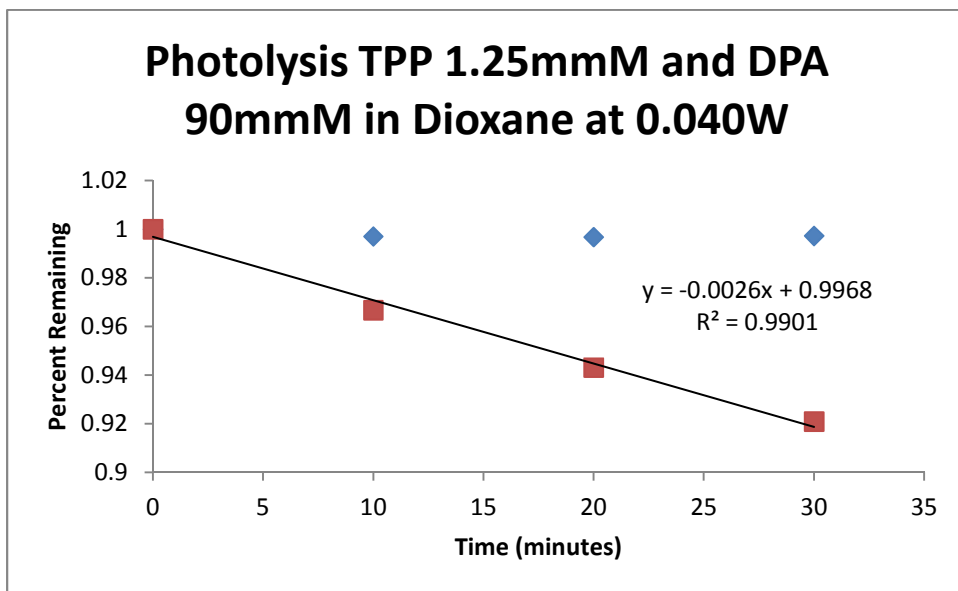


Figure 22. Light Intensity Rate Experiment – Laser set at 0.040W

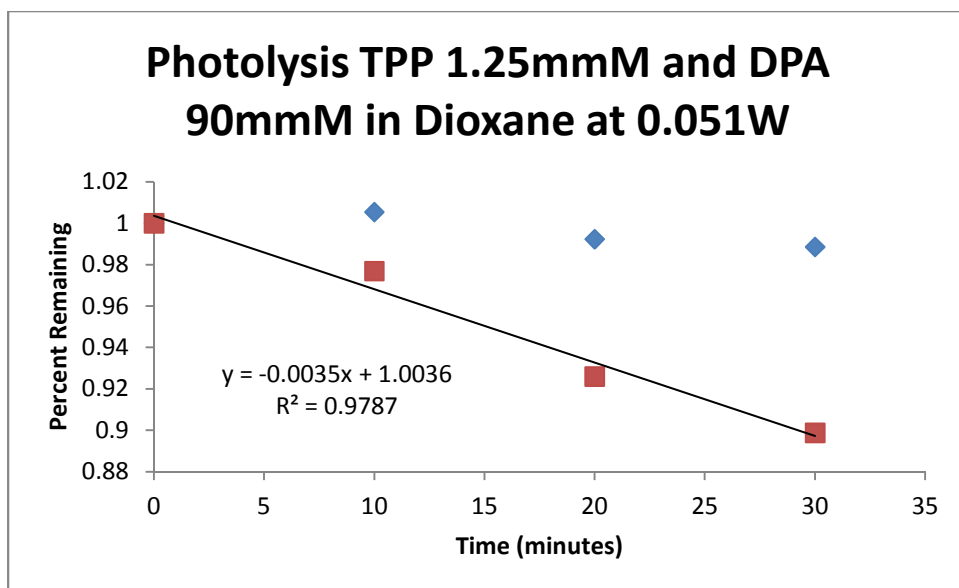


Figure 23. Light Intensity Rate Experiment – Laser set at 0.051W

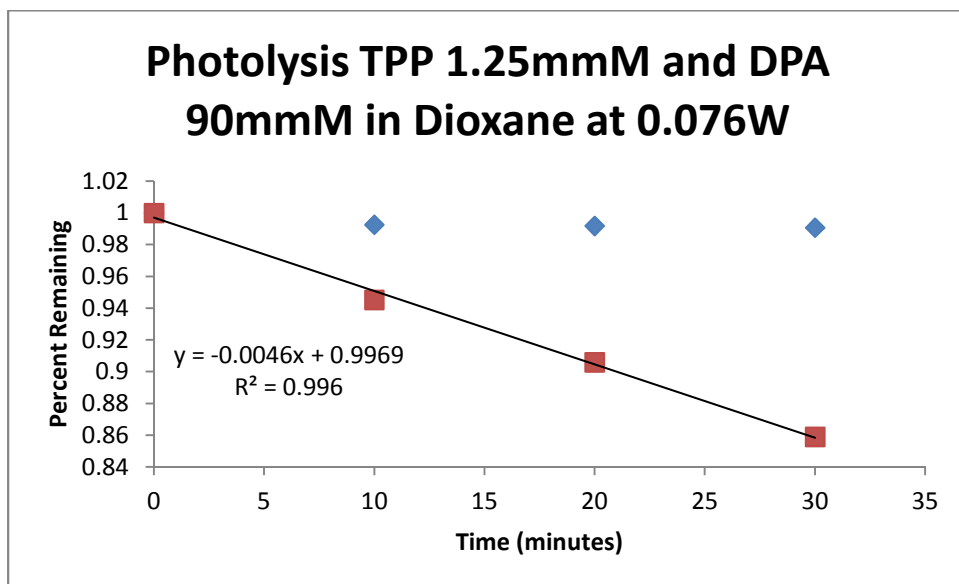


Figure 24. Light Intensity Rate Experiment – Laser set at 0.076W

The slopes (which are summarized in Table 2) of the photolyses were then graphed against their corresponding wattages in Figure 25, and a linear relation was noted. This indicates that the reaction is first order with respect to light intensity. The final reactant, oxygen, was shown to be essential to the reaction by my fellow research

student April Berlyoung. April removed the oxygen from a solution containing TPP and DPA by an involved process in which an unphotolyzed mixture of DPA and TPP in dioxane was frozen at liquid nitrogen temperature. The mixture was then pumped on using a vacuum pump and then thawed. This “freeze-pump-thaw” process was repeated three times and the solution was then photolyzed. Very little destruction of DPA occurred.

Table 2. Slopes of Photolyses in Light Intensity Rate Experiments

Watts	Slope of Photolysis
0.025	-0.001766
0.04	-0.002607
0.051	-0.003545
0.076	-0.004620

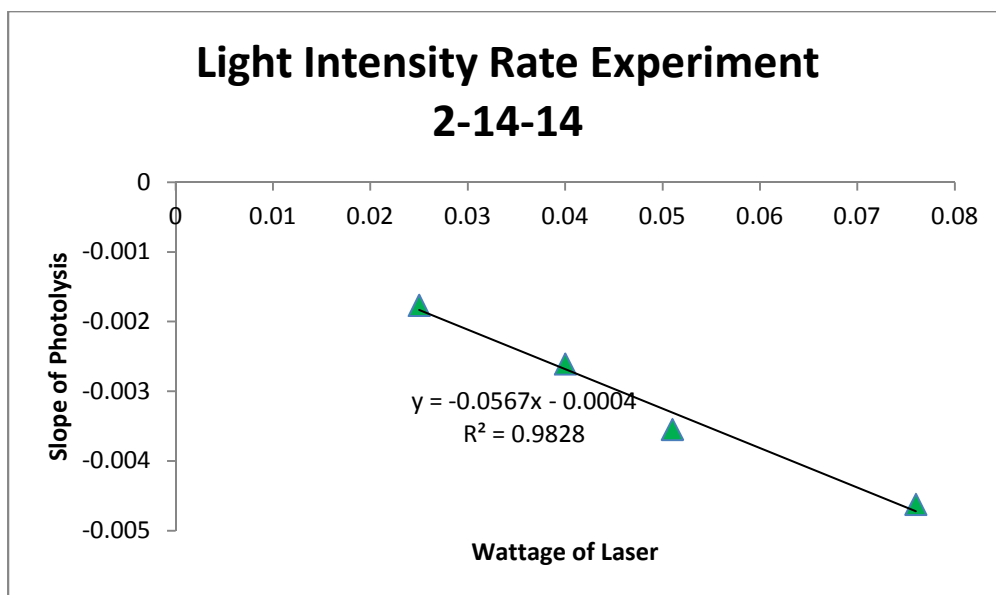


Figure 25. Slope of Photolysis vs. Wattage of Laser

Figure 26 shows carbon 13 NMR spectra for DPA before and after photolysis. Carbon-13 NMR spectra are useful in determining the different “types” of carbon in a molecule. This method is able to identify carbons that are dissimilar. Carbons that are symmetrically the same in a molecule show up as the same peak in a C13 NMR spectrum. From Image 1, it is known that all carbons in DPA are sp² hybridized. In the top spectrum in Figure 26, there are two groups of peaks. The peaks near 77 ppm are from deuteriochloroform, and these peaks appear in the aliphatic region. The peaks on the left between 140 and 125 ppm represent DPA, and they appear in the aromatic region. After the photolysis, a new peak at 84 ppm emerges. This is thought to be the new sp³ hybridized carbons in DPA EPO as seen in Image 3 since these peaks are in the aliphatic region. The sp³ hybridized carbons are the ones that are bound to the endoperoxide bridge. Also, numerous peaks between 141 and 123 ppm are in the second spectrum that are not in the first. This is thought to be due to the fact that the photolysis was not complete leaving behind original DPA peaks. But also some of the peaks due to carbon atoms near the bridgehead carbon atoms would be expected to shift giving rise to new peaks seen in the lower spectrum of Figure 26. In short, the C13 NMR spectra indicate that a new type of carbon is being produced during photolysis, and this is hypothesized to be sp³ hybridized carbons bound to an endoperoxide bridge that is a result of reacting with singlet oxygen.

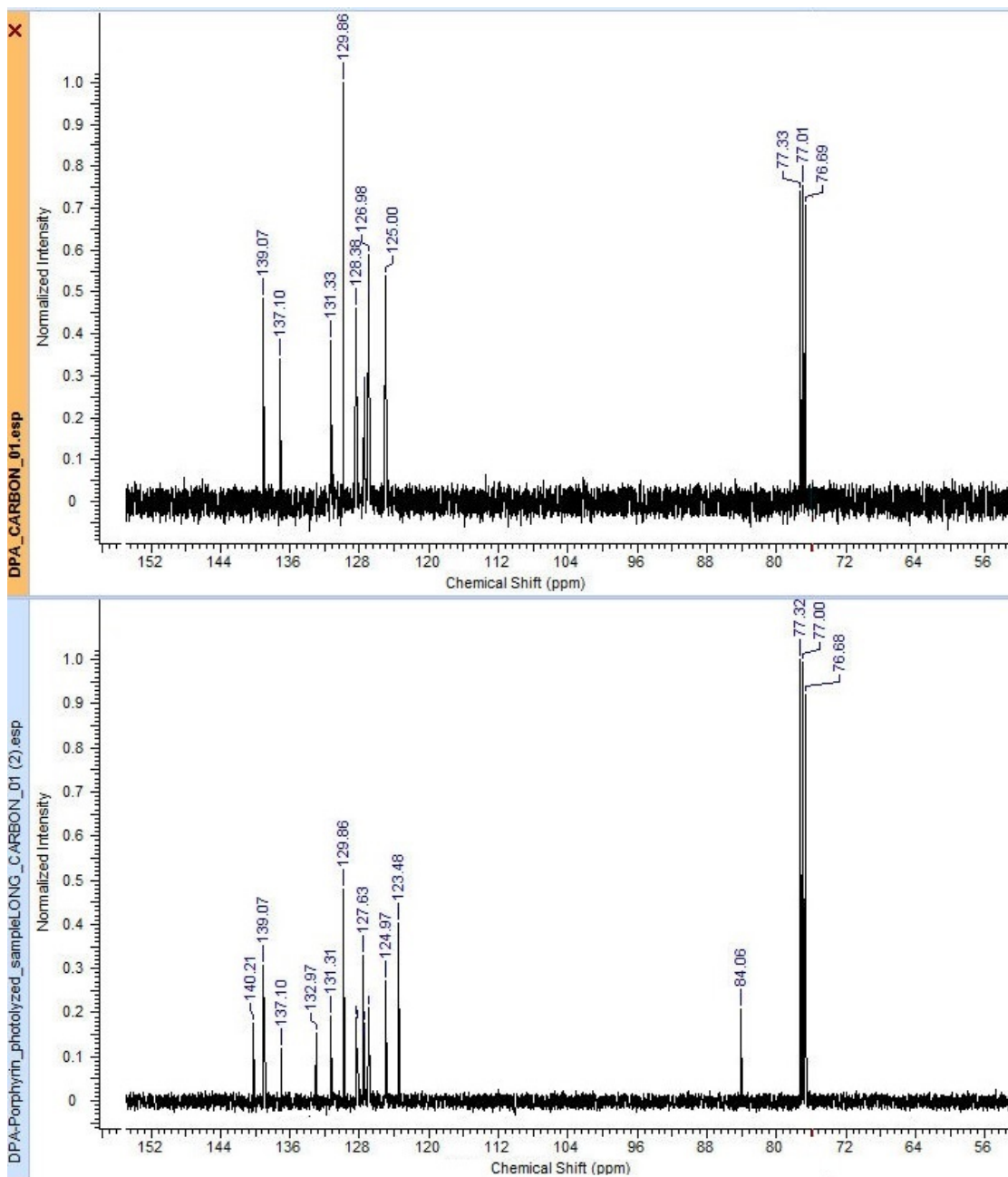


Figure 26. NMR Spectra of DPA before and after a photolysis

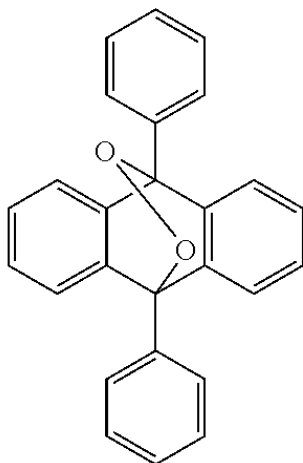


Image 3. 9,10-diphenylanthracene endoperoxide (DPA EPO)

Conclusion

The focus of this study was to find the kinetic orders of the reactants in a particular photolysis reaction. The kinetic orders of TPP, DPA and light intensity were all found to be one. The only reactant that was not studied was oxygen. Since it is known that oxygen is essential to the photolysis reaction, further research is needed to determine the order of oxygen. Purely to learn more about the chemistry, more data should be taken to learn about the DPA EPO product of this photolysis reaction. Further PDT research dealing with porphyrin molecules should focus on finding a method of delivery into the body because TPP only dissolves in nonpolar solutions.

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