Laser Flash Photolysis Study of Hydroxyl Radical Reactions

An Honors Thesis (HONR 499)

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Abstract

Reactive oxygen species (ROS) are a family of chemical compounds that are physiologically and environmentally important. One member of this family is the hydroxyl radical (HO•). A previous study[1] explored the rates of HO• reactions with aromatic substrates in a nonaqueous solution and correlated these rates with the ionization potential of those substrates. Developing this correlation is the first step in being able to estimate how fast HO• will react in nonaqueous physiological or environmental systems. The following research is a continuation of this primary study and expands upon it with a wider variety of activated and deactivated ring structures. The experimental procedure included sample preparation, laser-flash photolysis (LFP), and data analysis. The findings of this study were subsequently published in the *Journal of Physical Chemistry* in 2018 (Appendix A).

Acknowledgments

I would primarily like to thank Dr. James Poole for allowing me to work with him in his lab, the CRISP program for providing the internship opportunity, and the Honors Undergraduate Fellows Program for funding me.

I also want to thank Dr. Patricia Lang for overseeing my thesis work, Abigail Waggoner for helping orient me into the lab, and Dr. John Emert for helping me complete my honors degree during a difficult time.

Process Analysis Statement

The goal of our research was to determine if there is a correlation between the rate of the reaction between hydroxyl radical and ionization potential of the compound with which it reacts. We ultimately found that this relationship does exist, at least for the compounds included in the procedure. My role in this experiment consisted of thee main parts: sample preparation, laser-flash photolysis, and data analysis. Over the course of a few months, I collected data for 13 different individual compounds. This new data was added to an existing body of data to create a manuscript that was published in the *Journal of Physical Chemistry* in 2018.

I learned a lot of skills and lessons during my few months working on this project. First, I became familiar with using equipment such as a rotovap, two types of vacuum pumps, a glass oven distillation system, Luzchem LFP spectrometer, Continuum laser, NMR, UV-vis spectroscopy, and Igor data analysis software. I learned how to run the collected data through the appropriate equations, produce usable graphs, and interpret results. However, I believe my most valuable lesson was in problem solving and patience.

The equipment we were using was very old. I had problems with the LFP instrument almost every day. There were a few shutter components of the instrument, and one of them was triggering improperly. Whenever this would happen, the entire system would stop working. Sometimes I was able to fix it, but many times my work for the day was done as soon as it happened. Additionally, my entire data set for that day would be unusable. There were three entire weeks where I could not do anything at all, because the instrument was in pieces across the work bench. The chemistry department's instrument technician and Dr. Poole

worked hard to find a solution, even calling the manufacturer and replacing parts, but no one was able to fix it permanently. Even through all this, I was able to obtain results. It was an extremely frustrating process to get consistent data, and it took the entire summer to run 13 compounds. Without the technical issues, I estimate that I could have run 20 or more. I am extremely proud of what I was able to do with the equipment I had.

Finally, I learned about the tribulations of the peer review process. We initially tried to publish in the Journal of Organic Chemistry. Two reviewers approved our paper with minor notes, but a third reviewer did not like it at all. Even after editing the document to address this person's concerns, we were denied. It was very disappointing to have our hard work criticized and rejected, but that is the whole point of peer review. Instead of giving up, we reorganized and reframed the paper, and it was approved for publication by the Journal of Physical Chemistry.

Background

The hydroxyl radical (HO•) is composed of an oxygen atom bonded to a hydrogen atom and contains an unpaired electron. This makes HO• very reactive and short-lived. In the atmosphere, reactions between ozone, solar radiation, and water produce HO• [2]. Oxidation chain reactions with carbon monoxide, methane, and ozone produce more stable products like hydrogen peroxide, formaldehyde, and nitric acid (**figure 1**).

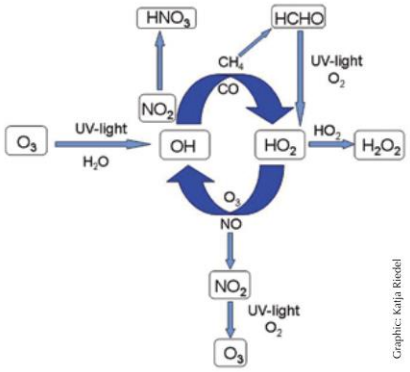

Figure 1 – OH• interacts with light and other gases in the atmosphere. Image from Riedel and Lassey.

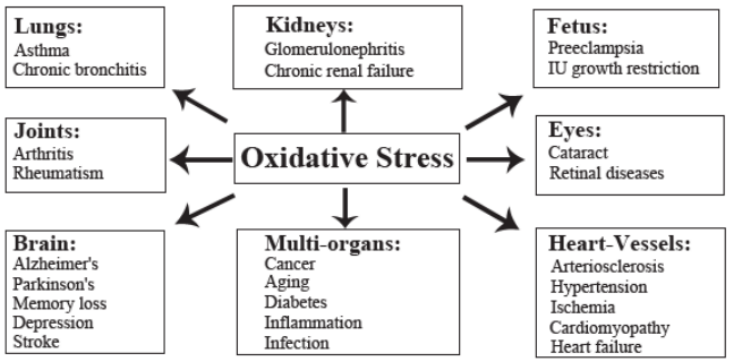

Figure 2 – ROS cause oxidative stress that have pathological effects on many body systems. Image from Pham-Huey et al.

HO• also plays a role in human health. It is part of a family of compounds called Reactive Oxygen Species (ROS) that includes superoxide(O_2^-•), hydrogen peroxide (H_2O_2), and nitric oxide (NO). The body is exposed to ROS through external factors such as pollution, radiation, and other chemicals, but it also produces its own[3]. At low concentrations, ROS can be beneficial. For example, phagocytes produce ROS in order to defend against pathogens [3]. They are also involved in signaling pathways and apoptosis [4]. At higher concentrations, ROS can be destructive because they create oxidative stress. At a cellular level, oxidative stress can damage to lipids, proteins, and nucleic acids. This is problematic as oxidative stress has been linked to a variety of diseases (**figure 2**). A quick internet search will reveal that oxidative stress and the potential benefits of antioxidants are of great concern to many people and are the subject of many mainstream health articles.

Since these oxidation reactions happen so quickly, they can be difficult to study in their actual environment. Additionally, the substances with which they react may be present in tiny volumes that are hard to measure or hard to synthesize. However, if we can produce a reliable method of predicting rates based on ionization potential, we could estimate how fast these

reactions occur in nature as long as we know the substrate with which HO• is reacting. We began as simple as possible, using monosubstituted benzene rings. We tried to sample a range of activated and deactivated ring structures. We also tried a few heterocycles and polysubstituted rings. The aim is to build up to larger organic molecules that may be affected by ROS in the body, such as nitrogenous bases or amino acids.

Experimental

Each day that I ran samples, I had to collect or prepare four solutions. The first was HPLC grade acetonitrile, which I took directly from the bottle. Acetonitrile was used as the nonaqueous solvent, as it has been shown to react relatively slowly with HO•. Next, I prepared a solution of roughly $8x10^{-4}$ M N-hydroxypyridine-2-thione (NPS) in acetonitrile, which is the compound that produces HO• when hit by a 355nm laser pulse. The third solution I prepared was 0.010-0.015 M *trans*-stilbene in acetonitrile, which was used as our reporter. *Trans*-stilbene reacts with HO• to produce a compound that absorbs at 395 nm.

Finally, I prepared the substrate solution. Substrates that I used in this study were N,N-dimethylaniline, anisole, diphenyl ether, acetanilide, phenyl acetate, ethylbenzene, cumene, t-butylbenzene, benzene, chlorobenzene, methyl benzoate, α,α,α-trifluorotoluene, and benzonitrile. All substrate solutions had to be soluble in acetonitrile, could not absorb at 355 nm, and could not produce a product with HO• that absorbed at 355 nm. All substrate compounds were monosubstituted aromatic ring structures, which spanned the spectrum of activation and deactivation. Most substrates were purchased and used as-is, with a few

exceptions. I made phenyl acetate using a procedure from existing literature [5]. I also distilled the phenyl acetate, *N,N*-dimethylaniline, and methyl benzoate before using them.

Next, I combined different proportions of these solutions to run through LFP. Samples were prepared in 3.5mL quartz cuvettes. Sample solutions totaled 2100µL each. Every sample contained 100 µL NPS. Samples were run in duplicate, with each pair containing a progressive volume of substrate. One sample per duplicate contained 500 µL *trans*-stilbene, and the other had no *trans*-stilbene.

Samples A, M, and T were negative controls that contained no arene substrate. Their average represented the absorbance of the trans-stilbene when no arene was present to react with the hydroxyl radical. A, M, and T each had the same reagent volumes and were used to make sure that readings were consistent throughout the course of the experiment. Sample B acted as a positive control and contained no trans-stilbene. It represented the absorbance when only arene substrate was present to react with the hydroxyl radical. Samples U-Z acted as extra points that were used to fill in gaps only when the data was unclear without them.

After my solutions were prepared, LFP was used to analyze the rates of hydroxyl radical reactions. In LFP, a cuvette containing the experimental sample is held in an LFP instrument that shines light through the sample. A laser pulse is sent through the sample perpendicular to the light source. The instrument detects the absorbance of the light over time, and the information is sent to the computer. In this case, the monitor light wavelength was 395 nm and the laser pulse was 355 nm. The instrument collected data for 1.8 µs post-pulse. Laser pulses were between 20 and 30 mJ/pulse, with a 6-10ns nominal pulse width.

Each full reading consisted of 2500 individual data points. These were color-coded and plotted on the same graph (**figure 3**) where the y-axis was the absorbance at 395 nm, and the x-axis was time after the laser pulse. Using data analysis software, I recorded the average absorbance between 40-100 ns post-pulse, which consisted of 50 data points. This information was plotted as A_{395} initial over A_{395} along a y-axis. I also ran the data through multiple equations to determine the ratio of the concentration of arene to stilbene, which was plotted along an x-axis. The result was a Stern-Volmer plot that could be used to determine the average rate of the reaction (**figure 3 inset**). The rate *k* was calculated using the following equation.

$$\frac{A_{395_0}}{A_{395}} = 1 + \frac{k_{arene}[arene]}{k_{stilbene}[stilbene]}$$

The rates determined using my data were then plotted against the calculated and experimental gas phase ionization potentials of the substrate (**figure 4**).

Discussion

The final data (**figures 4 and 5**) show that there is a correlation between the rate of the HO• reaction and the IP of the arene substrate. We found that this correlation was stronger for monosubstituted arenes, bicyclic systems, and alkenes. Polysubstituted arenes and heterocycles show a weaker correlation, and additional data would be helpful to determine if there is a clearer pattern. These results are important because they suggest that we may be able to estimate HO• reaction rates simply by knowing the gas phase IP of the substrate. This data can be applied to atmospheric and environmental reactions as well as physiological

reactions. With more time and data, we may be able to predict rates between HO• and specific amino acids, nucleotides, or phospholipids.

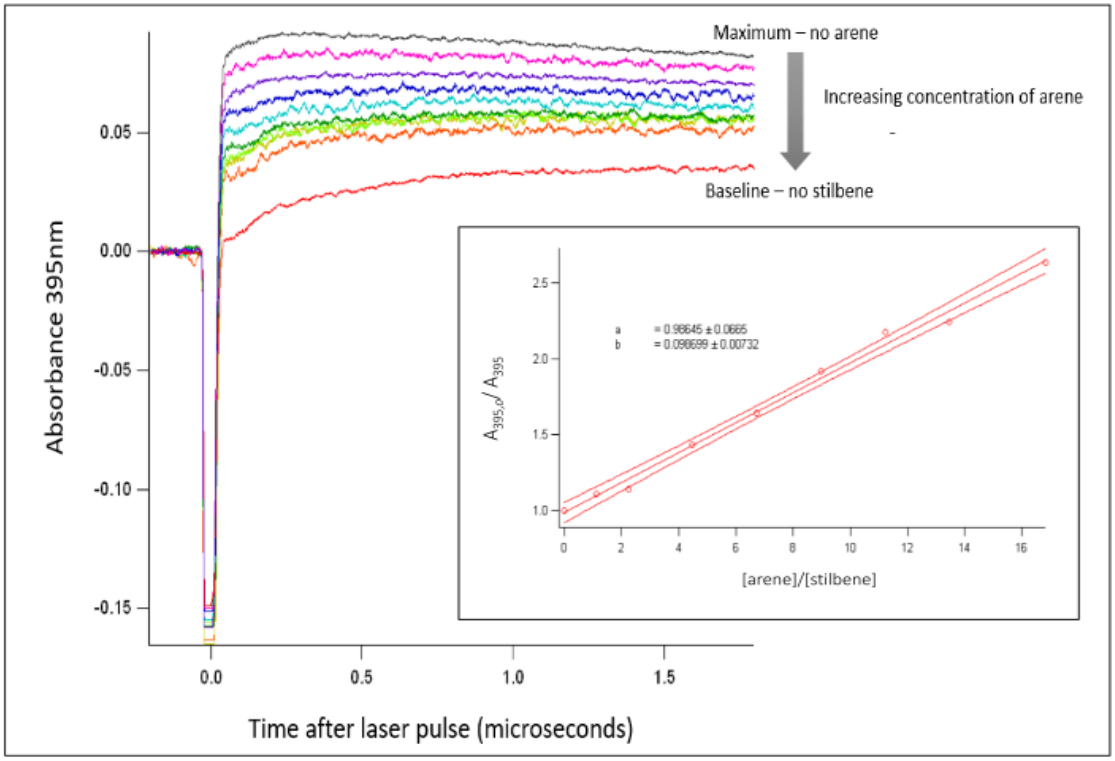

Figure 3 – Raw data collected from LFP procedure is color-coded to differentiate separate runs. The average change in rate is plotted against the ratio of the concentration of arene to stilbene (inset). From the slope of this data set we are able to calculate the rate of the reaction.

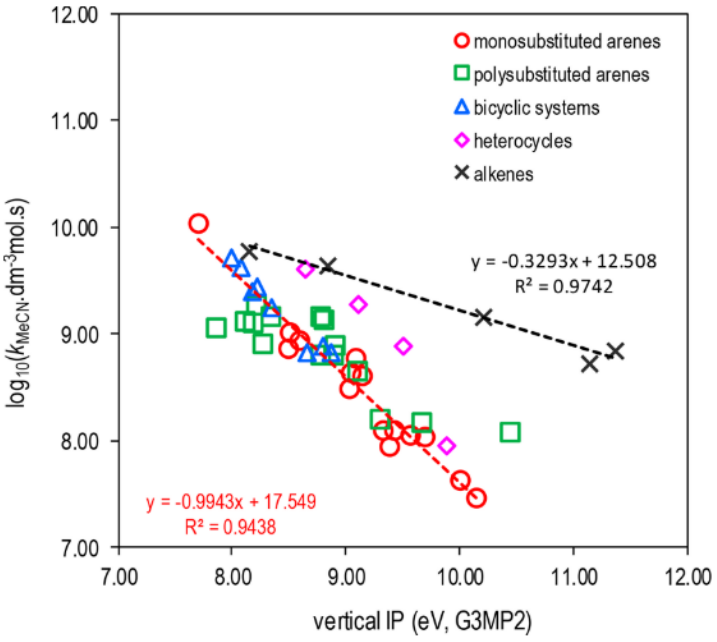

Figure 4 – All final data, including that from the existing literature, my contribution, and later work. There is a clear linear correlation between rate and vertical IP for monosubstituted arenes, bicyclic systems, and alkenes. More data would be useful for polysubstituted arenes and heterocycles.

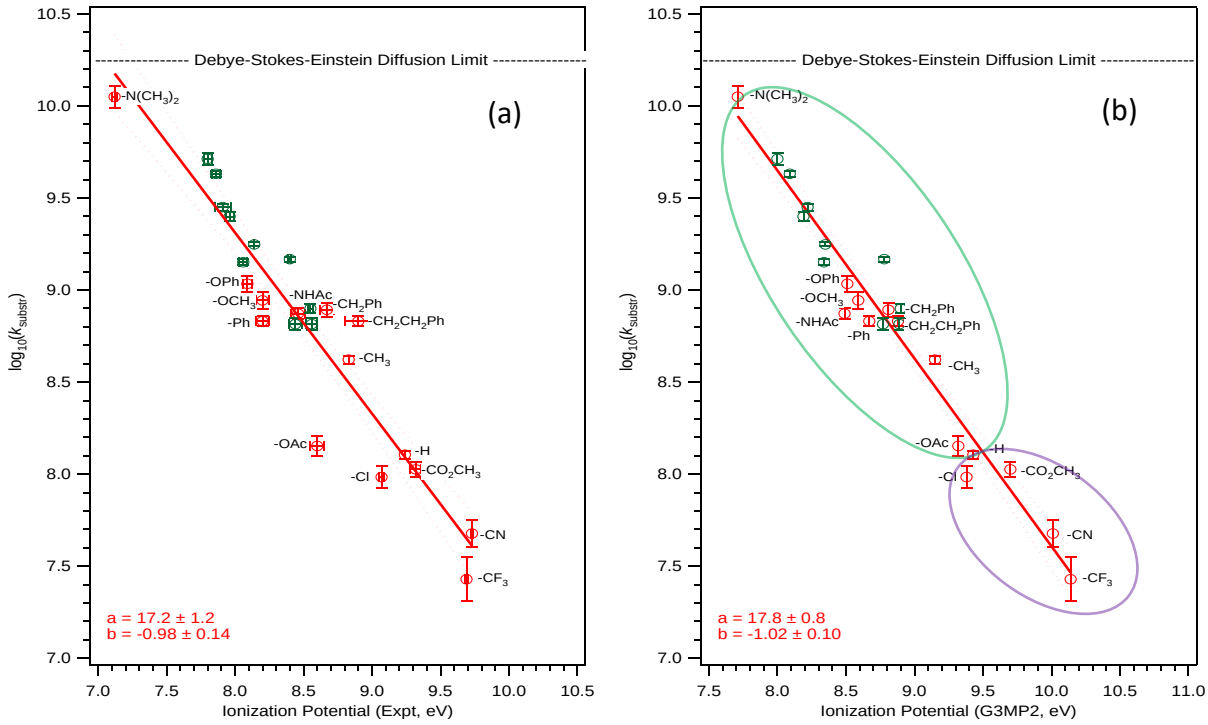

Figure 5- Rate compared with (a) experimental IP and (b) calculated IP. Labeled compounds are data from my work. The green oval shows activated ring structures, and the purple oval shows deactivated ring structures.

References

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Appendices

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