Syntheses of a Tin (IV) meso-Tetra(4-pyridyl) Porphyrin Dichloride-Tetrachlorobis (Bipy)2 Ruthenium (II) Complex and Studies of Photophysical Properties of 1-Nitropyrene

Phillip Sharp
sharppb@jacks.sfasu.edu

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Syntheses of a Tin (IV) meso-Tetra(4-pyridyl) Porphyrin Dichloride-Tetrachlorobis (Bipy)$_2$ Ruthenium (II) Complex and Studies of Photophysical Properties of 1-Nitropyrene

By

Phillip Brandon Sharp

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Syntheses of a Tin (IV) meso-Tetra(4-pyridyl) Porphyrin Dichloride-Tetrachlorobis (Bipy)₂ Ruthenium (II) Complex and Studies of Photophysical Properties of 1-Nitropyrene

By

Phillip B. Sharp, B.S.

APPROVED:

____________________________
Dr. Matibur Zamadar, Thesis Director

____________________________
Dr. Michele Harris, Committee Member

____________________________
Dr. Russell Franks, Committee Member

____________________________
Dr. Josephine Taylor, Committee Member

____________________________
Pauline M. Sampson, Ph. D.
Dean of Research and Graduate Studies
ABSTRACT

Development of a photosensitizer that can work in both aerobic and anaerobic environments would increase the robustness of the cancer treatment known as photodynamic therapy. The development of a photosensitizer was first attempted by synthesizing a tin (IV) meso-tetra (4-pyridyl) porphyrin dichloride-tetrachlorobis (Bipy)2 ruthenium (II) complex. The synthesize of the tin porphyrin was done by a method from literature and had a percent error of 31.58 and 37.31 when comparing the theoretical percentages of carbon (55.39) and nitrogen (12.92) meaning that photosensitizer was not synthesized.

The second type of chromophore that was studied was 1-nitropyrene do determine if it can act as a possible photosensitizer. As a proof of concept, 1-nitropyrene can act as a photosensitizer by using visible light. Upon visible light irradiation 1-nitropyrene can produce 1O2, OH, and possibly NO species under aerobic conditions and can produce OH and possibly NO under anaerobic conditions.
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CHAPTER 1: SYNTHESIZE OF A TIN (IV) MESO-TETRA(4-PYRIDYL) PORPHYRIN DICHLORIDE-TETRACHLOROBIS (BIPY)$_2$ RUTHENIUM (II)

1.1. Introduction

Recently, there has been a growing interest in the design and synthesis of new type of porphyrin based supramolecular complexes for a number of applications. These include the capturing of solar energy to split water into oxygen and hydrogen gas to store solar energy in a fuel (1) and the production of reactive oxygen species for the photodynamic therapy medical application of treating cancer (2). Several studies on visible light induced water oxidation have been done in the past where ruthenium metal complexes and other earth-abundant metal complexes have been used as a homogeneous catalyst (3). In almost all cases, a combination of a visible light photosensitizer, oxygen evolving complex and a sacrificial electron acceptor were used to produce oxygen gas (O$_2$) from water. Recently, Licheng Sun et al. reported a ruthenium based supramolecular complex, a covalently linked photosensitizer and catalyst for visible light–driven water oxidation (4). However, this supramolecular complex is unable to produce any oxygen gas from
water if there is no sacrificial electron acceptor molecule, such as sodium thiosulfate, in the solution. A solution’s pH sometimes plays a very important role for the catalyst being in active form in the catalytic solution (5). Inspired by the structures of biological chlorophyll pigments and their light absorbing and electron transfer roles in the water oxidation process, a number of artificial photosynthetic systems were developed (6). For the last couple of years, there has been growing interest in tin (IV) porphyrin based photocatalysts for splitting water into hydrogen and oxygen gas (7). The major advantages of using tin(IV) porphyrins are as follows: they are usually six coordinated with trans-diaxial ligands around a Sn^{4+} ion with a redox inert electron configuration ([Kr]4d^{10}5S^0) similar to the Mg^{2+} center of chlorophylls ([Ne]3S^0); they are known to have suitable redox potentials for water splitting; they are easily accessible and stable against acid-induced demetallation (8,9). Water oxidation by tin (IV) porphyrins has not been thoroughly explored.

Over the past decades, photodynamic therapy (PDT) has become an increasingly powerful method for the treatment for cancers, such as prostate, skin and lung cancers (10). It is recognized as a promising new, effective, and noninvasive chemotherapeutic treatment. Traditionally, photodynamic therapy involves the localization of photosensitizers in the target tissue. Upon illumination with visible light, photosensitizers generate singlet oxygen (^1O_2) which is a critical intermediate involved in PDT, by reacting with dissolved oxygen in tissue. Singlet oxygen causes damage to adjacent tissues (6) but ^1O_2 has a short life span, 10~300 ns (11) with most of the damage
to DNA localized. These treatments are better tolerated as they can treat diseased tissues effectively and non-invasively causing minimal nonspecific damage to other tissues (7) meaning that most of the damage is to cancer cells. However, the current PDT method is facing some fundamental challenges, such as: (1) poor solubility of hydrophobic photosensitizers in biological media, (2) insufficient photosensitizer concentration at the target sites, (3) tumor hypoxia and (4) low selectivity of photosensitizers to the target sites. Among these issues, tumor hypoxia, a state of low oxygen concentration in the tumor cells, is recognized as the greatest challenge that limits its clinical utility. PDT-photochemistry highly depends on the presence of oxygen (O$_2$) for producing lethal singlet oxygen ($^1$O$_2$) (8). A considerable amount of research has been carried out on methods for delivering cisplatin or other platinum metal-based chemotherapy drugs to the tumor sites. However, despite its tremendous success, its use is limited because of the poor target selectivity and serious side-effects that occur during long-term traditional chemotherapy treatment. Studies also indicate that hypoxic cells can exhibit considerable resistance to chemotherapy via several mechanisms one being inhibiting ROS development because of a lack of available oxygen (9).

Thus, development of a water-soluble photosensitizer, tin (IV) meso-tetra(4-pyridyl) porphyrin dichloride-tetrachlorobis(bipy)$_2$ ruthenium (II), that will kill hypoxic cancer cells by splitting water and photoreacting with oxygen to form singlet oxygen is highly desirable. Tin (IV) meso-tetra(4-pyridyl) porphyrin dichloride-tetrachlorobis(bipy)$_2$ ruthenium (II) is referred as the tin porphyrin-ruthenium catalyst, TinPorRuthCatalyst.
The proposed TinPorRuthCatalyst is an assembly of Sn (IV) meso-tetra(4-pyridyl) porphyrin dichloride (Sn-TPyP) and four [Ru(bipy)$_2$Cl] substituents, shown in Figure 1.

**Figure 1:** Proposed structure of the TinPorRuthCatalyst.

The *meso*-tetra(4-pyridyl) porphyrin (TPyP) and the ruthenium complexes consist of aromatic rings that absorb throughout the visible spectrum. Specifically, the wavelength of one of the Q-bands of the porphyrin, 630 nm, is the light used for most PDT treatments (12). Since it is well known that longer wavelengths ($\lambda$) of light penetrate deeper into biological tissue, the longer $\lambda$ at 630 nm allows for better penetration than at the porphyrin’s Soret band, which is around 405 nm. Since the ruthenium complex attaches to the outer nitrogens on TPyP, it increases the water solubility of the catalyst because of the positive charges. It is important for a photosensitizer to be water soluble because
water insoluble photosensitizers are poorly absorbed into the bloodstream, causing a loss of its activity. This loss of activity suggests that a stronger dose of the drug is needed to be effective, which in turn increases the toxicity of the photosensitizer (13).

It is not currently known if the complex in Figure 1 can be synthesized. While the synthesis of the proposed catalyst has not been previously carried out, Araki et Al. (14) have synthesized a tetra-ruthenated porphyrin (TRuP), shown in Figure 2.

![Figure 2: TPyP[Ru(bipy)2Cl4] synthesized by Araki (14).](image)

The porphyrin was synthesized by refluxing [Ru(bipy)2Cl2], with 5,10,15,20-Tetra(4-pyridyl) porphyrin (TPyP) in glacial acetic acid with a molar ratio of 4:1. The excess acetic acid was removed under vacuum and the solution was refluxed again with MeOH. Then the solution was precipitated by adding a saturated solution of LiCl/acetone. The solid was washed with acetone and then dried under vacuum. A CHN analysis, UV-Vis and IR spectroscopies were used to determine if the correct tetra-ruthenated porphyrin
product was synthesized. No crystal structure of TRuP nor a percent yield for the synthesis were reported. The theoretical percentage of carbon, hydrogen, nitrogen in TRuP were calculated, which was 52.67, 4.05, and 12.28 %, respectively. The percentage of C, H, and N obtained for TRuP corresponded well with theoretical percentage of C, H, and N. The percentage of C, H, and N obtained for TRuP were 51.96, 4.08, and 12.32 %, respectively. The UV-Vis spectra for TRuP exhibited absorption bands at 292, 356, 414, 470, 518, 584 and 642 nm. The IR spectrum that was reported had peaks at 3060 w, 3008 w, 1600 s, 1560 w, 1551 w, 1470 sh, 1457 s, 1440 s, 1412 s, 1398 sh, 1380 sh, 1345 w, 1304 s, 1260 m, 1235 w, 1231 sh, 1227 sh, 1225 m, 1207 w, 1183 w, 1150 m, 1098 w, 1015 m, 975 w, 964 s, 879 m, 870 s, 845 w, 795 s, 760 s, 722 s, 652 w, 560 w, 460 w, 420 w, 410 sh, and 390 w (m=medium, sh=shoulder, s=strong, w=weak). All IR spectrum peaks are in cm\(^{-1}\). Analysis of the IR spectrum revealed that a conjugated ring system was created. The wide peaks at 3060 cm\(^{-1}\) and 3008 cm\(^{-1}\) were probably due to sp\(^2\) C-H symmetrical and asymmetrical stretches while the strong peak at 1600 cm\(^{-1}\) and the shoulder peak at 1470 cm\(^{-1}\) were an indication of an aromatic C=C stretch. Assigning the peaks of 1560 cm\(^{-1}\), 1551 cm\(^{-1}\) and the peaks in the lower portion of the spectrum, from 1440 cm\(^{-1}\) through 390 cm\(^{-1}\), was difficult since many peaks fall within multiple ranges of many different types of modes and cannot be effectively interpreted. The C-N stretch appeared within this region, 1350-1000 cm\(^{-1}\). There was no indication of a sp or sp\(^3\) C-H stretch, C=N stretch, C=C stretch nor any stretches involving carbon with oxygen, which is expected for TPyP. What was interesting was
that there was no N-H stretch (3500-3300 cm⁻¹) but it could be easily overshadowed by its central location in TPyP (15). Another synthesis of a ruthenated-porphyrin was done by Mei (16). In a study of DNA binding of porphyrin-ruthenium complexes, Mei et al used MPyTPP (5-Pyridine-10,15,20-triphenylporphyrin) and cis-Ru(pip)₂Cl₂ in a 1:1.5 ratio refluxed with acetic acid under an argon atmosphere. The solvent was removed and MeOH was added to the solid where it was once again refluxed for another 45 min. The solid was precipitated in a similar method to that of Araki (14) by using a saturated solution of acetone/LiCl. Mei and coworkers were able to obtain a percent yield of 56 % for the product [MPyTPP-Ru(pip)₂Cl]⁺. The product is shown in Figure 3.

![Figure 3: [MPyTPP-Ru(pip)₂Cl]⁺ structure synthesized by Mei (16).](image)

Araki was also able to synthesize a metalloporphyrin derivative of TPyP by reacting CoTPyP (Cobalt TPyP) with [Ru(bipy)₂Cl₂] in a 1:4 molar ratio (17). The procedure was the same as in his earlier work, with the only difference being the starting material, CoTPyP, instead of TPyP.

The splitting of water is a major hurdle that must be overcome for the
TinPorRuthCatalyst to be able to work in low oxygen environments. The redox reactions and the overall reaction for water splitting are shown in equations (1)-{3}. (18)

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Equation</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidation half reaction</td>
<td>$2H_2O \rightleftharpoons O_2 + 4H^+ + 4e^-$</td>
<td>(1)</td>
</tr>
<tr>
<td>Reduction half reaction</td>
<td>$4H^+ + 4e^- \rightleftharpoons 2H_2$</td>
<td>(2)</td>
</tr>
<tr>
<td>Overall reaction</td>
<td>$2H_2O \rightleftharpoons O_2 + 2H_2$</td>
<td>(3)</td>
</tr>
</tbody>
</table>

The splitting of water involves two half reactions: the oxidation reaction that forms O$_2$ and the reduction reaction that forms H$_2$. The main reaction that needs to be considered is the oxidation reaction because of the overpotential needed (1.23 V) compared with the reduction potential (0 V). The overpotential can be supplied by a current, like in electrolysis, but this is not feasible within the human body. Therefore, a catalyst would be needed to lower the energy necessary for this reaction. Experiments with ruthenium complexes have been able to produce oxygen from water. For example, in an article by Fei, (19) a triruthenium complex was synthesized and used as a photosensitizer with Na$_2$S$_2$O$_8$ as the electron acceptor. The redox potential of Ru$^{2+}$/Ru$^{3+}$ was determined by cyclic voltammetry and was 0.74 V. This is lower than the 1.23 V needed but only a ruthenium complex was used. A porphyrin connected to multiple ruthenium complexes increases the catalytic activity of each individual molecule (20). The redox potential indicates that it is thermodynamically possible to split water by a method that is similar to what Fei accomplished. The O$_2$ production was measured by an oxygen electrode, and the oxygen turnover frequency was determined to be 4.7 min$^{-1}$. It should be noted from
this experiment that all three components, a photosensitizer, light source, and electron acceptor, were needed to oxidize water. This was determined by conducting control experiments that measured O₂ production as the photosensitizer was irradiated with light and without light. It was found that when light irradiated the sample it produced O₂ and without light, O₂ production stopped. In another experiment, Juris and coworkers determined that the reduction potential of [Ru^{III}(bpy)₃]^{3+} was 1.21 V vs NHE (21). In another study by Kim (22) the reduction potential of a tin porphyrin [Sn^{IV}(OH)₂TPyP] attached with hydroxyl groups was measured in tetrahydrofuran (THF). Its potential was determined to be 1.10 V. The inclusion of both the tin porphyrin and ruthenium complexes should give enough energy to overcome the energy barrier for water splitting. Redox potentials of tin (IV) porphyrin complexes in aqueous media (pH = 7) reveal that the excited state of the tin (IV) porphyrins (E_{ox}*S = -0.57 V, E_{ox}*T = 0.19 V vs. NHE) can oxidize water into oxygen (9). This process is thermodynamically favorable.

Recently, porphyrins and ruthenium complexes are shown to produce singlet oxygen from dioxygen upon with visible light irradiation (23). Having a metal complexed at the center of a porphyrin has also been shown to increase the production of singlet oxygen. There are two types of pathways that are responsible for photooxidation reactions with DNA (Figure 4). Type I involves a hydrogen abstraction from 2′ deoxyguanosine, a DNA base, with the excited photosensitizer (photo*) forming 8-oxo-2′ deoxyguanosine (24). The other pathway, Type II, involves the use of singlet oxygen (24). Visible light hits the
photosensitizer, exciting the photosensitizer to a higher energy state (photo\(^*\)) then the excess energy is transferred to oxygen, forming singlet oxygen.

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Type I reaction" /></td>
<td><img src="image2.png" alt="Type II reaction" /></td>
</tr>
</tbody>
</table>

**Figure 4:** Type I & Type II photooxidation reactions with DNA.

The transition from the triplet state of oxygen to the singlet state of oxygen is considered a “forbidden” transition as stated by the spin conservation principle. This principle states that any transition that involves a change of multiplicity is forbidden. A forbidden transition does not mean that the transition is impossible but means that the transition is improbable and not as likely to happen as a spin allowed transition. Due to the ground state of molecular oxygen being paramagnetic, having unpaired electrons, the likelihood of these forbidden transitions increases. This is because unpaired electrons can be perturbed more easily than spin paired electrons, diamagnetic.

The viability of Type II over Type I for tetra-ruthenated porphyrins was shown by Araki (24). Researchers calculated the ratio of Type II/Type I with calf-thymus DNA. The regular supramolecular assembly, Tetra-ruthenated porphyrin species (TRP), had a
value of 2.3 and the Zinc tetra-ruthenated porphyrin species (ZnTRP) was 5.6. See Figure 5.

![Figure 5](image.png)

**Figure 5:** Structure of the superamolecule, ZnTRP.

Apparently, the Zn coordinated porphyrin caused an increase in singlet oxygen production. The main issue with the singlet oxygen pathway is that it requires ground state oxygen to produce singlet oxygen.

Singlet oxygen can be determined by its emission band at around 1270 nm (25). This band is usually very weak and can be missed so the measurement of singlet oxygen will be determined by two other methods. The first method, used by Chen, (26) involves the oxidation of dihydroxynaphthalene (DHN) to juglone, see Figure 6.
Figure 6: Reaction of singlet oxygen with DHN.

The production of juglone from DHN can be observed by UV-Visible spectroscopy by monitoring the maximum absorbance of both DHN (310 nm) and juglone (427 nm). So, as DHN decreases, absorbance at 310 nm also decreases and as juglone increases the absorbance at 427 nm increases. The two changes in absorbance indicate that singlet oxygen was produced. The second method uses N-benzoyl-DL-methionine (BZ-DL-MET). Zamadar and coworkers demonstrated that in the presence of singlet oxygen, BZ-DL-MET oxidized and formed sulfoxide products (Figure 7) (27). Compressed O₂ was passed through a porous Vycor glass hollow core fiber optic coated with TPyP that was attached to a water solution containing BZ-DL-MET anion. The proximal end of the fiber was irradiated with 532 nm light and the sulfoxide anion product formed at the distal end of the fiber with a percent yield of 5%. The yield was improved to 11 % when the reaction was carried out in D₂O. An increase in yield was expected based on work by Lindig, because of the increased lifetime of singlet oxygen in D₂O over H₂O. However, the lifetime of the singlet oxygen in D₂O was less than the expected 20-fold increase (28).
The less than expected increase was thought to be a surface effect involving adsorption or quenching of singlet oxygen by the silicon probe.

![Figure 7: Reaction of BZ-DL-MET anion in presence of singlet oxygen.](image)

1.2. Experimental Section

### 1.2.1. Materials and Instruments. All reagent obtained were of the highest quality and used without further purification. A Water Technologies deionization system was used to obtain deionized H₂O. Sn (IV) meso-tetra-(4-pyridyl) porphine dichloride (Sn Por) was purchased from Frontier Scientific, USA and Cis-dichlorobis(2,2’-bipyridine) Ru (II) (Ru(bipy)₂ Cl₂) was purchased from Sigma Aldrich, USA. Methanol (MeOH) and acetone was obtained from VWR International, USA. Acetic acid (12 M) was purchased from Flinn Scientific, USA. Lithium chloride was purchased from Sigma Aldrich, USA.
Argon gas was obtained from Redball, USA. CHN analysis was performed using a CN 628 analyzer manufactured by Leco Corp. USA.

1.2.2. Synthesis of TinPorRuthCat. In a glove box, under argon atmosphere and at room temperature, approx. 0.156 g of Ru(bipy)$_2$Cl$_2$ was placed into a 50 mL two-neck round bottom flask (RBF). The RBF was then sealed with a septum and removed from the glove box. A reflux apparatus (Figure 8) was set up where the RBF containing Ru(bipy)$_2$Cl$_2$ was connected to a closed condenser and placed in a Thermowell wired to a Variac set to 100 volts.

![Figure 8: Setup for reflux apparatus.](image)

The reflux apparatus was kept under positive argon pressure. Then 0.050 g of the Sn (IV) meso-tetra-(4-pyridyl) porphine dichloride and 5 mL of acetic acid were added to the RBF under positive argon pressure. The resulting solution was refluxed for about one
hour and cooled to room temperature. The acetic acid solution from the RBF was removed via distillation under reduced pressure (Figure 9).

![Distillation setup](image)

**Figure 9**: Distillation setup for removal of acetic acid.

After removal of acetic acid from the RBF, 5 mL of MeOH was added to the flask and the resulting solution was refluxed again for about 45 mins. A saturated LiCl/acetone solution was prepared by mixing 1.0 g of LiCl into 55 mL of acetone for 20 mins. The LiCl/acetone solution was added drop wise to the RBF which resulted in the formation of a black precipitate. The precipitate was then collected by vacuum filtration and stored in a vial under dark for future use.
1.3. Results and Discussion

1.3.1. Synthesis of TinPorRuthCat. The characterization of the TinPorRuthCat was done using a CN 628 analyzer housed in the SFASU Soils Lab. The results yielded a carbon % of 37.896 and a nitrogen % of 8.0995 (Table 1). This is compared with the theoretical percent of carbon at 55.39 % and for nitrogen, 12.92 %. Experimental values were different for carbon and nitrogen than expected with a percent error of 31.58 % and 34.99%, respectively. Since the % N and % C did not match the expected values for the TinPorRuthCat, it was evident that the catalyst was not synthesized.

<table>
<thead>
<tr>
<th>Theoretical Molecular Formula</th>
<th>Theoretical Molecular Weight (g/mol)</th>
<th>Actual</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>SnC_{120}N_{24}H_{88}Ru_{4}Cl_{6}</td>
<td>2601.90</td>
<td>37.896</td>
<td>31.58</td>
</tr>
<tr>
<td>% C (by weight)</td>
<td>55.39</td>
<td>37.896</td>
<td>31.58</td>
</tr>
<tr>
<td>% N (by weight)</td>
<td>12.92</td>
<td>8.0995</td>
<td>37.31</td>
</tr>
</tbody>
</table>

Table 1: Theoretical % of carbon and nitrogen compared with data obtained from a Leco CN 628 analyzer.

Researchers have been able to synthesize a tin porphyrin and ruthenium complexes in glacial acetic acid (29) but sensitive characterization techniques, i.e. NMR, were not performed and purity was not determined. Engelmann et al. (20) reported difficulty with using glacial acetic but was able to synthesize a meso-tetra(4-pyridyl) porphyrin with
ruthenium complexes after the solvent was changed from glacial acetic acid to an organic solvent mixture (CH$_2$Cl$_2$/ DMF) (10:1) with the addition of [Ru(bipy)$_2$Cl(H$_2$O)(NO$_3$)] as a reagent. From this procedure Engelmann et al. were able to get a yield of 90%. It seemed that by refluxing with glacial acetic acid the pyridyl groups competed with the ruthenium complexes for solvent protons. The competition for solvent protons resulted in compounds with different types of substituents which caused a loss in purity that affected the percent composition of the final product. The UV-Vis spectrum of the synthesized product in ultrapure H$_2$O are shown in Figure 10 with absorptions at 293, 347, 418, 483, 563, and 606 nm.

![UV-Vis spectrum](image)  

**Figure 10:** UV-Vis spectrum of TinPorRuthCat (8.0 x 10$^{-6}$ M) in Ultrapure H$_2$O.
The $\lambda$ max is located at 418 nm and is consistent with unmetalated meso-tetra(4-pyridyl) porphyrins, along with absorptions of 563 and at 606 nm (30). The absorption bands at 293, 347 and 483 nm could be absorptions from ruthenium complexes (20). From this data it can be concluded that the product synthesized is probably a tin porphyrin with various pyridyl and various amounts of ruthenium bipyridine complexes. Further characterizations are needed to determine or speculate on the actual structure of the tin porphyrin complex.

1.4. Conclusion

The synthesis of the TinPorRuthCat was unsuccessful. Theoretically the percentage of carbon and nitrogen, by weight, should be about 55.39 and 12.92 respectively. The actual percentages were determined by CHN analysis to be 37.896 for carbon and 8.0995 for nitrogen. The method of synthesizing the TinPorRuthCat was the same method that Araki et al. developed and although a meso-tetraruthenated product was synthesized (14), many researchers were not able to synthesize a product with good purity. Engelmann reported that using glacial acetic acid competed with pyridyl groups for solvent protons that caused the formations of other products with different substituents. 12 M acetic acid was used instead of glacial acetic acid (17.4 M) to prevent pyridyl groups competing for
solvent protons, but this probably still a problem with the TinPorRuthCat and would cause the error in the percentages of carbon and nitrogen. The TinPorRuthCat that was synthesized is probably a porphyrin that has unknown amounts of ruthenium complexes, but the complete composition of the product cannot be determined without further analysis.
CHAPTER 2: PHOTO-TRANSFORMATION OF 1-NITROPYRENE

2.1. Introduction

There are approximately 15.5 million people (31) living in the United States today suffering from cancer. This number is expected to rise to 17 million by the end of this year. Due to the widespread occurrence and the severity of cancer, scientists from all over the world are conducting a lot of research towards treating and detecting the various forms of cancer. Many of the existing treatments for cancer are almost as bad as the disease itself because of the detrimental side effects (32). In addition to side effects, another major challenge is that these treatments are not very target selective, meaning that they attack normal cells as well as cancer cells (33). Photodynamic therapy (PDT) was developed in the 1990’s (34) to provide a cancer treatment that did not have the side effects and selectivity issues usually associated with other cancer treatments. PDT uses a visible light source, usually a longer wavelength laser (35), to excite a photosensitive molecule, called a photosensitizer. Photosensitizers are injected into the body where it is localized in cancer cells. Then a laser irradiates the photosensitizer,
exciting the molecule to a higher energy state. The excited photosensitizer molecules then release the excess energy into the surroundings. Dissolved oxygen (O$_2$) in the bodily fluid at the target plays an important role in PDT because the excess energy released from excited photosensitizer usually will excite ground state dissolved O$_2$ to form reactive singlet oxygen ($^1$O$_2$). Singlet oxygen is a short-lived and very fast, reactive oxygen species (ROS) that has been shown to cause cell death (36). The current photosensitizers that are approved by the FDA are Photofrin®, Levulan® and Matvix® (37). The main one that is used is Photofrin® with Levulan® and Matvix® used topically to treat skin cancers (37). Although PDT has several advantages, there are some disadvantages that need to be improved upon to make PDT a viable clinical method for cancer treatment. The major challenges of the current PDT treatment are dependent on the specific wavelength of light and tumor hypoxia. The specific wavelength of light that is used to excite the photosensitizer molecules are important because longer wavelengths are able to penetrate body tissue better than shorter wavelengths (12). This restricts the type of light that could be used; the light source needs enough energy to excite the molecule but needs to be of long enough wavelength to penetrate through the skin to reach cancerous tumors. The major problem which limits PDT in clinical applications is tumor hypoxia, lack of adequate oxygen (O$_2$) at the target site. PDT can only be used in areas of the body that have access to oxygen which is a major problem in large cancer growths because cancer cells tend to create an oxygen poor environment (38). Thus, researchers around the world are trying to develop treatment methods that can be
effective for hypoxic tumors. There is a considerable amount of literature in which PDT is combined with other chemotherapeutic methods for targeting hypoxic tumors. Currently, there is no successful method that addresses all the major challenges of PDT.

We investigated, 1-nitropyrene (Figure 11), to determine if it can act as a photosensitizer and as a chemotherapeutic agent. 1-nitropyrene is a polyaromatic hydrocarbon (PAH) and is mostly found in the incomplete combustion of diesel exhaust.

![Figure 11: Structure of 1-nitropyrene.](image)

1-nitropyrene has been shown to be photo-transformed by visible light (39). Zulma et al. reported that 1-nitropyrene’s photodegradation as well, by UV light in various organic solvents including nonpolar, protic and aprotic polar solvents (40). Several photodegraded products have been identified (1-hydroxypyrene, methoxy pyrene, etc.) in polar organic solvents. Several mechanisms have been proposed for explaining the different product formations (40). For example, upon absorbing UV radiation, 1-nitropyrene forms a nitrosopyrene intermediate, which subsequently converts into hydroxypyrene (41).
As stated above, photodynamic therapy (PDT) requires three components to effectively kill cancer cells. It needs a photosensitizer that will localize in cancer cells, a visible light source that can irradiate the photosensitizer, and an excess of O\textsubscript{2} to create singlet oxygen (\textsuperscript{1}O\textsubscript{2}) which is responsible for apoptosis of cancer cells (42). If all three cannot be achieved, singlet oxygen will not be created, and PDT would be essentially ineffective as a cancer treatment method.

Singlet oxygen (\textsuperscript{1}O\textsubscript{2}) is a reactive oxygen species that is higher in energy than O\textsubscript{2}. The energy difference occurs because both electrons in the singlet state are spin paired in the antibonding molecular orbitals while the ground state has the electrons non-spin paired, shown in Figure 12. This spin-pairing makes singlet oxygen 95 kJ/mol higher in energy compared to ground state oxygen (43) and is responsible for the increase in reactivity of \textsuperscript{1}O\textsubscript{2}.

![Molecular orbital diagram comparison of the valence electrons of oxygen to singlet oxygen.](image)

**Figure 12:** Molecular orbital diagram comparison of the valence electrons of oxygen to singlet oxygen.
In quantum mechanics, the singlet state refers to the multiplicity of the oxygen molecule and is determined by the formula $2S + 1$ where $S$ refers to the total spin angular momentum. The spin of electrons can only be $\pm \frac{1}{2}$; therefore, the total spin is equal to the total spin amount for all the electrons. For singlet oxygen one electron is spin up ($+\frac{1}{2}$) and one electron is spin down ($-\frac{1}{2}$) so $2*(0) + 1 = 1$. For ground state oxygen both electrons are spin up ($+\frac{1}{2}$) so $S = \frac{2}{2}$, giving a multiplicity of 3 which is referred to as a triplet state.

Singlet oxygen can be determined by its emission band at around 1270 nm (43). Since the 1270 nm emission is usually very weak and can be missed, the measurement of singlet oxygen will be determined by using the fluorescent probe Singlet Oxygen Sensor Green (SOSG®). The probe consists of a dyad of fluorescein and anthracene moieties that is non-fluorescent, Figure 13. In the presence of singlet oxygen the non-fluorescent probe becomes fluorescent and can be detected around 525 nm. SOSG is a very selective for singlet oxygen and negligibly reacts with other ROS (44).

![SOSG reaction with singlet oxygen to form a fluorescent product.](image)

**Figure 13:** SOSG reaction with singlet oxygen to form a fluorescent product.
Hydroxy radicals (ȮH) are another type of ROS that have an unpaired electron. This makes ȮH very reactive (10^9s^-1 in vivo) (45) and very destructive to any macromolecules. Recent literature (46) indicated that the generation of ȮH in solution can be detected by using the fluorescence probes 3’-(p-aminophenyl) fluorescein (APF) and 3’-(p-hydroxyphenyl) fluorescein (HPF). Both probes have a marked response for detecting ROS (Table 2) (47) with APF usually having a greater response than HPF. These probes have very small responses to light and large responses to ȮH and ONOO⁻ making them useful in detection of ȮH. However, they cannot be used to quantify the amount of ȮH being produced without minimizing the other possible ROS in solution.

<table>
<thead>
<tr>
<th>ROS</th>
<th>ROS Generation Method</th>
<th>APF</th>
<th>HPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ȮH</td>
<td>100 μM of ferrous perchlorate (III) and 1 mM H₂O₂</td>
<td>1200</td>
<td>730</td>
</tr>
<tr>
<td>ONOO⁻</td>
<td>3 μM (final) of ONOO⁻</td>
<td>560</td>
<td>120</td>
</tr>
<tr>
<td>OCl⁻</td>
<td>3 μM (final) of -OCl</td>
<td>3600</td>
<td>6</td>
</tr>
<tr>
<td>¹O₂</td>
<td>100 μM of 3-(1,4-dihydro-1,4-epidioxy-1-naphthyl) propionic acid</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Ȯ₂⁻</td>
<td>100 μM of KO2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>100 μM of H₂O₂</td>
<td>&lt;1</td>
<td>2</td>
</tr>
<tr>
<td>NO</td>
<td>100 μM of 1-hydroxy-2-oxo-3(3aminopropyl)-3-methyl-1-</td>
<td>&lt;1</td>
<td>6</td>
</tr>
<tr>
<td>ROO⁻</td>
<td>100 μM of 2,2’-azobis(2-amidinopropane), dhidrochloride (AAPH)</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Auto-oxidation</td>
<td>2.5 hours exposure to fluorescent light source</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

**Table 2:** Fluorescence response of HPF and APF to various reactive oxygen species (ROS).
Huang, et. al. summarized the recent development of reactive nitrogen donors and the anticancer drugs that are used in conjunction with nitrogen oxygen (NO) donors (48). Singh et al. showed that N-Nitrosothioproline (NTHP) conjugated magnetic nanoparticles can release a small amount of NO for killing cancerous cells without any toxic effect (49). Despite some success, there is still a great need for a new generation of NO donor-based therapy with controlled release of appropriate levels of NO at the target site to kill the cancer cells. The generation of NO can be detected by using the DAR-4M probe which is another type of fluorescent probe when excited 560 nm in the presence of NO species will fluoresce at 575 nm (50).

There is a need for a dual photosensitizer that can generate multiple reactive species. Fukuzumi, et al. reported that Metal Bacteriochlorins photosensitizers are dual photosensitizers. These photosensitizers can produce singlet oxygen and superoxide reactive oxygen species (51). However, these photosensitizers are ineffective in producing singlet oxygen and superoxide in absence of oxygen (O2) under visible light irradiation. Thus, there is an increasing interest in finding photosensitizers which are capable of generating singlet oxygen and hydroxyl radicals in both aerobic and anaerobic conditions under visible light irradiation. That is the reason for the research on 1-nitropyrene to determine if it is capable of producing singlet oxygen, hydroxy radicals and NO species in solution.
Currently there is no literature on the photodegradation of 1-nitropyrene directly producing singlet oxygen, hydroxyl radicals or NO species. However, there are indirect methods that indicate that the photodegradation of 1-nitropyrene and its derivatives go through a likely singlet oxygen and free radical process. This is shown by Zang et al. (52) where it was reported that 1-aminopyrene is likely undergoing a singlet oxygen and free radical process by using histidine, a singlet oxygen quencher, upon light irradiation. In the light irradiation of 1-aminopyrene the photo-transformation rate decreased in the presence of histidine, implying that singlet oxygen was being produced. This was further analyzed by adding both histidine and NaN$_3$ a radical quencher, to the photo-transformation of 1-aminopyrene, slowing the process 2.5 times the rate of just histidine, implying that singlet oxygen and other radicals were being produced. Another article by Muck et. al (53) used three concentrations of H$_2$O$_2$ ($5.0 \times 10^{-4}$, $5.0 \times 10^{-3}$ and $5.0 \times 10^{-2}$ M) to study indirectly the photo-transformation of 1-nitropyrene upon visible light irradiation. H$_2$O$_2$ is known to go through a direct photolysis (wavelength <340 nm) to produce hydroxy radicals. These extra radicals should increase the rate of photo-transformations if the reaction goes through a radical process. The photo-transformation rate was shown to increase in the presence of H$_2$O$_2$ ($1.0 \times 10^{-4}$ M) from $1.41 \times 10^{-4}$ s$^{-1}$ to $2.8 \times 10^{-4}$ s$^{-1}$ mean while the reaction with the higher concentrations ($5.0 \times 10^{-3}$ and $5.0 \times 10^{-2}$ M) produced rates that were slightly faster with a rate of $1.7 \times 10^{-4}$ s$^{-1}$ for both concentrations.
2.2. Experimental Section

2.2.1. Materials and Instruments. All reagents obtained were HPLC quality and used without any purification. A Water Technologies deionization system was used to obtain deionized H$_2$O. Most reagents were purchased from Sigma Aldrich, USA including; 1-nitropyrene, 1-hydroxypyrene, dimethylformamide (DMF), dimethylsulfonate (DMSO), ethanol (EtOH), chloroform, dichloromethane (DCM), 1,2-dichloroethane (DCE), 35 % H$_2$O$_2$ and sodium azide (NaN$_3$). Hexane, acetone, and acetonitrile were purchased from VWR International, USA. Methanol (MeOH) and the fluorescent probe; diaminohodamine-4M (DAR-4M) were purchased from EMD Millipore Corp, USA. Iron (III) chloride, Iron (II) chloride and cyclohexane were purchased from Flinn Scientific, USA. The other fluorescent probes; singlet oxygen green sensor (SOSG), 3’-(p-aminophenyl) fluorescein (APF), and 3’-(p-hydroxyphenyl) fluorescein (HPF), were obtained from Invitrogen, USA. UV-Visible spectra were recorded from a double beam spectrophotometer model number UV-2550( PC) by the Shimadzu Corporation, Japan. Fluorescent spectra were recorded by a Perkin-Elmer, USA LS-55 at room temperature. Photo-transformation experiments were carried out with a Rayonet photochemical reactor model number RPR-100 manufactured by The Southern New England Ultraviolet Company, USA (wavelength range 185-575 nm). Two green lasers (447 and 473 nm;
model numbers; MDL-III-447-20mW-15067137 and MBL-III-473nm-20mW-15050686) and two blue lasers (532 and 556 model numbers; MGL-III-532-20mW-15077107 and MGL-FN-556-20mW-15070827) purchased from Changchun New Industries Optoelectronics Tech. Co, Ltd. China.

2.2.2. Photo-transformation of 1-Nitropyrene in Visible Light in

**Dimethylformamide (DMF) in Aerobic Conditions.** A 3 mL solution of 1-nitropyrene (1.0 x 10^{-4} M) in DMF was prepared at room temperature (RT) under normal atmospheric pressure. The photo-transformation of 1-nitropyrene was carried out in a Rayonet photochemical reactor (185 nm - 575 nm) for approximately 30 mins. The photo-transformation of 1-nitropyrene was monitored by UV-Vis spectra obtained in 2 min intervals.

2.2.3. Photo-transformation of 1-Nitropyrene in Visible Light in

**Dimethylformamide (DMF) in Anaerobic Conditions.** A 3 mL solution of 1-nitropyrene (1.0 x 10^{-4} M) in DMF was prepared at room temperature (RT) under normal atmospheric pressure. The resulting solution was purged with argon gas for 20 mins to remove any traces of oxygen in solution. The photo-transformation of 1-nitropyrene was carried out in a Rayonet photochemical reactor (185 nm - 575 nm) for approximately 30 min. The photo-transformation of 1-nitropyrene was monitored by UV-Vis spectra obtained in 2 min intervals.
2.2.4. The Effect of Various Solvents on the Rate of Photo-transformation of 1-Nitropyrene. A 3 mL solution of 1-nitropyrene (1.0 x 10^{-4} M) was prepared in various solvents that include nonpolar (hexane and cyclohexane), polar aprotic (acetone, DMF, acetonitrile, and DMSO), halogenated (chloroform, DCM, and DCE) and polar protic solvent (ethanol) at room temperature and normal atmospheric environment. The 1-nitropyrene solution was photo-transformed with a Rayonet photochemical reactor for about 20 mins. UV-Vis absorption was recorded in intervals of 2 min. A similar experimental procedure was followed for the photo-transformation under anaerobic conditions. A 3 mL solution of 1-nitropyrene (1.0 x 10^{-4} M) was prepared in various solvents and then each solution was purged with argon for 20 mins in a sealed cuvette. The solution was then irradiated with a Rayonet photochemical reactor for 20 mins with the UV-Vis spectrum recorded every 2 mins. The experiment was done in triplicate for aerobic/anaerobic to establish a reasonable first order photo-transformation rate of 1-nitropyrene in each solvent.

2.2.5. Photo-transformation of 1-Nitropyrene at Different Wavelengths of Excitation. A 3 ml solution of 1-nitropyrene (1.0 x 10^{-4} M) was prepared in either DMF or EtOH. The cuvette was then irradiated continuously with a laser of a specific wavelength (447, 473, 532 and 556 nm) for 20 mins. The UV-Vis spectrum was recorded in 2 min intervals for 20 mins. Photo-transformation of 1-nitropyrene in anaerobic conditions was monitored similarly. The solution of 1-nitropyrene was purged with argon in a sealed cuvette for 20 mins before exposing to light. Aerobic and
anaerobic experiments were done in triplicate with the spectra used to calculate reasonable photo-transformation rates of 1-nitropyrene for each wavelength of light used.

2.2.6. Effect of H$_2$O$_2$ on the Photodegradation of 1-Nitropyrene. A stock solution of H$_2$O$_2$ (3.6 x 10$^{-2}$ M) was prepared with ultrapure H$_2$O by adding 31.3 μL of 35 % (w/w) H$_2$O$_2$ diluted with 10 mL of ultrapure H$_2$O. The H$_2$O$_2$ stock solution was then added at 0.83 μL, 83.3 μL and 833 μL to make three experimental concentrations of H$_2$O$_2$, 1.0 x 10$^{-2}$, 1.0 x 10$^{-3}$ and 1.0 x 10$^{-5}$ M, respectively in a 3 mL solution of 1-nitropyrene (1.0 x 10$^{-4}$ M) in EtOH. The solutions were photo-transformed with a Rayonet photochemical reactor for about 20 mins. UV-Vis absorption was recorded in 2 min intervals. For anaerobic conditions, solutions were purged with argon for 20 mins in a sealed cuvette before a visible spectrum was recorded. The experiment was done in triplicate for both aerobic/anaerobic to establish a suitable rate for the photodegradation of 1-nitropyrene with different concentrations of H$_2$O$_2$ in EtOH.

2.2.7. Detection of Singlet Oxygen. To measure singlet oxygen, the fluorescent probe SOSG by Invitrogen was used. The SOSG solution was prepared by adding 33 μL of MeOH to a single vial of 100 μg of SOSG as by following the direction as stated in the product’s manual. The experimental solution was prepared by taking 6 μL of SOSG solution and placing it in a 3 mL solution of 1-nitropyrene (1.0 x 10$^{-4}$ M) in MeOH. Another solution was prepared in a similar manner but with 1-hydroxypyrene (1.0 x 10$^{-4}$ M). All solutions were prepared at room temperature and normal atmospheric pressure.
Solutions were then irradiated with a 447 nm laser. The fluorometer was set to excite at a wavelength of 504 nm with the excitation slit width set at 8 nm and an emission slit width of 5 nm. Fluorescent spectra were obtained in 5 min intervals for 30 mins.

2.2.8. Detection of Hydroxyl Radicals. Hydroxyl radicals were measured by using two different fluorescent probes, APF and HPF by Invitrogen. The probes were received in a 5mM solution in DMF. The experimental solution was prepared by taking 2 μL of HPF or APF solution and placing it in a 3 mL solution of 1-nitropyrene (1.0 x 10^{-4} M) in DMF. Another solution was prepared in a similar manner with 1-hydroxypyrene (1.0 x 10^{-4} M). All solutions were prepared at room temperature and normal atmospheric pressure. Solution were then irradiated with a 447 nm laser. The fluorometer was set to excite at a wavelength of 490 nm with the excitation slit width set at 10 nm and an emission slit width of 7 nm. Fluorescent spectra were obtained for 30 mins in 5 min intervals.

2.2.9. In Vitro Effects of 1-Nitropyrene and 1-Hydroxypyrene in BL21 E. coli. The toxicity of singlet oxygen generated from photosensitization reaction of 1-nitropyrene (1.0 x 10^{-4} M) was studied by comparing cell growth of BL21 E. coli. bacteria under light irradiation with a control under dark conditions. E. coli cells in Luria Broth (LB) Lennox formulation were grown in an incubator set at 28 °C (to match the measured temperature of the Rayonet reaction) until the beginning of the E. coli exponential growth phase (A_{600} ≈ 0.2). A 1.0 mL aliquot was centrifuged for 5 mins at 10,000 x g. The supernatant was removed and 500 μL of sterile water was used to wash the cells to remove the broth. The
sample was centrifuged again for 5 mins at 10,000 x g. We resuspended in 495 μL of sterile water and then added 5 μL of sterile water (control), DMF (control), 1-nitropyrene or 1-hydroxypyrene. Two aliquots (495 μL sterile water + 5 μL of sample) were taken with one being placed in a photochemical reactor and the other aliquot in the dark, to prevent light irradiation, for 30 mins. After 30 mins, 20 μL of the aliquots from both the photochemical reactor and in the dark was spread evenly onto a LB agar plate. The samples were labeled as “light” for the aliquot that was in the reactor or as “dark” for the aliquot that was placed in the dark. Both plates were inverted and placed in an incubator set at 28°C for up to 48 hours. Each plate was qualitatively analyzed by visual inspection of the relative number of E. coli colonies.

2.3. Results and Discussion

2.3.1. Photo-Transformation of 1-Nitropyrene in Visible Light in

Dimethylformamide (DMF) in Aerobic Conditions. Figure 14 showed the absorption of 1-nitropyrene (1.0 x 10^{-4} M) in DMF before and after the solution was irradiated with visible light. The absorption spectrum of 1-nitropyrene has three major absorptions at 292, 375, and 408 nm. Upon illumination of the 1-nitropyrene solution, the absorbance at 292, 375, and 408 nm began to decrease with new absorption peaks increasing at 281 nm and in the range of 324-355 nm. The change in absorbance suggests that 1-nitropyrene was photo-transformed into other derivatives of pyrene under visible light irradiation.
Zulma et al (40) reported that 1-nitropyrene produced hydroxypyrenes, hydroxynitropyrenens, and/or pyrenedions upon UV light irradiation. Further studies suggested that the major product in the photo-transformation of 1-nitropyrene is 1-hydroxypyrene. There are three isosbestic points located at 284, 323, and 388 nm, which have the absorptions overlap each other. This is a good indicator that the reaction converts directly into the product without going through an intermediate. These points represent where the reactant and product absorptions and molar absorptivity are the same but the ratio between reactants and products are changing. In this specific it case 1-nitropyrene is directly being photo-transformed into 1-hydroxypyrene (54). In order to characterize the photo-transformation product of 1-nitropyrene, the absorption spectra of commercially available 1-hydroxypyrene was recorded. As depicted in Figure 15, the absorption peaks of 1-hydroxypyrene very closely overlaid with the absorption peaks of the photo-transformation product of 1-nitropyrene. Therefore, it appears that 1-hydroxypyrene is one of the major photo-transformed products under stated reaction conditions. Further characterization of the product would need to be carried out to show 1-hydroxypyrene is the product. An experiment was carried out to determine the stability of 1-hydroxypyrene under visible light irradiation. Figure 16 showed a very negligible change in absorption of 1-hydroxypyrene under visible light irradiation. This data suggests that 1-hydroxypyrene is stable under stated reaction conditions. Other photo-transformed products might have been formed in small quantities, however, the full
characterization of other products is out of the scope of this study due to lack of proper instruments in the department.

2.3.2. Photo-Transformation of 1-Nitropyrene in Visible Light in Dimethylformamide (DMF) in Anaerobic Conditions. To determine the necessity of oxygen for photo-transformation of 1-nitropyrene, an experiment was carried out in which the solution of 1-nitropyrene was purged with argon and then irradiated with visible light. Figure 17 shows the changes of absorbance of 1-nitropyrene under visible light in anaerobic conditions. Results were similar to those obtained in the photo-transformation of 1-nitropyrene in aerobic conditions. Upon visible light irradiation, under anaerobic conditions the solution of 1-nitropyrene most likely produced 1-hydroxypyrene as a major product as evidenced by the gradual reduction of absorptions at 292, 375, and 408 nm and increases in absorptions at 281, in the range of 324-355 nm and at 388 nm, matching the same absorptions in 1-hydroxypyrene. There were also three isosbestic points at 286, 315, and 370 nm similar to the 1-nitropyrene spectra under aerobic conditions. These points represent 1-nitropyrene is likely forming 1-hydroxypyrene but isosbestic points are a good indicator that there is one to one conversion of reactants to products (54).

2.3.3. The Effect of Various Solvents on the Rate of Photo-Transformation of 1-Nitropyrene. To examine the solvent effect on the photo-transformation of 1-nitropyrene, several photo-transformation experiments were conducted in various types
of organic solvents (protic and nonprotic solvents) under both aerobic and anaerobic conditions. Figures 18-37 show the changes of absorption of 1-nitropyrene in organic solvents in aerobic and anaerobic conditions. The loss of absorption of 1-nitropyrene (monitored at 388 nm) was observed to follow first order kinetics for most solvents. The rate constants were calculated by linear regression fitting of the experimental data (calculated absorbance values as $ln(A_0/A)$ vs $t$, where $A_0$ is the absorbance at time 0, and $A$ is the absorbance at time $t$). Table 3 lists all the rate constants ($k_{obs}$ (s$^{-1}$)) of photo-transformation of 1-nitropyrene studied in various solvents under aerobic and anaerobic conditions.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Aerobic $k_{obs}$(s$^{-1}$)</th>
<th>R$^2$</th>
<th>Anaerobic $k_{obs}$(s$^{-1}$)</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>$8.667 \times 10^{-5} \pm 0.0005$</td>
<td>0.9891</td>
<td>$8.333 \times 10^{-5} \pm 0.0004$</td>
<td>0.9998</td>
</tr>
<tr>
<td>DCE</td>
<td>$1.933 \times 10^{-4} \pm 0.0003$</td>
<td>0.9997</td>
<td>$1.317 \times 10^{-4} \pm 0.0005$</td>
<td>0.9974</td>
</tr>
<tr>
<td>chloroform</td>
<td>$2.000 \times 10^{-5} \pm 0.0005$</td>
<td>0.9575</td>
<td>$1.167 \times 10^{-4} \pm 0.0004$</td>
<td>0.9984</td>
</tr>
<tr>
<td>cyclohexane</td>
<td>$4.833 \times 10^{-5} \pm 0.0004$</td>
<td>0.9920</td>
<td>$3.167 \times 10^{-5} \pm 0.0002$</td>
<td>0.9474</td>
</tr>
<tr>
<td>hexane</td>
<td>$1.083 \times 10^{-4} \pm 0.0010$</td>
<td>0.9963</td>
<td>$2.667 \times 10^{-5} \pm 0.0002$</td>
<td>0.9826</td>
</tr>
<tr>
<td>acetone</td>
<td>$4.167 \times 10^{-5} \pm 0.0003$</td>
<td>0.9372</td>
<td>$5.667 \times 10^{-5} \pm 0.0006$</td>
<td>0.9877</td>
</tr>
<tr>
<td>DMF</td>
<td>$4.500 \times 10^{-5} \pm 0.0002$</td>
<td>0.9996</td>
<td>$4.667 \times 10^{-5} \pm 0.0002$</td>
<td>0.9897</td>
</tr>
<tr>
<td>DMSO</td>
<td>$7.333 \times 10^{-5} \pm 0.0001$</td>
<td>0.9977</td>
<td>$4.500 \times 10^{-5} \pm 0.0005$</td>
<td>0.9957</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>$1.083 \times 10^{-4} \pm 0.0010$</td>
<td>0.9963</td>
<td>$6.833 \times 10^{-5} \pm 0.0001$</td>
<td>0.9907</td>
</tr>
</tbody>
</table>

Table 3: Solvent effects of photo-transformation of 1-nitropyrene at 388 nm at RT and atmospheric pressure.

As shown in Table 3, the rate of photo-transformation of 1-nitropyrene had a maximum in the chlorinated solvent DCE with $1.933 \times 10^{-4}$ s$^{-1}$ and $1.317 \times 10^{-4}$ s$^{-1}$ under aerobic
and anaerobic conditions, respectively. The aprotic solvent acetone had the second highest rates of $1.083 \times 10^4$ s$^{-1}$ and $1.167 \times 10^4$ s$^{-1}$. The other aprotic solvents, DMF, acetonitrile, and DMSO, had rates of approximately $4.722 \times 10^5$ s$^{-1}$. The protic solvent, ethanol had rates of $7.333 \times 10^5$ s$^{-1}$ and $6.833 \times 10^5$ s$^{-1}$ in aerobic and anaerobic conditions. Interestingly, cyclohexane and chloroform does not follow first order kinetics under aerobic conditions (Figure 24 & 22) but first order kinetics were observed under anaerobic conditions (Figure 25 & 23).

2.3.4. Photo-Transformation of 1-Nitropyrene in DMF and EtOH at Different Wavelengths of Excitation. In order to examine the light effect on the photo-transformation of 1-nitropyrene, a solution of 1-nitropyrene in DMF was irradiated at various wavelengths (Figures 38-45). Table 4 shows the rate of photo-transformation of 1-nitropyrene at four different excitation wavelengths; 447, 473, 532 and 556 nm in DMF.

<table>
<thead>
<tr>
<th>Laser Wavelength (nm)</th>
<th>DMF</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic</td>
<td>Anaerobic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rate Constant, $k_{obs}$ (s$^{-1}$)</td>
<td>$R^2$</td>
<td>Rate Constant, $k_{obs}$ (s$^{-1}$)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>447</td>
<td>$3.500 \times 10^{-5} \pm 0.0004$</td>
<td>0.9930</td>
<td>$3.000 \times 10^{-5} \pm 0.0003$</td>
<td>0.9701</td>
</tr>
<tr>
<td>473</td>
<td>$5.000 \times 10^{-6} \pm 0.0001$</td>
<td>0.9040</td>
<td>$3.333 \times 10^{-6} \pm 0.0018$</td>
<td>0.9258</td>
</tr>
<tr>
<td>532</td>
<td>$3.333 \times 10^{-6} \pm 0.0001$</td>
<td>0.8417</td>
<td>$1.667 \times 10^{-6} \pm 0.0001$</td>
<td>0.5662</td>
</tr>
<tr>
<td>556</td>
<td>$3.333 \times 10^{-6} \pm 0.0002$</td>
<td>0.9049</td>
<td>$3.333 \times 10^{-6} \pm 0.0002$</td>
<td>0.9391</td>
</tr>
</tbody>
</table>

Table 4: Photo-transformation rates of 1-nitropyrene at 388 nm with different excitation wavelengths in DMF.
The fastest rate observed was for 447 nm (3.500 x 10^-5 s^-1). This was seen with an ethanol solution of 1-nitropyrene as well (Table 5 and Figures 46-53) with 447 nm having the highest rate (2.167 x 10^-5 s^-1). For the higher wavelengths, 473, 532, and 556, the rate of photo-transformation of 1-nitropyrene was observed to drop drastically.

<table>
<thead>
<tr>
<th>Laser Wavelength (nm)</th>
<th>EtOH</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic</td>
<td>Rate Constant, k_{obs}(s^{-1})</td>
<td>R^2</td>
<td>Anaerobic</td>
<td>Rate Constant, k_{obs}(s^{-1})</td>
<td>R^2</td>
</tr>
<tr>
<td>447</td>
<td>2.167 x 10^{-5} ± 0.0001</td>
<td>0.9975</td>
<td>2.667 x 10^{-5} ± 0.0001</td>
<td>0.9931</td>
<td></td>
<td></td>
</tr>
<tr>
<td>473</td>
<td>6.667 x 10^{-6} ± 0.0002</td>
<td>0.8949</td>
<td>3.333 x 10^{-6} ± 0.00</td>
<td>0.7881</td>
<td></td>
<td></td>
</tr>
<tr>
<td>532</td>
<td>3.333 x 10^{-6} ± 0.0001</td>
<td>0.9404</td>
<td>5.000 x 10^{-6} ± 0.0002</td>
<td>0.8615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>556</td>
<td>1.667 x 10^{-6} ± 0.0001</td>
<td>0.9200</td>
<td>5.000 x 10^{-6} ± 0.0001</td>
<td>0.8749</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 5: Photo-transformation rates of 1-nitropyrene at 388 nm with different excitation wavelengths in ethanol.*

For example, the rate of photo-transformation of 1-nitropyrene in DMF at 556 nm was measured to be 3.333 x 10^{-6} s^{-1}. This is probably due to the fact that 1-nitropyrene absorbs strongly at 294, 381 and 407 nm and after 470 nm the absorption of 1-nitropyrene practically drops to zero. This was done to compare a protic and aprotic solvent as a representation of all solvents with DMF and EtOH being chosen because they both had high rates and followed first order kinetics.

### 2.3.5. Effect of H₂O₂ on the Photo-Transformation of 1-Nitropyrene in Ethanol.

In order to examine the photo-transformation properties of 1-nitropyrene in a hydrogen peroxide rich environment, a solution of 1-nitropyrene was irradiated at various concentrations under aerobic and anaerobic conditions. H₂O₂ undergoes a photolysis to produce hydroxy radicals that should increase the photo-transformation rate of 1-
nitropyrene upon light irradiation. Three different concentrations of H$_2$O$_2$ (1.0 x 10$^{-2}$, 1.0 x 10$^{-3}$ and 1.0 x 10$^{-5}$ M) in EtOH were tested (Figures 54-59). The rates of photo-transformation of 1-nitropyrene in the presence of hydrogen peroxide are summarized in Table 6.

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Aerobic</th>
<th></th>
<th></th>
<th>Anaerobic</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate Constant, k$_{obs}$(s$^{-1}$)</td>
<td>R$^2$</td>
<td>Rate Constant, k$_{obs}$(s$^{-1}$)</td>
<td>R$^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 x 10$^{-2}$</td>
<td>4.333 x 10$^{-5}$ ± 0.0003</td>
<td>0.9940</td>
<td>4.500 x 10$^{-5}$ ± 0.0001</td>
<td>0.996</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 x 10$^{-3}$</td>
<td>6.667 x 10$^{-5}$ ± 0.0005</td>
<td>0.9944</td>
<td>5.667 x 10$^{-5}$ ± 0.0001</td>
<td>0.995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 x 10$^{-5}$</td>
<td>1.067 x 10$^{-4}$ ± 0.0024</td>
<td>0.9960</td>
<td>5.167 x 10$^{-5}$ ± 0.0014</td>
<td>0.997</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Effects of three different concentrations of hydrogen peroxide on photo-transformation of 1-nitropyrene at 388 nm in EtOH.

At the highest H$_2$O$_2$ concentration (1.0 x 10$^{-2}$ M), the rate of photo-transformation of 1-nitropyrene was slowed to 4.333 x 10$^{-5}$ s$^{-1}$ under aerobic and 4.500 x 10$^{-5}$ s$^{-1}$ under anaerobic conditions. This was compared to the rate of 1-nitropyrene in ethanol, 7.333 x 10$^{-5}$ s$^{-1}$ under aerobic and 6.833 x 10$^{-5}$ s$^{-1}$ under anaerobic conditions (Table 3).

Interestingly, an increased rate of photo-transformation of 1-nitropyrene was observed at the lower hydrogen peroxide concentration (1.0 x 10$^{-5}$ M) with an aerobic rate constant of 1.067 x 10$^{-4}$ s$^{-1}$ and a rate constant of 5.167 x 10$^{-5}$ s$^{-1}$ under anaerobic conditions. The intermediate H$_2$O$_2$ concentration (1.0 x 10$^{-3}$ M) rate was in between 1.0 x 10$^{-5}$ and 1.0 x 10$^{-2}$ M with an aerobic rate of 6.667 x 10$^{-5}$ s$^{-1}$ and an anaerobic rate of 5.667 x 10$^{-5}$ s$^{-1}$.

This rate trend was also seen in the article by Muck (53) where the lowest concentration
(5.0 x 10^{-4} M) increased the rate meanwhile the higher concentrations (5.0 x 10^{-3} and 5.0 x 10^{-2} M) where about the same rate as without H_2O_2. Hydrogen peroxide, after creating hydroxy radicals, can further react with itself to form a superoxide which is less reactive than hydroxy radicals and thereby not changing the rate or even inhibiting the reaction (55).

2.3.6. Detection of Singlet Oxygen. The generation of \(^1\)O_2 was detected by using singlet oxygen sensor green (SOSG). Figure 60 shows that the fluorescence intensity of SOSG gradually increased with increasing amount of irradiation time, indicating \(^1\)O_2 generation in the methanol solution of 1-nitropyrene. The fluorescence spectra of SOSG was recorded immediately after irradiation. When the 1-nitropyrene solution was irradiated with a 447 nm laser, the fluorescence intensity at 525 nm increased, indicating generation of singlet oxygen in solution. Additional blank experiments revealed that the SOSG’s fluorescence intensity at 525 nm did not increase in the absence of light. The fluorescence data gives a strong indication that the solution of 1-nitropyrene generates singlet oxygen (\(^1\)O_2) under visible light irradiation. Figure 61 shows the fluorescent spectra of 1-nitropyrene in MeOH excited at 407 nm. The fluorescent spectrum has a \(\lambda_{max}\) around 496 with decreasing intensities until about 600 nm. This means 1-nitropyrene does have some emission with SOSG (525 nm) emission wavelength. This could be further tested by conducting another experiment of monitoring the fluorescence of 1-nitropyrene after irradiating it with light to see if the intensity of 1-nitropyrene increased but this was not possible at the time. Similar experiments were carried out to
detect the generation of \( ^1\text{O}_2 \) by 1-hydroxypyrene (Figure 62). The fluorescence intensity of SOSG gradually increased with increasing irradiation time, indicating the generation of \( ^1\text{O}_2 \) in the methanol solution of 1-hydroxypyrene. An increase in the fluorescence intensity of SOSG at 520 nm was observed, indicating that 1-hydroxypyrene is capable of generating singlet oxygen in solution under visible light irradiation. Figure 63 shows the fluorescent spectrum of 1-hydroxypyrene excited at 387 nm. It clearly shows that 1-hydroxypyrene does not emit light in the range of 500 – 800 nm so the emission at 525 is solely due to SOSG reacting with singlet oxygen generated by irradiating 1-hydroxypyrene with visible light.

2.3.7. Detection of Hydroxyl Radicals. To detect hydroxyl radicals, the fluorescence probes 3’-p-(aminophenyl) fluorescein (APF) and 3’-p-(hydroxyphenyl) fluorescein (HPF) were used. Both of the probes become fluorescent when they are in contact with hydroxyl radicals. Figure 64 shows that the fluorescence intensity of APF gradually increased with increasing amount of irradiation time of a solution of 1-nitropyrene in DMF. The fluorescence intensity at 539 nm gradually increased with increasing amount of irradiation time under aerobic conditions. No evidence of increasing fluorescence intensity of APF at 539 nm was observed in the dark. This data indicates that the solution of 1-nitropyrene generates hydroxyl radicals under visible light irradiation in aerobic conditions. Similar experiments were carried out to determine if the solution of 1-nitropyrene produces hydroxyl radicals in situ in anaerobic conditions under visible light irradiation. Figure 65 shows the change in fluorescence intensity of APF in a 1-
nitropyrene solution when irradiated under anaerobic conditions. The fluorescence intensity of APF shifted to 560 nm and gradually increased in intensity with the amount of irradiation time. A control reaction showed that the fluorescence intensity of APF significantly decreased when the solution of 1-nitropyrene and APF was irradiated in the presence of 1% DMSO (Figure 66). DMSO is usually used to quench hydroxyl radicals in the solution. A similar reaction was carried out by using sodium azide as a hydroxyl radical quencher. As seen in other literature (17), a significant increase in fluorescence intensity of APF at 530 nm was observed upon visible light irradiation of the solution of 1-nitropyrene, APF, and 1 mM NaN₃ (Figure 67). This data clearly indicates that the portion of the fluorescence of APF quenched by DMSO represents the contribution of hydroxyl radicals. In order to determine the generation of hydroxyl radicals produced by 1-hydroxypyrene, a similar APF experiment was conducted. Figure 68 and Figure 69 show the change of fluorescence intensity of APF when the solutions of 1-hydroxypyrene and APF were irradiated with visible light in aerobic and anaerobic conditions, respectively. As seen for 1-nitropyrene, a gradual increase in fluorescence intensity of APF as observed in aerobic and anaerobic solutions. This data suggests that the 1-hydroxypyrene solution generates hydroxyl radicals in situ in aerobic and anaerobic solution. Additional control experiments were conducted to confirm the hydroxyl radical’s generation by using 1% DMSO solution as a hydroxyl radical’s quencher. Figure 70 showed a decrease in fluorescence intensity of the APF/1-hydroxypyrene
solution in the presence of 1% DMSO, similar to the observation with 1-nitropyrene. The fluorescence intensity of APF showed an increase in the presence of NaN₃ (Figure 71).

All experiments were repeated using another type of hydroxyl radical quencher, 3’-p-(hydroxyphenyl) fluorescein (HPF), to detect the generation of hydroxyl radicals (Figures 72-79). Although both probes, APF and HPF, react with the same ROS their reactivity is different (Table 2) with APF having the strongest reactivity with hydroxy radicals but reacts stronger with other ROS than HPF. However, HPF has less reactivity but reacts the strongest with hydroxy radicals. 1-nitropyrene produced similar changes in fluorescence intensity with HPF with increasing intensity at 539 nm under aerobic (Figure 72) and at 560 nm under anaerobic conditions (Figure 73). There is an increase in fluorescence intensity of HPF with 1% DMSO and sodium azide in Figures 74 & 75. 1-hydroxypyrene with HPF exhibited similar trends in aerobic an anaerobic condition (Figures 76-79). These results collectively indicate that hydroxy radicals are produced in the photoinitiated reaction of 1-nitropyrene.

2.3.8. In Vitro Effects of 1-Nitropyrene and 1-Hydroxypyrene in BL21 E. coli. To determine the antibacterial properties of 1-nitropyrene and 1-hydroxypyrene, solutions of 1-nitropyrene and 1-hydroxypyrene were tested for their effects on the growth of E. coli bacteria as a model organism (Figure 80). Figures 80-1 and 80-1a, show E. coli with just water, under dark and light conditions. There is no inhibition of E. coli with sterile water whether under light or dark conditions. Figures 80-C and 80-D show E. coli with the
solvent, DMF. Similar results were obtained with no inhibition of E. coli. with DMF under dark or light conditions. A significant inhibition of the growth of E. coli bacteria was observed when the 1-nitropyrene solution, in DMF, was illuminated with visible light (Figure 80- F). Under dark conditions (Figure 80-E) 1-nitropyrene had negligible inhibition of E. coli bacteria, indicating that 1-nitropyrene is non-toxic to E. coli under dark conditions. Similarly, the treatment of E. coli bacteria with 1-hydroxypyrene showed almost 100% inhibition of growth under visible light irradiation (Figure 80-H). Interestingly, a significant inhibition of the growth was also observed with 1-hydroxypyrene under dark conditions (Figure 80-G). This data suggests that 1-nitropyrene as well as 1-hydroxypyrene are potential antibacterial agents and can inhibit the growth of bacteria, particularly E. coli. Furthermore, this data leads us to conclude that the solution of 1-hydroxypyrene can possess antibacterial properties under dark conditions.

2.4. Conclusion

The product that is formed from the photo-transformation of 1-nitropyrene is likely 1-hydroxypyrene. (Figure 15) The overlay of 1-hydroxypyrene with 1-nitropyrene UV-Vis spectrums after irradiation with visible light, clearly shows an absorption decrease for 1-nitropyrene (294, 378, 409 nm) and an increase in the absorptions associated with 1-hydroxypyrene (281, 352, 368, 388 nm). The overlap of the spectra also has three
distinct isosbestic points which indicates that there is one major product. The SOSG fluorescent probe was used to determine if singlet oxygen was produced from irradiating 1-nitropyrene with visible light. The specific fluorescent intensity of SOSG with 1-nitropyrene increased with irradiation time indicating that singlet oxygen was produced. APF and HPF was used to determine if hydroxy radicals were produced with irradiation of 1-nitropyrene. Like SOSG, fluorescence intensity of APF and HPF with 1-nitropyrene increased with irradiation time, an indicator that hydroxy radicals were produced, although there could be other ROS interacting with APF or HPF. A study of the effect of 1-nitropyrene on the inhibition of E. coli bacteria growth was done. It was determined that 1-nitropyrene did not inhibit growth but only if irradiated with visible light did 1-nitropyrene inhibited bacterial growth.

This proof of concept project provides preliminary data which indicates the photo-transformation of 1-nitropyrene produces 1-hydroxypyrene, 1O2, as well as OH radicals in both aerobic and anaerobic conditions. In additions, this preliminary data provides a potential application for use as an antibacterial method.
Appendix

**Figure 14:** Photo-transformation of 1-nitropyrene (1.0 x 10^{-4} M) in DMF with visible light for 55 min. Blue (0 min)

**Figure 15:** Overlay of absorption spectra of 1-nitropyrene (1.0 x 10^{-4} M) after 28 minutes of light irradiation (yellow) with 1-hydroxypyrene (5.0 x 10^{-5} M) in DMF (green).
Figure 16: The absorption of 1-hydroxypyrene ($5.0 \times 10^{-5}$ M) in DMF vs irradiation time (30 mins).

Figure 17: Photo-transformation of 1-nitropyrene ($1.0 \times 10^{-4}$ M) under anaerobic conditions in DMF for 55 min. Blue (0 min)
Figure 18: Rate constant (k_{obs} \text{ (min}^{-1}) for 1-nitropyrene in DCM at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light.

Figure 19: Rate constant (k_{obs} \text{ (min}^{-1}) for 1-nitropyrene in DCM at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.
Figure 20: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in DCE at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light.

Figure 21: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in DCE at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.
**Figure 22:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in CHCl$_3$ at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light. Did not follow first order kinetics.

**Figure 23:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in CHCl$_3$ at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.
Figure 24: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in cyclohexane at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light. Did not follow first order kinetics.

Figure 25: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in cyclohexane at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.
**Figure 26**: Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in hexane at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light.

**Figure 27**: Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in hexane at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.
Figure 28: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in acetone at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light

Figure 29: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in acetone at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light
Figure 30: Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in DMF at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light.

Figure 31: Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in DMF at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.
Figure 32: Rate constant \( (k_{\text{obs}} \text{ (min}^{-1}) \) for 1-nitropyrene in DMSO at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light.

\[
y = 0.0025x + 0.0042 \\
R^2 = 0.9372
\]

Figure 33: Rate constant \( (k_{\text{obs}} \text{ (min}^{-1}) \) for 1-nitropyrene in DMSO at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.

\[
y = 0.0028x + 0.0048 \\
R^2 = 0.9897
\]
**Figure 34:** Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in acetonitrile at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light.

**Figure 35:** Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in acetonitrile at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light.
**Figure 36:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in aerobic conditions. Monitored for 20 min with irradiation with visible light

**Figure 37:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in anaerobic conditions. Monitored for 20 min with irradiation with visible light
**Figure 38:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in DMF at 388 nm in aerobic conditions. Monitored for 20 min with 447 nm light irradiation.

**Figure 39:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in DMF at 388 nm in anaerobic conditions. Monitored for 20 min with 447 nm light irradiation.
**Figure 40:** Rate constant ($k_{\text{obs}} \text{ (min}^{-1}\text{)}$) for 1-nitropyrene in DMF at 388 nm in aerobic conditions. Monitored for 20 min with 473 nm light irradiation.

**Figure 41:** Rate constant ($k_{\text{obs}} \text{ (min}^{-1}\text{)}$) for 1-nitropyrene in DMF at 388 nm in anaerobic conditions. Monitored for 20 min with 473 nm light irradiation.
**Figure 42:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in DMF at 388 nm in aerobic conditions. Monitored for 20 min with 532 nm light irradiation.

**Figure 43:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in DMF at 388 nm in anaerobic conditions. Monitored for 20 min with 532 nm light irradiation.
**Figure 44:** Rate constant (k<sub>obs</sub> (min<sup>-1</sup>)) for 1-nitropyrene in DMF at 388 nm in aerobic conditions. Monitored for 20 min with 556 nm light irradiation

**Figure 45:** Rate constant (k<sub>obs</sub> (min<sup>-1</sup>)) for 1-nitropyrene in DMF at 388 nm in anaerobic conditions. Monitored for 20 min with 556 nm light irradiation
Figure 46: Rate constant ($k_{\text{obs}} (\text{min}^{-1})$) for 1-nitropyrene in ethanol at 388 nm in aerobic conditions. Monitored for 20 min with 447 nm light irradiation.

![Graph showing the rate constant for 1-nitropyrene in aerobic conditions.]

$y = 0.0013x - 1E-06$
$R^2 = 0.9975$

Figure 47: Rate constant ($k_{\text{obs}} (\text{min}^{-1})$) for 1-nitropyrene in ethanol at 388 nm in anaerobic conditions. Monitored for 20 min with 447 nm light irradiation.

![Graph showing the rate constant for 1-nitropyrene in anaerobic conditions.]

$y = 0.0015x + 0.0018$
$R^2 = 0.9931$
**Figure 48:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in aerobic conditions. Monitored for 20 min with 473 nm light irradiation

**Figure 49:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in anaerobic conditions. Monitored for 20 min with 473 nm light irradiation
**Figure 50:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in aerobic conditions. Monitored for 20 min with 532 nm light irradiation.

**Figure 51:** Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in anaerobic conditions. Monitored for 20 min with 532 nm light irradiation.
**Figure 52:** Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in aerobic conditions. Monitored for 20 min with 556 nm light irradiation.

**Figure 53:** Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene in ethanol at 388 nm in anaerobic conditions. Monitored for 20 min with 556 nm light irradiation.
**Figure 54:** Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene with $1.0 \times 10^{-2}$ M concentration of H$_2$O$_2$ in ethanol at 388 nm in aerobic conditions. Monitored for 20 min with visible light irradiation.

**Figure 55:** Rate constant ($k_{\text{obs}}$ (min$^{-1}$)) for 1-nitropyrene with $1.0 \times 10^{-2}$ M concentration of H$_2$O$_2$ in ethanol at 388 nm in anaerobic conditions. Monitored for 20 min with visible light irradiation.
Figure 56: Rate constant ($k_{obs} \text{ (min}^{-1}\text{)}$) for 1-nitropyrene with $1.0 \times 10^{-3}$ M concentration of H$_2$O$_2$ in ethanol at 388 nm in aerobic conditions. Monitored 20 min with visible light irradiation.

Figure 57: Rate constant ($k_{obs} \text{ (min}^{-1}\text{)}$) for 1-nitropyrene with $1.0 \times 10^{-3}$ M concentration of H$_2$O$_2$ in ethanol at 388 nm in anaerobic conditions. Monitored 20 min with visible light irradiation.
Figure 58: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene with 1.0 x 10$^{-5}$ M concentration of H$_2$O$_2$ in ethanol at 388 nm in aerobic conditions. Monitored 20 min with visible light irradiation

Figure 59: Rate constant ($k_{obs}$ (min$^{-1}$)) for 1-nitropyrene with 1.0 x 10$^{-5}$ M concentration of H$_2$O$_2$ in ethanol at 388 nm in anaerobic conditions. Monitored 20 min with visible light irradiation
**Figure 60:** Emission spectrum of 1-nitropyrene with SOSG in DMF with irradiation at 447 nm. Monitored for 55 min in 5 min intervals. 0 min (light blue)

**Figure 61:** Emission spectrum of 1-nitropyrene (1.0 x 10^{-4} M) in DMF excited at 406 nm.
**Figure 62:** Emission spectrum of 1-hydroxypyrene with SOSG in DMF with irradiation at 447 nm. Monitored for 30 min in 5 min intervals. 0 min (light blue)

**Figure 63:** Emission spectrum of 1-hydroxypyrene in DMF excited at 387 nm.
Figure 64: Emission spectrum of 1-nitropyrene with APF in DMF irradiated with 447 nm under aerobic conditions.

Figure 65: Emission spectrum of 1-nitropyrene with APF in DMF irradiated with 447 nm under anaerobic conditions.
**Figure 66:** Emission spectrum of 1-nitropyrene with APF & 1% DMSO in DMF irradiated at 447 nm. Blue (0 min)

**Figure 67:** Emission spectrum of 1-nitropyrene with APF and sodium azide in DMF irradiated with 447 nm. Blue (0 min)
**Figure 68**: Emission spectrum of 1-hydroxypyrene with APF in DMF irradiated at 447 nm under aerobic conditions. Orange (0 min)

**Figure 69**: Emission spectrum of 1HP with APF in DMF irradiated at 447 nm under anaerobic conditions. Orange (0 min)
Figure 70: Emission spectrum of 1HP with APF & 1% DMSO in DMF irradiated at 447 nm. Gray (0 min)

Figure 71: Emission spectrum of 1HP with APF & sodium azide in DMF irradiated at 447 nm. Gray (0 min)
Figure 72: Emission spectrum of 1-nitropyrene with HPF in DMF irradiated at 447 nm under aerobic conditions. Orange (0 min)

Figure 73: Emission spectrum of 1-nitropyrene with HPF in DMF irradiated at 447 nm under anaerobic conditions. Blue (0 min)
Figure 74: Emission spectrum of 1-nitropyrene with HPF & 1% DMSO in DMF irradiated at 447 nm. Gray (0 min)

Figure 75: Emission spectrum of 1-nitropyrene with HPF & sodium azide in DMF irradiated at 447 nm. Gray (0 min)
Figure 76: Emission spectrum of 1HP with HPF in DMF irradiated at 447 nm under aerobic conditions. Orange (0 min)

Figure 77: Emission spectrum of 1HP with HPF in DMF irradiated at 447 nm under anaerobic conditions. Orange (0 min)
Figure 78: Emission spectrum of 1HP with HPF & 1% DMSO in DMF irradiated at 447 nm. Gray (0 min)

Figure 79: Emission spectrum of 1HP with HPF & sodium azide in DMF irradiated at 447 nm. Gray (0 min)
After 48 Hours

Control 1: 500 uL H$_2$O under dark

Control 1a: 500 uL H$_2$O under light

C: 495 uL H$_2$O; 5 uL DMF under dark

D: 495 uL H$_2$O; 5 uL DMF under light
Figure 80: Bacteria study of 1-nitropyrene & 1-hydroxypyrene with E. coli under dark and light conditions.
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VITA

After completing his work at Central High School, Central Texas, in 2002, Phillip Sharp attended Angelina college in Lufkin, Texas. In 2007, he joined the U.S. Army and served for four years. After the army, he attended Stephen F. Austin State University during 2012-2016 and obtained a degree of Bachelor of Science. In the spring of 2017, he started attending graduate school and began working as a graduate assistant at Stephen F. Austin State University. In December 2018 he received a degree of Master of Science in Natural Science.

Permanent Address: 457 Higgins Lane
Pollok, Tx 75969

ACS style

This thesis was typed by Phillip B. Sharp