TOWARDS PATHWAY ANALYSIS OF OXYGEN PHOTOSENSITIZATION
BY POLYCYCLIC AROMATIC HYDROCARBONS IN
MICROHETEROGENEOUS MEDIA

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Chemistry and Biochemistry

Fall 2018

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ACKNOWLEDGEMENTS

I would like to thank my research advisor Dr. Sharon L. Neal, for her continued support, patience, guidance, and opportunities in helping develop a direction that has and will always influence my outlook in life.

I would also like to thank all the members of the Neal lab, both past and present, in their assistance and efforts in completing and being successful in my research and teaching endeavors. I would like to especially thank Olga Dmytrenko for her assistance and guidance with molecular modeling and Baogan Huynh for her dedicated assistance in acquiring data for the Neal lab. If it had not been for the continued support and encouragement of the Neal lab, as well as other University of Delaware chemistry research group and staff members, I do not think my journey would have been as gratifying and educational as it has.

I would like to thank my family, especially my parents, who have supported me emotionally as well as giving helping hands in ensuring I am successful in my future endeavors. I believe that through their support and teachings, despite the sacrifices and many struggles I have faced up to accomplishing this feat, I would not have grown into the individual that I am today.
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ABSTRACT

Advancing analysis on the mechanisms behind reactive oxygen species (ROS) production through photosensitization has applications within areas of synthesis, toxicology, and photodynamic therapy. Current direct analysis methods require costly instrumentation, which have led to the development of cheaper analysis methods with spectroscopic techniques such as luminescent, colorimetric and spectral probes. However, these spectroscopic techniques face difficulty in measuring ROS in natural and complex environments due to spectral overlap and the production of multiple components from side reactions. The focus herein is to introduce methodology and assemble automated instrumentation that can be used as a foundation to investigate the mechanisms of photosensitization of oxygen by polycyclic aromatic hydrocarbons (PAHs) in natural and complex environments by reaction progress monitoring. The preliminary studies described here were conducted in octanol, which is reportedly a microheterogeneous solvent. In the future, the methodology introduced can be combined with colorimetric or spectral probe techniques and numerical techniques, such as self-modeling curve resolution and reaction progress kinetic analysis, to monitor and separate individual spectral components to determine mechanistic and kinetic information. Actinometer measurements indicated the instrumental setup irradiates samples at approximately 10 times that of surface solar irradiance on a midsummer day at 400 nm, without irradiation in the area of direct sensitization of molecular oxygen. PAH photodegradation measurements in octanol show a relationship between the rate of degradation to the activation energy barrier of the formation of the endoperoxide transition state in the formation of ROS and the irradiation power.
Chapter 1

INTRODUCTION

Molecular oxygen is a ubiquitous part of our environment above and below water surfaces, making it a readily available reagent for reactions. Photochemical reactions in particular are quite common between oxygen and organic molecules leading to many oxygenated products and reactive oxygen species (ROS).\textsuperscript{1-4} While ROS are vital to many natural biological processes, in excess these molecules can be toxic.\textsuperscript{5} A class of chemicals that are widely studied for their toxicity and reactions with molecular oxygen are polycyclic aromatic hydrocarbons (PAHs), which are molecules that consist of two or more benzene rings bonded in a linear or clustered arrangement. These molecules are produced daily through both natural and anthropogenic means, such as forest fires or the incomplete combustion of fossil fuels, and have also been linked to a variety of health-related issues.\textsuperscript{6-8} In particular the photosensitization of these molecules, not only leads to various reaction by-products and the production of ROS, but also the degradation of the parent PAH molecules. This has been noted as an area of concern as the by-products from these types of reactions can be more environmentally toxic than the parent compounds themselves.\textsuperscript{9-11} In recent years, the impact of post bioaccumulation of PAHs on plants and marine life, as well as the photosensitization of these compounds, has been of recent interest due to the mutagenic effects, cancer development, and ultimately death of the related species. In particular the mechanisms for singlet oxygen generation, in relation to photodynamic therapy, have led researchers to use PAHs as model compounds to study.
photosensitized ROS production mechanisms in natural water and isotropic environments.\textsuperscript{3,12,13} Methods of detection of ROS can be challenging as they are typically short lived molecules and evolve in complex environments. Spectrophotometric probes such as p-nitrosodimethylaniline (RNO) allow for indirect measurements of singlet oxygen production, through chemical sensor disappearance.\textsuperscript{14} Combined with numerical techniques such as self-modeling curve resolution (SMCR) and reaction progress kinetic analysis, measurements can be completed utilizing less specialized equipment, fewer measurements, and yield mechanistic and kinetic information on ROS production through PAH sensitization.\textsuperscript{15,16}

In this presented work, an instrumental setup and methodology that can be utilized to measure and monitor PAH photodegradation for mechanistic pathway analysis will be discussed. Automated measurement adaptations to increase data acquisition rates and decrease acquisition time for future experiments will also be mentioned. The methodology included will focus the use of the solvent octanol to assist in the simulation of a microheterogeneous environment.

1.1 Oxygen Photosensitization Reactions

Oxygen photosensitization reactions occur when photoexcited organic molecules interact with molecular oxygen. This process occurs naturally as oxygen is ubiquitous in the environment, and has potential to quench excited organic molecules due to its unique ground state energy level being in the triplet state. Studies have shown that the process by which this quenching can occur can be through either direct energy transfer or through electron transfer.\textsuperscript{1,3,4,13} It is known that the charge transfer interaction between molecular oxygen and an excited sensitizer molecule in the singlet
or triplet state tends to produce singlet oxygen, the lowest excited state of molecular oxygen.¹⁷

Singlet oxygen can also be directly generated through excitation of oxygen near 1270 nm, but requires high power lasers.¹⁸ Singlet oxygen is a strong oxidizing agent and reacts readily with nearby alkenes and aromatic compounds leading to new oxygenated products, damage of biomolecules, and has been shown to complete cycloaddition reactions with aromatic compounds to produce endoperoxide complexes. Endoperoxide complexes have been shown to reproduce singlet oxygen on further photo-irradiation and also superoxide during thermolysis.¹⁹ Singlet oxygen has been a wide area of study over the years due to its many applications in synthesis and medical uses, such as photodynamic therapy.²⁰,²¹ However, it has a short lifetime in pure water at about 5 µs, and even lower lifetime in the presence of other molecules.⁴ Superoxide, which is an ROS that also can be produced through this process, is known to be toxic within cells and contribute to oxidative stress and cell aging. Superoxide is normally produced through the electron reduction of oxygen, but it is also enzymatically produced among plants, fungi, bacteria and phytoplankton.²² Previous studies have suggested that the mechanism behind oxygen photosensitization reactions show the possibility of the production of either singlet oxygen or superoxide depending on the sensitizers triplet energy and oxidation potential, where some PAHs can produce mixtures of both.³,¹⁰

Anthracene and a few selected derivatives, which are a part of a class of organic molecules connected by fused benzene rings and known as polycyclic aromatic hydrocarbons (PAHs), will be used as sensitizers in monitoring oxygen photosensitization reactions and will be the focused PAHs within this document.
PAHs are considered as pollutants ranging from highly toxic or carcinogenic to benign and are known to be found in significant levels in surface and ground water, as well as particulate matter in the atmosphere. As such, these molecules have been studied for decades based on their toxic and carcinogenic behavior.\textsuperscript{5,11,23–25} PAHs with their large aromatic structures can absorb visible light and undergo several different photochemical transformations depending on their surrounding environment. Due to interest in the formation of singlet oxygen and other by-products, a common studied reaction is the reversible formation of endoperoxides between excited PAHs and molecular oxygen.\textsuperscript{9,26–28} While current studies have developed an understanding behind the general mechanisms of oxygen photosensitization by sensitizers, more research is required to determine the impact of different environmental settings such as biorelevant media in order to analyze the mechanism and kinetic rates of ROS production.

1.2 Complex Systems Undergoing Photosensitization

There are many natural systems, such as plants and animals, that utilize photosensitization reactions with oxygen in many different ways. Plants in particular have developed complex systems that utilize reactions that produce ROS to participate in defense against pathogens. Many of these reactions occur through the use of the production of phytoalexins in order to trigger light activated production of ROS. Where the generated singlet oxygen can be used for signaling apoptosis, directly damaging threatening species by oxidation, and or the production of hydrogen peroxide as another signaling agent that plants use to activate their defenses against parasitic species.\textsuperscript{29} However, many different microbes attached to plants have also developed the ability to counter these defenses by using ROS to invade and colonize
plants, as many plant cells only have a few defense mechanisms against singlet oxygen.\textsuperscript{30}

There are many natural products found in plants and animals that can be used as photosensitizers, as well as many already commonly used dyes. Example dyes such as methylene blue and rose bengal are well known photosensitizers. From previous studies with singlet oxygen signaling of apoptosis in plant cells, these same mechanisms are also being purposely used for the advancement of medicine in regards to photodynamic therapy (PDT) treatments. This is due to the amount of control one can have over PDT treatments, in that it has the ability to be tuned so it is only activated by a specific type of illumination at a target site for a given treatment time. This can be controlled based on the sensitizer required for the type of treatment and administration of the sensitizer through ingestion, injection or topical application. Tuning the sensitizers for all these factors includes the development of different sensitizers such as dye tagged zinc oxide nanoparticles\textsuperscript{31} and hybrid lipid-polymer nanoparticles conjugated with specific nucleic ligand targets to bind with target biomolecules.\textsuperscript{32}

Many photosensitization reactions are also occurring within the atmosphere on aerosol particles. Organic aerosol particles have air/water surfaces in the bulk phase which provides a unique surface for photochemical reactions to occur. It has been shown, that if the aerosols contain light absorbing compounds they can undergo production of ROS and can further produce secondary aerosols that remain as aerosols or deposit on the ground. The new particle compositions were reported to be based on the environment and particle makeup.\textsuperscript{33} This environmental dependency can be seen from one study that used common organic aerosols to react with the PAH
benz[a]anthracene, which rapidly reacted and produced multiple different by-products.\textsuperscript{34} Some of these many photosensitization reactions with aerosols are known as aerosol aging, due to the change in particle composition through radical production and interparticle reactions. These reactions have been shown to affect the properties of the particles, by reducing or increasing the amount of light absorption, by influencing the amount of volatile organics, and or through the production of hydroperoxyl radicals which are released into the air.\textsuperscript{35}

Air and seawater surfaces are also a place for oxygen photosensitization reactions, as well as within water. This is especially the case where there is a presence of large amounts of dissolved organic matter which act as sensitizers. This organic matter can originate from natural sources, chemical waste, and or any introduction of large amounts of PAHs or other petroleum hydrocarbons such as oils spills. While PAHs do affect aquamarine life based on bioaccumulation\textsuperscript{36,37}, a bigger concern is when these cells undergo photosensitization because of the production of ROS and the resulting effects on cellular health.\textsuperscript{5,38} Further understanding behind oxygen photosensitization is required in order to lead to improvements in the determination of the impact of oxygen sensitization, on our ecosystems.

1.3 Analysis of Photooxidation Reactions

Direct detection of singlet oxygen is possible through direct emission at \(~1268\text{ nm}, but is expected have very poor quantum yields due to weak signals and the short lifetime of singlet oxygen, primarily due to its reactive nature.\textsuperscript{4,39} Singlet oxygen can also be measured at 1920 nm using time resolved absorption experiments, but are not as sensitive as luminescence techniques.\textsuperscript{12} Superoxide can be directly detected by its absorbance spectrum as it strongly absorbs in the 230-350 nm regime. However,
measuring superoxide presents significant challenges, in that it does not remain stable in pure water samples for more than a few seconds to minutes, while other components normally present within solutions that generate superoxide tend to absorb strongly in the same region.\textsuperscript{39}

Due to the requirement of costly instrumentation and challenges in direct detection, as well as challenges faced in utilizing mass spectrometry and chromatographic methods\textsuperscript{40} to isolate ROS generated compounds, many photooxidation reactions with ROS monitoring have moved to indirect methods using chemical reagents and spectrophotometric probe monitoring.\textsuperscript{41} This has led to an advancement of ROS measurements with luminescent,\textsuperscript{42} colorimetric,\textsuperscript{43} and probe techniques.\textsuperscript{44,45} However, many of these techniques face difficulties in measuring ROS production in natural environments, such as the microheterogeneous environment within cells, and require methodology to focus on the produced signal from the selected analyte. Having the ability to monitor the multicomponent production of ROS \textit{in situ} and simultaneously, is the key to a better understanding of these systems and the mechanisms behind ROS generation.

Spectrophotometric data obtained from these complex systems can also be difficult to analyze due to component overlap. However due to the nature of spectra being bilinear data sets, methods of self-modeling curve resolution (SMCR) can be applied in order to isolate the different components present.\textsuperscript{16} This methodology can also be combined with techniques such as reaction progress kinetic analysis to assist in identifying individual component profiles as well as mechanistic and kinetic information.\textsuperscript{15} This process is done through the addition of taking multiple profiles of data with small controlled perturbations or controlled systematic changes to the
reaction system. Utilizing these techniques with spectrophotometric probes can improve and allow for reaction monitoring that originally required specialized equipment to be completed with less expensive and easier methodology.

However, with the addition of more components into a reaction system, it can cause different side reactions to occur. This has been noted with charged surfactants by Martinez et al., where it decreased production of singlet oxygen and changed the resulting photoproducts. Octanol, a solvent that has a reverse micellar nature and can contain hydration, has been used extensively in regards to the partition coefficients of hydrophobic and hydrophilic environments and to simulate the lipid compartmentalization within cells. This suggests that octanol could be used as a viable solvent to study the mechanisms of ROS production within a microheterogenic environment, without the further addition of other components.
Chapter 2

MATERIALS AND METHODS

2.1 Chemicals

Anthracene (AN, 99%) and 9-methylanthracene (9-MA, 99.9%) were purchased from Fluka Analytical. 2,3-dimethylanthracene (2,3-DMA, 98%) and 9,10-dimethylanthracene (9,10-DMA, 98%) were purchased from TCI America. 1-octanol (OctOH, 99%) (certified ACS), and p-nitroanisole (PNA, 99%) were purchased from Acros Organics. Pyridine (PYD, 99.9%) (certified ACS), Optima water (HPLC Grade), Acetonitrile (ACN, HPLC Grade), and ethanol (EtOH, 200 proof) (USP) were obtained from Thermo Fischer Scientific. All compounds were used without further purification.

2.2 Instrumentation

2.2.1 Photodegradation Instrumental Design

Photodegradation experiments were completed using the completed setup shown below in Figure 2.1. The irradiation light source is a 300 W Xenon-arc lamp powered by an ILC Technology PS300-1 power supply, and housed in an ILC Technology R400. Source light was passed through an in-house fabricated 18 cm long water filled glass cylinder with a collimating quartz lens and quartz window to provide UV and IR filtering, preventing direct activation of molecular oxygen. The output power of the light source at the sample location was measured before and after each experiment with a Newport 843-R power meter equipped with a 919P Thermopile Sensor (190 -11000 nm) to confirm a consistent output. Additional UV filtering was provided by the walls of the reaction vessel, which exhibited a 50% cut-on wavelength.
of 290 nm. The sample solution is held in a glass temperature-controlled vessel under constant stirring. Absorption spectra of aliquots of the sample in the vessel were taken in quartz cuvettes (Firefly Sci, Brooklyn, NY) using a diode array spectrophotometer (Agilent Technologies, Model 8452A, Palo Alto, CA) at specific time intervals against an atmospheric or solvent background.

Figure 2.1 Instrumental scheme used for monitoring PAH photodegradation. The light of a xenon arc lamp is filtered and columnated through water towards the sample in a temperature-controlled vessel.

2.2.2 Automated Instrumental Design

Automated instrument design includes the same setup of illumination to reaction vessel as discussed in Section 2.2.1 and is shown below in Figure 2.2. The source irradiation and analysis setup are separated by a black dividing wall for isolation from stray source irradiation. The sample solution within the reaction vessel is extracted using 4x6 mm Everprene-60 tubing (Grayline LLC., Waukesha, Wisconsin) attached to glass elbow tubes, through the use of a Dynamax peristaltic pump (Rainin Instrument Co. Inc., Model RP-1, Emeryville, CA) at a rate of 28 rpm. The extracted solution was measured to have a flow rate of about 25 mL/min, resulting in a full solution refresh rate of once per minute. Absorption spectra of the
flowing sample were taken through a quartz semi-micro flow cell (Starna Cells Inc., Atascadero, CA) using a charged-coupled device (CCD) array compact spectrophotometer (Ocean Optics, FLAME-CHEM-UV-VIS, Largo, FL) at specific time intervals against a solvent background.

Figure 2.2 Instrumental scheme adapted for automatic monitoring of PAH photodegradation solutions. Sample solution is cycled through a flow cell by use of a peristaltic pump.

2.3 Molar Absorptivity Determinations

Multiple sets of broadband (190 – 810 nm) molar absorptivity spectra were determined for the compounds presented in Section 2.1 in quartz cuvettes using the diode array spectrophotometer. Each absorptivity spectrum was determined through the measurement of the absorbances of the selected compound with exponentially increasing concentrations for a set of 8 to 25 spectra from a minimum absorbance of 0.03 to a maximum absorbance of approximately 2.50. The multiple absorbance
spectra were then analyzed and averaged per compound per solvent, using (*Matlab
ver. R2017A) and are used for all analyses presented in this work.

2.4 Illumination Source Calibration Measurements

2.4.1 Actinometer Methodology

Power source measurements were completed using the p-nitroanisole (PNA)
and pyridine (PYD) sunlight actinometer method described by D. Dulin & T. Mill48
and updated by Laszakovits et al. in 2017.49 PNA stock solutions were made in ACN,
and for long term storage were covered and stored in brown bottles in a chemical
refrigerator at 5 °C and allowed to equilibrate to room temperature for 30 minutes
before use. The absorbance of diluted stock solutions was checked prior to each use to
validate stock integrity, those that had absorbance values of less than 10% original
starting absorbance or exhibited shifted spectra were discarded. PNA sample solution
volumes of 200 mL were prepared in HPLC-grade water at a concentration of
1.24 \times 10^{-5} \text{ M}, with the addition of PYD at a concentration of 1.24 \times 10^{-2} \text{ M} to maintain
a 1:1000 concentration ratio of PNA: PYD. Absorption spectra of the sample solution
was irradiated using the setup described in Section 2.2.1. Initial PNA decay rates were
obtained by monitoring the change in absorbance of PNA at 320 nm.

2.4.2 Photon Irradiance Determination

Total photon irradiance of the 300 W xenon arc lamp on the reaction vessel
was calculated using Equation 1, where \( E_{p,\text{tot}}^{0} \) represents the total photon irradiance, \( k' \)
is the initial rate, \( [\text{PNA}]_{0} \) is the initial PNA molarity, \( l \) is the optical path length, \( \rho_{\lambda} \)
is the relative spectral photon irradiance, \( A_{0} \) is the initial absorbance spectrum of PNA,
\( \Delta \lambda \) is the wavelength resolution of the instrument, and \( \varnothing \) is the PNA quantum yield which is determined using the updated equation from Laszakovits et al. (Equation 2).\(^{49}\)

\[
E_{p,\text{tot}}^0 = \frac{k'[\text{PNA}]_p l}{10000\Sigma_{A} \rho \lambda (1 - 10^{A_{0}}) \Delta \lambda} \tag{1}
\]

\[
\varnothing_{\text{PNA}} = 0.29 \text{ [Pyd]} = 0.00029 \tag{2}
\]

Relative spectral photon intensity was calculated based on the effective spectral photon intensity on the sample after transmission through the water filter and the glass reaction vessel following Equation 3, where \( E_{p,\lambda}^0 \) is the effective spectral photon intensity.

\[
\rho_{\lambda} = \frac{E_{p,\lambda}^0}{\sum_{E} E_{p,\lambda}^0 \Delta \lambda} \tag{3}
\]

Total photon irradiance can be converted to units of power following a method described by Zepp & Cline,\(^{50}\) which can be used to adjust lamp output based on the readings from the power meter. Photon irradiance along with PNA degradation rates can assist in determining the relative quantum yield of PNA in a different solvent through the use of the relative actinometric method shown by Equation 4.\(^{51}\) Where \( \varnothing \) represents the quantum yield of the compound (\( X \)), in this case PNA, and each solvent (\( S \)), and \( k \) represents the compound’s rate of degradation or production in the same solvent.

\[
\varnothing_{X,S_2} = \varnothing_{X,S_1} \ast \frac{k_{X,S_2}}{k_{X,S_1}} \tag{4}
\]
Following the relative actinometric method allows for the relative quantum yield of other compounds to be calculated within different solvents from reference data.

2.5 PAH Photodegradation Measurements

PAH stock solutions (0.1 – 10 mM) were prepared in ethanol and stored in amber vials at room temperature before use. For long term storage, solutions were stored in brown bottles in a chemical refrigerator at 5 °C and allowed to equilibrate to room temperature for 30 minutes before use. The absorbance of diluted stock solutions was checked prior to each use to validate stock solution integrity, those that had absorbance values of less than 10% original starting absorbance or exhibited shifted spectra were discarded. PAH sample solution volumes of 200 mL were prepared using OctOH and a stock solution to achieve the desired PAH concentration of 50 or 200 µM. Samples were held at a constant 25 °C and were continuously stirred during the irradiation process. Samples were irradiated at constant power readings of 130 or 180 mW for the duration of the measurement. Absorption measurements were taken at specific time intervals required to collect at least 20 spectra or until there was approximately less than 10% of the original PAH concentration remaining. Concentrations for each absorption measurement were computed at the wavelength of maximum absorbance using molar absorptivity data. Linear regression of these concentration profiles or their logs was completed to determine the rate of PAH decomposition and kinetic order based on the fit of the profile.
Chapter 3

RESULTS

3.1 Molar Absorptivity Determinations

Molar absorptivity measurements were compiled and averaged for each compound and solvent. As discussed in Section 2.3, 25 measurements of exponentially increasing concentration are measured for each molar absorptivity data set acquired. The average of the compilation of these measurements is used for absorbance to concentration calculations, as well as provide information about the compound’s absorbance spectrum in each solvent.

Shown below in Figure 3.1, is an example of the compiled molar absorptivity measurements of p-nitroanisole (PNA) in water. The insert in Figure 3.1 shows the molar absorptivity at the wavelength of maximum absorbance at 320 nm as it scales with molar concentration of PNA. In the insert of Figure 3.1 the raw molar absorptivity shows small differences for each data set before the concentration of 5.0×10^{-5} M. The spike around 660 nm is caused due to a damaged diode on the diode array spectrometer, where it remained present for all measurements at varying degrees. However, the spike around 490 nm was not expected, and only appeared during the PNA molar absorptivity measurements in water.

Figure 3.2 is the compiled molar absorptivity measurements of PNA in octanol. In octanol the maximum wavelength of absorbance of PNA blue shifted to 304 nm. The insert of Figure 3.2 shows that at low concentration the molar absorptivity is higher and with increasing concentration the molar absorptivity decreases, which was not represented in the absorptivity measurements in water. However, at higher concentrations the molar absorptivity appears to be similar to the
value that was measured in water. The solvent octanol simulates a microheterogenic environment and is reported to form reverse micelles in solution.\textsuperscript{52} These micelles could, in theory, cause a compartmentalization effect with PNA at low concentration levels, which could be the cause behind the increase in molar absorbance. However, further studies on the effect of concentration and molar absorptivity in octanol would need to be conducted to confirm if this effect is due to the compartmentalization of PNA in octanol.

![Figure 3.1 Compilation of raw molar absorptivity measurements of PNA in water with an insert detailing the molar absorptivity as a function of concentration at 320 nm.](image-url)

Figure 3.1 Compilation of raw molar absorptivity measurements of PNA in water with an insert detailing the molar absorptivity as a function of concentration at 320 nm.
Figure 3.2 Compilation of raw molar absorptivity measurements of PNA in octanol with an insert detailing the molar absorptivity as a function of concentration at 304 nm.

3.2 Actinometer Measurements

The photodegradation of PNA with pyridine (PYD) was monitored in water and octanol following the methods described in Section 2.4.1 for both instrumental methods described in Section 2.2. An example of a photodegradation set of PNA in water and octanol and are shown below in Figure 3.3 and Figure 3.4 respectively. Linear regression of the concentration profiles of the initial PNA measurements at the respective solvent wavelengths were calculated for water and octanol, which were used as outlined in Section 2.4.2. The average initial rate of PNA photodegradation in water for the instrumental setup described in Section 2.2.1 was calculated to be $6.98 \pm 0.17 \times 10^{-9} (\text{M} \cdot \text{s}^{-1})$, while the automated instrumental setup resulted in an initial rate of $1.16 \times 10^{-9} (\text{M} \cdot \text{s}^{-1})$. The decrease in rate could be caused by some of the solution not remaining irradiated during circulation and or due to increased data
collection rates providing a clearer view of the initial rate change. Further
measurements are required to determine the exact cause for the decrease in rate. The
resulting irradiance from the degradation in water following the instrumental setup
described in Section 2.2.1 showed that the xenon arc lamp output at the sample’s
surface was $4.40 \times 10^{16}$ (photons $\cdot$ cm$^{-2}$$\cdot$s$^{-1}$$\cdot$nm$^{-1}$). The irradiation compared to that of
the surface solar irradiance at 400 nm for a midsummer day at 40 °N latitude was
approximately 10 – 14 times.$^{50}$

![Absorbance spectra of the photodegradation of PNA with PYD in water, following the maximum wavelength of PNA at 320 nm.](image)

![Absorbance spectra of the photodegradation of PNA with PYD in octanol, showing the maximum wavelength of PNA at 304 nm.](image)

Figure 3.4 shows little to no deviation from the expected peak of PNA in
octanol at 304 nm. However, Figure 3.3 shows a shifting PNA peak from 320 nm to
304 nm over time, which was not described in literature references for this method,
and appeared constant over all 3 trials in water. In these studies the photodegradation rate of PNA in water was found to be approximately 40 times faster than the photodegradation in octanol. Following Equation 2 in Section 2.4.2 the average quantum yield of PNA in water was found to be $1.83 \times 10^{-2}$. By using Equation 4 the relative quantum yield of PNA in octanol was found to be $4.71 \times 10^{-4}$. This value may be used to compute relative quantum yields of other compounds in octanol.

### 3.3 Photodegradation Reaction Progress Measurements

#### 3.3.1 Photodegradation vs. Sensitizer Concentration

Anthracene (AN) and three anthracene derivatives 9-methylanthracene (9-MA), 2,3-dimethylanthracene (2,3-DMA), and 9,10-dimethylanthracene (9,10_DMA) were used as model PAHs to observe the reaction progress of their photodegradation in octanol held at a power reading of 180 mW on the thermopile sensor. Figure 3.5 below shows the spectra of a data set of the photodegradation of AN along with the calculated rate of change of the concentration of AN at 358 nm.

![Figure 3.5 Absorbance spectra of the photodegradation of anthracene in octanol shown with an insert of the calculated concentration change over time at the selected wavelength of 358 nm.](image)
The average first-order kinetic profiles calculated for each PAH at concentrations 50 and 200 µM are reported below in Table 3.1. The first-order kinetic profiles were used for comparison purposes, as the zero-order kinetic profile of 9-MA and 9,10-DMA did not follow a linear trend. The difference between the rates for the two concentrations of each compound in Table 3.1 show that with increased concentration there is no significant change in degradation rate for 9,10-DMA and 9-MA. Both the degradation of AN and 2,3-DMA show a small change with increasing concentration, but further analysis is required to show if there is a significant difference. Furthermore, there appears to be an increase in degradation rate by degree of methyl substitution on the central ring. It has been reported that the addition of electron-releasing groups at the site of molecular oxygen addition, in the formation of the endoperoxide transition state, increases the rate of degradation. This would match the observed trend of increasing photodegradation rate based on degree of substitution in the 9 and 10 positions of AN, and the unchanged rate for 2,3-DMA in comparison to AN.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>50 µM Photodegradation Rate (s(^{-1})) x10(^{-4})</th>
<th>200 µM Photodegradation Rate (s(^{-1})) x10(^{-4})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>0.26 ± 0.03</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>2,3-dimethylanthracene</td>
<td>0.27 ± 0.02</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>9-methylanthracene</td>
<td>1.37 ± 0.04</td>
<td>1.46 ± 0.05</td>
</tr>
<tr>
<td>9,10-dimethylanthracene</td>
<td>5.06 ± 0.43</td>
<td>5.28 ± 0.21</td>
</tr>
</tbody>
</table>

Table 3.1 Photodegradation comparison and summary of tested PAHs at 50 and 200 µM. All calculated degradation rates are 1\(^{st}\) order and listed with their 95% confidence intervals.
Anthracene was reported in 1999 by Dabestani et al. to have a biomolecular rate constant of $2.5 \times 10^{-10}$ and $3.1 \times 10^{-10} (\text{M} \cdot \text{s}^{-1})$ in cyclohexane and benzene respectively. In 2003 Fasnacht & Blaugh reported that the biomolecular rate constant of anthracene measured in methanol was $2.8 \times 10^{-10} (\text{M} \cdot \text{s}^{-1})$, where the irradiance was reported to be approximately half of what is reported in Section 3.2 at 400 nm. In this work the zero-order rate constants of anthracene following the average degradation rate in octanol was found to be $8.99 \times 10^{-10} (\text{M} \cdot \text{s}^{-1})$. In comparison to methanol and the other isotropic solvents the rate in octanol appears to be approximately three times as slow, this could be the result of the microheterogeneous nature of octanol, but will require further testing.

The $[4+2]$ cycloaddition transition state of a PAH and molecular oxygen in the formation of an endoperoxide is reported to be responsible for the formation of singlet oxygen, after which the reaction between excited oxygen species and PAHs causes degradation of the sensitizer. Gaussian09 calculations for the zero point energy activation barriers for the endoperoxide transition state for each PAH were obtained and calculated using the CAM-B3LYP/6-311+G(d,p) basis set by Dmytrenko, O. The resulting zero-point energy barriers are shown in Table 3.2 below, are ordered based on highest to lowest, and compared with the photodegradation rates of the selected PAHs at 50 µM. 9,10-DMA was reported to not have a zero-point energy activation barrier.

The comparison of the degradation rates to the activation energy barrier in Table 3.2 reveals an inversely proportional trend, that as the activation barrier energy decreases the rate of degradation increases. This trend coincides with the previous observation, which showed that the addition of electron-releasing groups at the point
of addition of molecular oxygen for the endoperoxide transition complex increases the photodegradation rate. These results indicate that the formation of the endoperoxide transition state is a key part of the mechanism of PAH photodegradation, ROS production, and is influenced by the addition of electron-releasing groups at the site of the [4+2] cycloaddition with molecular oxygen.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>50 µM Photodegradation Rate (s(^{-1})) x10(^{-4})</th>
<th>Zero-point Energy Activation Barrier (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>0.26 ± 0.03</td>
<td>4.56</td>
</tr>
<tr>
<td>2,3-dimethylantracene</td>
<td>0.27 ± 0.02</td>
<td>4.53</td>
</tr>
<tr>
<td>9-methylantracene</td>
<td>1.37 ± 0.04</td>
<td>1.95</td>
</tr>
<tr>
<td>9,10-dimethylantracene</td>
<td>5.06 ± 0.43</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.2 Summary of the rate of PAH photodegradation at 50 µM and calculated zero-point energy barrier for the formation of the endoperoxide intermediate. No energy barrier was reported to be present for the formation of the endoperoxide intermediate with 9,10-DMA.

**3.3.2 Photodegradation vs. Source Power**

The photodegradation of each PAH at 50 µM, as shown in Section 3.3.1, was also completed with a lower lamp power reading of 130 mW on the thermopile sensor as mentioned in Section 2.2.1. Table 3.3 below shows the average first-order rate constants of each PAH at both power readings. All rates conducted at 130 mW power appear slower than those at 180 mW by approximately 60%. These results indicate that through the adjustment of the power of the irradiation source, the rate of the reaction could be controlled. It also shows that there is a possibility to use reaction progress kinetic analysis to find the relationship between the source irradiation and the
degradation rate of PAHs, which could be related to future quantum yield calculations for the production of ROS.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>130 mW, 50 µM Photodegradation Rate (s⁻¹) x10⁻⁴</th>
<th>180 mW, 50 µM Photodegradation Rate (s⁻¹) x10⁻⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>0.16 ± 0.01</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>2,3-dimethylanthracene</td>
<td>0.18 ± 0.04</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>9-methylantracene</td>
<td>0.78 ± 0.02</td>
<td>1.37 ± 0.04</td>
</tr>
<tr>
<td>9,10-dimethylantracene</td>
<td>3.09 ± 0.24</td>
<td>5.06 ± 0.43</td>
</tr>
</tbody>
</table>

Table 3.3 Photodegradation comparison and summary of tested PAHs at 50 µM at different power readings. All calculated degradation rates are 1st order and listed with their 95% confidence intervals.
Chapter 4

CONCLUSIONS

An instrumental methodology was introduced to assist in the observation of the pathways of ROS production by the photosensitization of PAHs in octanol. Molar absorptivity measurements of the actinometer, PNA, in water and octanol showed evidence that microheterogenic media may have an effect on the observed molar absorption of the compound at lower concentrations. Photodegradation measurements of PNA showed that the introduced instrumental methods, irradiated the sample with more light than the sun at the earth’s surface during a midsummer day at 400 nm. There is no irradiation present that will directly excite molecular oxygen due to water filtering. Kinetic analysis by linear regression of the absorbance of PNA through both introduced instrumental setups were able to monitor the photodegradation of PNA in the presence of PYD with the automated instrumental method showing a decrease in the initial photodegradation rate from the non-automated setup. Full spectrum observations with both instrumental setups noticed a wavelength shift of PNA in water from 320 nm to 304 nm, not reported in literature.

PAH photodegradation observations showed slower degradation occurring in the microheterogeneous solvent octanol. It also revealed a dependence on the formation of the endoperoxide transition state complex. Through monitoring the degradation of the selected PAHs, a relationship between the degree of methyl substitution and the activation energy barrier of the endoperoxide transition state was observed. PAH photodegradation rates were also observed to be dependent on the power of the irradiation source. The data collected through the methodology discussed allows for the addition of further numerical techniques and spectral probe analysis.
Based on this work's observations; for future spectral probe analysis of ROS production, the zero-point activation energy barrier between a ROS acceptor and sensitiser should be considered to be in competition with each other, unless a significant difference in activation barrier energy is observed.
REFERENCES

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(49) Laszakovits, J. R.; Berg, S. M.; Anderson, B. G.; O’Brien, J. E.; Wammer, K.


