ABSTRACT


Azobenzene derivatives are the largest class of dyes for synthetic textile fibers. Their color arises from the azo chromophore, which has the molecular structure -N=N-. The azo group also acts as a torsional axis for quick and reversible isomerization between the trans and cis conformations, where the trans conformation is more thermodynamically stable in the ground electronic state. Isomerization can be triggered by exposure to ultraviolet light and reversed by relaxation, heat, or visible light. Light can also break down the compounds by photodegradation, in which the azo bond is generally reported to be cleaved by a photoreductive pathway. This process produces toxic, mutagenic, and carcinogenic aromatic amines, and it destroys the color and photoswitching capability of the molecule. However, other analyses of C.I. Disperse Red 1 (DR1) have revealed that the dye molecule degrades at either its hydroxyethyl group or at the azo linkage. In the present work, I investigated DR1 fragmentation and photodegradation mechanisms through density functional theory (DFT) calculations, as well as high resolution mass spectrometry (MS), tandem MS (MS/MS or MSⁿ), and high-performance liquid chromatography-MS (HPLC-MS).

The goal of the DFT calculations was to determine the weakest bonds and reactive sites within DR1 in various states of isomerization and hydrogenation, in order to simulate photoswitching and photoreduction. The isomerization process was simulated, and the transition state was identified. Bond lengths and orders were calculated, along with the torsional angle about the azo bond along atoms C-N=N-C. Condensed-to-atom Fukui reactivity indices were calculated to determine the susceptibility of regions within the molecules to nucleophilic, electrophilic, and radical attack.

Experiments were performed to compare with the DFT results, elucidate DR1 fragmentation pathways, and identify photodegradation products. Solutions of DR1 in methanol were analyzed by quadrupole time-of-flight (Q-TOF) MS and MS/MS to identify fragmentation products. MSⁿ was performed using linear ion trap (LIT) MS to achieve more detailed fragmentation pathways. HPLC-MS was used to separate and analyze photodegradation products extracted from degraded DR1-dyed poly(ethylene terephthalate) fabric.
For all structures examined by DFT, the 2-(ethylamino)ethan-1-ol substituent was calculated to contain the most reactive regions of the DR1 molecule. Additionally, in the experiments, neither fragmentation nor photodegradation produced compounds corresponding to cleavage of the azo bond. This finding is contrary to previously proposed degradation and fragmentation mechanisms. Through DFT calculations, I found that reduction of the azo bond induces trans-to-cis isomerization. To my knowledge, this has not been reported previously. To that effect, it is likely that the photoreductive pathway of DR1 degradation involves isomerization. This relationship between these factors has not been previously illustrated.

This study provides a foundation for the possibility to avoid azo bond breakage during photodegradation. This would reduce the formation of harmful aromatic amines and maintain the chromophore and therefore the coloration properties of the molecule. An understanding of the contribution of isomerization to photodegradation allows for improvements in photoswitching performance. Future work to apply my methodology to other azobenzene derivatives, namely those in the Max Weaver Dye Library, will open doors to entirely new textile dyes and photoswitches.
The Multiple Mechanisms of Disperse Red 1 Photodegradation: A Theoretical and Experimental Understanding

by
Ciera Elyse Cipriani

A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Master of Science

Textile Chemistry

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APPROVED BY:

_______________________________
Dr. Melissa Pasquinelli
Committee Co-chair

_______________________________
Dr. Nelson Vinueza
Committee Co-chair

_______________________________
Dr. Erin Baker

_______________________________
Dr. Alan Tonelli
BIOGRAPHY

Ciera Elyse Cipriani was born on November 19, 1996 in Raleigh, North Carolina, to Zanna Swann and Don Cipriani. Throughout her life, she has developed a broad set of interests ranging from drumming to comedy to, fortunately, chemistry, which led her to the North Carolina State University Wilson College of Textiles in 2014. She began working in the Laboratory of Molecular Modeling from the Nanoscale under Dr. Melissa Pasquinelli as an undergraduate in 2015. Soon after, collaboration with Dr. Nelson Vinueza led to a co-advisorship. During her undergraduate career, Ciera presented her work at numerous conferences, including the 2017 Atlantic Coast Conference Meeting of the Minds and the 2018 National Conference on Undergraduate Research. Encouraged by her advisors, she enrolled in the Accelerated Bachelor’s/Master’s Program. She graduated summa cum laude and earned the Hoechst-Trevira Oligomer Award for Excellence in the Polymer and Color Chemistry Program in 2018. In her short ten months as a full-time master’s student in the Textile Chemistry Program, Ciera won the NC State University Three Minute Thesis Competition, competed at the Three Minute Thesis Regional Competition at the Conference of Southern Graduate Schools, and gave an invited Tech Talk on her research at Techtextil North America. In the fall, she will move to Texas A&M University to pursue a PhD in Materials Science and Engineering.
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CHAPTER 1. RESEARCH MOTIVATION AND OBJECTIVES

The goal of this research is to develop an understanding of the photodegradation mechanisms of the popular azo dye Disperse Red 1 (DR1). This work is motivated by three problems:

**Problem 1. Pollution of textile wastewater.** The textile industry is a large consumer of water and producer of effluent. The degradation of compounds within wastewater can increase its toxicity, carcinogenicity, and mutagenicity.

**Problem 2. Fading of textile products.** Lightfastness is an integral factor used to evaluate colorants on textile products, and photofading is an ongoing issue in the textile industry.

**Problem 3. Control of light-responsive materials.** Azobenzene derivatives are increasingly being used to imbue stimuli-responsive behavior to materials. Light is a common stimulus for these applications.

DR1 is a widely studied textile dye and light-responsive photoswitch. For that reason, the research problems are addressed by the following objectives:

**Objective 1.** Use density functional theory (DFT) to determine the factors which contribute to the photodegradation pathway followed by DR1.

**Objective 2.** Evaluate DFT results using tandem mass spectrometry (MS/MS and MS^n) experiments to identify fragmentation pathways of DR1.

**Objective 3.** Identify photodegradation products of DR1 using high performance liquid chromatography-MS (HPLC-MS).

Chapter 2 provides the background information required to understand the above problems and objectives. Chapter 3 addresses the objectives through a detailed study of DR1 photoswitching and photodegradation, which elucidates a previously unreported relationship between these behaviors. Chapter 4 summarizes the findings from Chapter 3 in context of the study problems and objectives, and it describes future topics to explore based on this work.
CHAPTER 2. BACKGROUND

2.1. Photochemistry

Light is an integral factor in the existence and perpetuation of life. Light contributes to important physical and chemical processes in nature, such as vision, photosynthesis, and maintenance of circadian rhythms. The study of chemical reactions enacted by light is termed photochemistry. These reactions are based upon the excitation of electrons within molecules as they absorb energy from light photons. Generally, this light is in the visible to ultraviolet range. Unlike thermochemical reactions, photochemical reactions do not require collision of chemical species, so these processes can be isolated to individual molecules. Photochemical processes are often reversible, a feature which is harnessed for research and industrial applications. Taking full advantage of photochemical processes is only possible by understanding their underlying electronic transitions.

2.1.1. Electronic Transitions

Electrons within molecules occupy orbitals with discrete energy levels. When irradiated with light to induce a photochemical change, electrons within a molecule move from the ground state highest occupied molecular orbital (HOMO) to the excited state, which is often the lowest unoccupied molecular orbital (LUMO),\textsuperscript{2,3} as illustrated in Figure 1.

![Figure 1](image)

**Figure 1.** An example energy diagram for a molecule, showing the process of electronic excitation by light from the HOMO to the LUMO, adapted from Ref. 4.
This electronic transition can induce a change in the molecular structure of the affected compound. Chatwal and Arora listed the following common photochemical reactions of a molecule: cleavage into radicals, decomposition into molecules, intramolecular rearrangement, photoisomerization, hydrogen atom abstraction, photodimerization, and photosensitization. Depending on the nature of this process, the electrons may release the absorbed energy in the form of heat or light to relax to their original energy level. Electrons which comprise covalent bonds in molecules may, however, be unable to maintain these bonds when irradiated with light. In this case, the covalent bonding will be destroyed, resulting in a change in the chemical structure of the affected compound.

### 2.1.2. Photoswitching

A photoswitching molecule undergoes a reversible change when it absorbs light. Examples of such changes include ring-opening or closing and isomerization. The behavior of photoswitching compounds can be harnessed to produce a desired effect. The process of photoisomerization is harnessed to create photoswitches, which act as “on-off” switches activated by light energy. Using light as a stimulus has several unique advantages. Light is inexpensive, and the application area and wavelengths are highly controllable. Photoswitching compounds have primarily been used for aesthetic purposes, with their functional uses gaining popularity only recently. One of the first applications for such molecules was for photochromic coloring of objects, specifically sunglasses, so that the lenses would darken when exposed to sunlight. The same mechanism that gives rise to this color change can be tailored to elicit a desired response in a material, colorant, or additive. Arthur Ashkin’s Nobel Prize-winning work on optical tweezers sparked interest in the functionality of photoswitches. From the 1960s through the 1980s, Ashkin developed a process which uses the radiation pressure of light to trap and manipulate small species like atoms, molecules, particles, and cells. His findings led to the development of DNA molecular tweezers, which harness the photoisomerization of azobenzene-based molecules to reversibly hybridize DNA strands to open and close the tweezers. This discovery brought attention to the enormous potential of azobenzene derivatives. Applications of azobenzenes photoswitches now span many fields, including microelectronics, material functionalization, nanomachines, and optical devices. Current work on photoswitches focuses on increasing the wavelength range of light activation, optimizing isomerization yield...
and behavior,\textsuperscript{5,11} producing larger-scale changes from these molecular-level transitions,\textsuperscript{5} and increasing switching efficiency on molecular and systemic levels.\textsuperscript{8}

2.1.3. Photodegradation

When light energy excites the electrons within a molecule, covalent bonds may be destroyed in a process known as photodegradation. Bond breakage occurs in two of the common photochemical reactions described in Section 2.1.1.: cleavage into radicals and decomposition into molecules.\textsuperscript{3} Generally, both of these processes alter the functionality of the original molecule. The radical case leaves behind free radical species that propagate further reactions, whereas general decomposition leaves only fragments of the original compound. The occurrence of other photochemical reactions can change a molecule’s chemical structure. Changes in bond order, length, and angle are interrelated and influence which bonds are more susceptible to breakage by photodegradation.

Industrial concern about photodegradation centers on product lifetimes and environmental impact. Materials, colorants, and finishes are designed to withstand an expected level of photodegradation. If photodegradation is not taken into account, products which are exposed to sunlight can lose their material properties, finish effectiveness, and color. Environmental efforts to reduce pollutants center around degrading them to eliminate them from the environment. Photodegradation can be useful in this sense, but it can also cause more harm than good. The environment, wildlife, and humans may be at risk of coming into contact with photodegradation reaction products. This poses a serious risk if the compounds are not well-known or are toxic. Therefore, it is prudent to further develop an understanding of photodegradation pathways for commonly used compounds.

2.2. Photochemistry of Azobenzene Derivatives

Azobenzene derivatives, which are based upon the structure in Figure 2, have a wide variety of applications because they have unique photochemical properties imbued by the azo group, -N=N-. It provides both color and the ability to photoswitch.\textsuperscript{13-15} By modifying the substituent groups, the properties of these molecules can be tailored.\textsuperscript{15} Azobenzene derivatives have long been used as colorants, particularly in the textile industry. Interest in the photoswitching capability of these compounds has increased in recent years because of the decreasing size of many technologies. The potential for molecular functionality to be applied and scaled up within these technologies is being realized.
Figure 2. Molecular structure of azobenzene. Azobenzene derivatives possess this core structure, with substituent groups bonded to the aromatic rings, most commonly at the para- or ortho-positions.

2.2.1. Azobenzene Derivatives as Disperse Dyes

The azobenzene molecular structure is found in most classes of textile dyes, as well as some pigments. Azo dyes can affix to fibers by either covalent bonds or intermolecular interactions. When affixed by van der Waals forces or hydrogen bonding, azo dyes are grouped within the disperse dye class. Disperse dyes are small, nonionic, water-insoluble molecules which have polar functional groups. These properties make them suitable for dyeing the two most important synthetic fibers: polyester and nylon. Disperse dyes are economical and exhibit high intensity and brightness. Generally, they are red, orange, or yellow, but it is possible to obtain a wide range of colors by modifying the substituent groups.

Though azobenzene derivatives offer many advantages as colorants, there are some concerns with their widespread application. Each year, hundreds of thousands of tons of colorants are used across the globe. As of 2000, between 60 and 70% of textile dyes used were based on the azo group, making it the most common chromophore in the industry. Up to 20% of the disperse dye added to a dyebath can be lost in effluent. Therefore, the environmental and toxicological impact of disperse dye molecules in textile wastewater is of utmost concern. Along with the dye molecules themselves, there are also concerns about the potential of dye precursors, intermediates, and degradation products to cause harm.

2.2.2. Azobenzene Derivatives as Photoswitches

Azobenzene derivatives exist as trans isomers in the ground electronic state. When electronically excited by ultraviolet light, they can isomerize to the cis form by out-of-plane torsional rotation about the azo bond. This photoisomerization occurs quickly, on the picosecond time scale. The cis isomer can thermally relax back to the trans structure on a timescale dependent upon the dye’s substituent groups. Isomerization back to the trans conformation can also be activated by the application of heat or light in the visible region.
Isomerization is also possible in the ground state, as has been demonstrated for the azo dye Disperse Orange 3. Azobenzene derivatives are often an ideal choice for photoswitch applications because they isomerize quickly and reversibly, demonstrate high conversion, and inherently undergo a relatively large change in shape and end-to-end distance during the process.

Since the development of DNA molecular tweezers, azobenzene derivatives have become the most popular class of compounds used as photoswitches in the fields of physics, chemistry, materials science, and biology. Recently, azobenzene photoswitches have been used to perform molecular-scale mechanical actions, control protein folding, store data, and functionalize materials. A wide variety of azobenzene-based compounds exist and are well documented as photoswitching molecules. However, photoinduced isomerization may contribute to and alter the photodegradation behavior of such compounds.

2.2.3. Photodegradation of Azobenzene Derivatives

Light energy can cause the photoisomerization of azo dyes, but it can also photodegrade them, fracturing covalent bonds. In this case, as bond conjugation is destroyed, dyes can lose their color. Furthermore, azobenzene derivatives can lose their photoswitching capability through cleavage of the azo bond. More concerning, however, is the potential to form harmful compounds as a result of this process. Fracturing the azo bond produces aromatic amines, which are toxic, carcinogenic, and mutagenic. Multiple studies on the photodegradation mechanisms of azobenzene derivatives have been published. Perhaps the most well-studied is C.I. Disperse Red 1. The potential for this molecule to follow multiple photodegradation pathways is of particular note, though little is known about the factors which contribute to this behavior.

2.3. Molecular Modeling

Molecular modeling is the use of computational methods to predict chemical, physical, and material properties. As computational power has greatly increased in the past few decades, molecular modeling has become a primary tool for chemists, physicists, and biologists. These techniques play a key role in the physical and life sciences by facilitating the development of new chemistries, drugs, materials, and products. In molecular modeling approaches, molecular systems are examined using computer simulations. Molecular modeling can provide specific information about a system that is difficult to determine experimentally. The system and
experimental approach can also be optimized prior to actually performing those experiments, which can save time, money, and samples. On a large scale, physical and material properties such as phase transformations, magnetism, equilibrium volume, and bulk modulus can be predicted.\textsuperscript{41} This is done through molecular simulations, which incorporate molecular configurations and interactions. Simulation approaches include molecular dynamics and Monte Carlo methods, which can model molecular motion.\textsuperscript{42} Quantum mechanical approaches can be used to calculate properties on the electron level, such as electronic transitions, molecular orbitals, electron density, and molecular geometry, including bond lengths, orders, and angles. Quantum mechanical methods involve explicit descriptions of electrons within a system. These include \textit{ab initio}, or “from first principles,” calculations which are computed using no empirical data. Semiempirical methods can also be employed, which incorporate some experimental data to describe systems. Density functional theory falls within this realm; certain treatments of DFT can include empirical data, though DFT is not inherently semiempirical. DFT describes electron density, rather than electrons themselves. In order to choose an appropriate model, the available computational resources, properties to be computed, and required level of precision must be taken into account.

\textbf{2.3.1. Quantum Mechanics}

David Young defines quantum mechanics as “the correct mathematical description of any behavior of electrons and thus of chemistry.”\textsuperscript{1} Quantum mechanics uses wave equations, which arose from the particle-wave duality of light, and is based upon two fundamental postulates.\textsuperscript{1} The first, presented by Planck, is that energy transmission is quantized. This means that energy is not transmitted continuously, but rather in discrete values. The second postulate was presented by de Broglie, building upon work by Hasenöhrl and Einstein; it relates the momentum of a particle to its wavelength. In 1926, Schrödinger applied these two postulates to the one-dimensional wave equation to obtain the time-dependent Schrödinger equation, as well as the energy and momentum operators. The operators are applied to the wave equation to produce the time-independent Schrödinger equation, commonly written as:

\[ H\psi = E\psi \]

where $H$ is the Hamiltonian operator, $E$ is the energy operator, and $\psi$ is the wavefunction. $\psi$ is a function describing the probability that a given particle will occupy a certain quantum state,
based on variables like time, position, and momentum.\textsuperscript{1} \( H \) is comprised of three terms: the kinetic energy, external potential energy, and electron-electron interaction, as given below:

\[
H = -\frac{1}{2} \sum_i^N \nabla_i^2 - \sum_{\alpha}^{N_{\text{at}}} \frac{Z_{\alpha}}{|r_i - R_{\alpha}|} + \sum_{i<j}^N \frac{1}{|r_i - r_j|}
\]  

(2)

Schrödinger’s equation is not exactly solvable for systems with more than one electron, but it can be approximated in such cases by using the Born-Oppenheimer approximation for subatomic particle motion.\textsuperscript{1,43,44} Molecules are composed of atoms, which are composed of protons, neutrons, and electrons. Changing the locations of these subatomic particles influences the total energy of the molecular system.\textsuperscript{45} The Born-Oppenheimer approximation splits the problem of determining molecular energy into two parts, based upon the fact that electrons have much lower mass than nuclei and therefore exhibit faster responses to changes in their surrounding environment. In this approximation, \( M \) nuclei are fixed at positions \( R_1, R_2, \ldots, R_M \), and electrons are allowed to move. The lowest energy arrangement of the atoms, or ground state of the molecule, is calculated by minimizing the adiabatic potential energy surface \( E(R_1, R_2, \ldots, R_M) \). The approximation of this minimum is the focus of molecular modeling techniques, wherein this minimization process is termed “optimization” of the molecular geometry.\textsuperscript{45}

2.3.2. Density Functional Theory

Density functional theory (DFT) is a method of predicting the properties of a system using the electron density, rather than the wavefunction.\textsuperscript{1,44} DFT employs functionals, or functions of functions, to calculate the energy of a system; the electron density is a function of the molecular orbitals, and the energy of a molecule is a function of the electron density. Though first explored in 1927 by Thomas and Fermi and further developed by Slater, DFT truly gained traction with the work of Hohenberg and Kohn in the 1960s.\textsuperscript{1,44,45} They developed the theory of DFT for the ground state electronic energy of a multi-electron system, expressing the total energy as a unique functional of the electron density.\textsuperscript{1,45} Therefore, the contributions to the total energy can be isolated as functionals of the electron density, as below:

\[
E_o[\rho_o] = T[\rho_o] + E_{ee}[\rho_o] + E_{Ne}[\rho_o]
\]  

(3)

where \( E_o[\rho_o] \) is the total ground state energy, \( T[\rho_o] \) is the ground state kinetic energy, \( E_{ee}[\rho_o] \) is the full electron-electron interaction, and \( E_{Ne}[\rho_o] \) is the electron-nucleus interaction. Hohenberg
and Kohn proved that all of the contributions to the total energy sum to a unique functional of the total electron density.¹

### 2.3.3.1. Determining the Ground State Energy

Since the true ground state electron density is unknown, in order to minimize the total energy, trial densities must be used by the variational principle. Hohenberg and Kohn examined this in their second theorem. Given a functional $F_{HK}$, dependent on the trial electron density $\rho_T$, a corresponding energy value $E[\rho_T]$ can be calculated. If $F_{HK}$ results in the true ground state energy $E_o$, then $\rho_T$ must be the true ground state density $\rho_0$. Any other $\rho_T$ will result in a higher calculated energy value and does not correspond to the energy minimum.¹

$$E_o \leq E[\rho_T] = T[\rho_T] + E_{ee}[\rho_T] + E_{Ne}[\rho_T] \quad (4)$$

The goal of DFT is to determine the functional which gives an accurate ground state energy through this method. Kohn and Sham contributed to this effort by combining the work of Thomas, Fermi, and Slater with Hartree-Fock orbital theory to develop the Kohn-Sham equations. These allow for the electron density to be computed using an exchange-correlation functional $E_{XC}$, which is a function of electron density. The exchange and correlation effects which arise from non-interacting electrons are approximated by $E_{XC}$:

$$E_{XC}[\rho] = (T_i[\rho] - T[\rho]) + (E_{ee}[\rho] - E_H[\rho]) \quad (5)$$

where $T_i[\rho]$ is the kinetic energy of interacting electrons and $E_H[\rho]$ is the Hartree electrostatic energy.

In order to approximate $E_o$, a trial density $\rho_T$ is input to equation 5, and the resulting value along with the external potential $E_{ext}[\rho]$ is input to equation 6:

$$E[\rho] = T[\rho] + E_{ext}[\rho] + E_H[\rho] + E_{XC}[\rho] \quad (6)$$

This calculation results in a set of molecular orbitals, which can be applied using an iterative procedure to determine another density, from which a new set of molecular orbitals is found. By repeating this process, the molecular orbitals obtained from one step will eventually show minimal change during the next iteration, within some defined tolerance. At this point, the calculation is converged, and the computed $E$ is determined to equal $E_o$. The determination of $E_o$ is the main goal of molecular modeling, and from this procedure, further steps can be taken to find related quantities. Some methods for doing so are described in Section 3.3.1.
2.3.3.2. Functionals

The selection of an appropriate functional for the approximation of $E_{XC}$ is an integral step in performing DFT calculations. Mathematically, a functional is a function of a function. DFT functionals are functions of electron density which output an $E_{XC}$ approximation. There are two classes of functionals: ab initio, which are directly derived from quantum mechanics, and semiempirical, which attempt to match experimental results as closely as possible. Within these classes are several methods of determining density functionals, including $X\alpha$, local density approximation (LDA), gradient-corrected, and hybrid.$^1$

The $X\alpha$ method, developed by Slater and Johnson,$^{46}$ incorporates exchange but not correlation arising from non-interacting electrons, making it the simplest approach. Moving up an accuracy level, the local density approximation (LDA) incorporates both exchange and correlation, but only considers electron density in the calculation. LDA is primarily used for band structures; it does a poor job in molecular calculations because it tends to overestimate bond strength and underestimate bond length. Gradient-corrected and hybrid methods are more accurate than LDA and, therefore, are the most popular choices for density functionals. Gradient-corrected methods incorporate both the electron density and the gradient of the electron density. Hybrid functionals add components from a Hartree-Fock calculation to other types of functionals. Gradient-corrected and hybrid functionals are generally preferred due to these improvements, but $X\alpha$ and LDA methods can sometimes give accurate results, depending on the system being studied.$^1$ Hybrid functional usage has surpassed that of gradient-corrected methods, with the Becke 3-parameter$^{47}$ Lee-Yang-Parr$^{48}$ (B3LYP) hybrid functional being the most commonly used for molecular level calculations. B3LYP incorporates the LDA and gradient-corrected local exchange and correlation terms to a small molecule test set using 3 fitting parameters, resulting in a functional which is accurate for organic systems.$^1$ Some common density functionals and their classifications are listed in Table 1.

Table 1. Commonly used density functionals and methods for deriving them, taken from Ref. 1.

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Name</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xα</td>
<td>X alpha</td>
<td>Exchange only</td>
</tr>
<tr>
<td>HFS</td>
<td>Hartree Fock Slater</td>
<td>Hartree-Fock with LDA exchange</td>
</tr>
<tr>
<td>VWN</td>
<td>Vosko, Wilks, and Nusair</td>
<td>LDA</td>
</tr>
</tbody>
</table>
Table 1 (continued).

<table>
<thead>
<tr>
<th>Functional</th>
<th>Description</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLYP</td>
<td>Becke correlation functional with Lee, Yang, Parr exchange</td>
<td>Gradient-corrected</td>
</tr>
<tr>
<td>B3LYP</td>
<td>Becke 3 term with Lee, Yang, Parr exchange</td>
<td>Hybrid</td>
</tr>
<tr>
<td>PW91</td>
<td>Perdue and Wang 1991</td>
<td>Gradient-corrected</td>
</tr>
<tr>
<td>G96</td>
<td>Gill 1996</td>
<td>Exchange</td>
</tr>
<tr>
<td>P86</td>
<td>Perdew 1986</td>
<td>Gradient-corrected</td>
</tr>
<tr>
<td>B96</td>
<td>Becke 1996</td>
<td>Gradient-corrected</td>
</tr>
<tr>
<td>B3P86</td>
<td>Becke exchange, Perdew correlation</td>
<td>Hybrid</td>
</tr>
<tr>
<td>B3PW91</td>
<td>Becke exchange, Perdew and Wang correlation</td>
<td>Hybrid</td>
</tr>
</tbody>
</table>

2.3.3.3. Basis Sets

Along with a functional, one must select the group of functions which comprise the approximation of the wavefunction, known as a basis set. Since the wavefunction describes electron probability density, the basis set attempts to describe the atomic orbitals representing that density. The orbitals incorporated in the basis set should correctly describe the atoms present in the system. These are called Gaussian type orbitals (GTO) and are generally of the form:

\[
\varphi = Y_{lm} \sum_l C_l \sum_j C_{ij} e^{-\zeta_{ij}r^2}
\]

(7)

\(Y_{lm}\) is a functional specifying the orbital symmetry, whether it is s, p, d, or f. The \(e^{-r^2}\) term is a Gaussian primitive function. The constant \(C_{ij}\) is a contraction coefficient, and \(\zeta_{ij}\) is a contraction exponent. The basis set defines these coefficients and exponents.\(^1\)

Many different basis sets exist which represent orbitals by different basis functions. The selection of a basis set depends on the system being examined, as well as the desired computational accuracy and efficiency. Computational efficiency has three factors in this case:\(^49\)

1. The number of basis functions. With \(N\) basis functions, the number of two-electron integrals, and thus the computation time, increases as \(N^4\).
2. The form of the basis set. The basis set should allow for efficient evaluation of integrals from the Hartree-Fock equations.
3. The function shapes. The function amplitudes should be large where the probability of finding electrons, described by the wavefunction, is large, and small where that probability is small.
The basis set should be selected to balance computational efficiency with accuracy for the specific molecular system being examined. The most common classes of basis sets are minimal and split-valence. Minimal basis sets are the simplest. They contract multiple GTO orbitals and apply a single contraction to describe each orbital. Minimal basis sets work well for large molecules but are usually not used to calculate quantitative results. Split-valence, or Pople, basis sets incorporate multiple contractions of GTOs and thus multiple basis functions for valence orbitals. Split-valence basis sets allow the electron density to change according to the molecular environment, which is not possible in minimal basis sets.\(^1\)

Split-valence basis sets have a standard nomenclature developed by the Pople group. For example, the simplest split-valence basis set commonly used is 3-21G. In it, three Gaussian primitives are contracted to form the basis function of the atomic core, the 1s orbital. The second and third numbers tell how many Gaussian primitives are contracted for the basis functions of each valence orbital. In the case of 3-21G, two contractions are given for each valence orbital, one contracting two Gaussian primitives and another with one Gaussian primitive. Additional notation includes plus signs, asterisks, and orbitals in parentheses. A basis set named with one plus sign, for example 6-31+G, has diffuse functions applied to some non-hydrogen atoms. When diffuse functions are applied to all atoms, the name includes two plus signs. The incorporated polarization functions are indicated by the number of asterisks. A single asterisk means that d primitives have been added to some non-hydrogen atoms, while two asterisks indicate that p primitives have been added to hydrogens, as in 6-31G**. Even more functions can be incorporated, indicated by a parenthetic notation. In it, the first value tells which functions have been added to non-hydrogen atoms, while the second value tells which functions have been added to hydrogens. For example, 6-31+G(d,p) has extra sets of d functions applied to non-hydrogen atoms and p functions applied to hydrogens. The most popular split-valence basis sets for organic systems are 6-31G and 6-311G, often with additional functions, because of their accurate quantitative results for a wide range of atoms. For this reason, the combination of B3LYP functional with the 6-31G basis set is often used in DFT calculations for organic molecules.\(^1\)

2.4. Mass Spectrometry

Mass spectrometry (MS) is an analytical chemistry technique which ionizes chemical compounds from a sample, manipulates them using electromagnetic fields, and detects the mass-
to-charge \((m/z)\) ratios and abundances of the ions. The MS field can be said to have begun in 1897, when Joseph J. Thomson discovered the electron and quantified its \(m/z\) ratio.\(^{50}\) The \(m/z\) ratio is used in MS to identify compounds present in a sample. In order to obtain \(m/z\) ratios, all mass spectrometers are built as illustrated in Figure 3. They must have an ionization source to create ions from the compounds in the sample, which are sent to the mass analyzer to be separated by their \(m/z\)’s. Then, the ions may be fragmented and analyzed again in additional mass analyzers. After separation and fragmentation, ions are detected and converted to electrical signals. These signals are transmitted to a data system, which produces the mass spectrum, measures instrumental parameters, and controls the mass spectrometer.\(^{50}\)

![Figure 3. General structure of a mass spectrometry system.](image)

There are many different types of ionization sources, mass analyzers, and detectors, which influence the sample state, sample preparation, ions formed, molecular fragmentation, detection limit, resolution, resolving power, and mass accuracy. Multiple mass analyzers can be combined in a system, or other techniques such as liquid and gas chromatography can be coupled with MS to elucidate further information about a sample.\(^{50}\) More information on chromatography is provided in Section 2.4.4. Instrumentation selection plays a critical role in the type and quality of results obtained from MS.

2.4.1. Ionization Sources

The ionization of compounds within a sample is required in order for them to be separated by electric and/or magnetic fields in the mass analyzer. Ionization methods are selected by evaluating the properties of the analyte and the acceptable intensity of the ionization process. Gas-phase ion sources (electron ionization, chemical ionization, and field ionization) can ionize only volatile and thermally stable compounds, whereas condensed-phase ion sources can ionize thermolabile compounds. Of the condensed-phase sources, there are two types: liquid-phase (electrospray ionization, atmospheric pressure chemical ionization, atmospheric pressure photoionization) and solid-state (matrix-assisted laser desorption ionization, secondary ion mass
spectrometry, plasma desorption, field desorption). Liquid-phase sources require the analyte to be in solution, whereas solid-state sources ionize the analyte from a nonvolatile deposit. Ionization sources can vary in intensity, described as “softness” or “hardness.” This property influences the types of ions that are formed and the extent of fragmentation of analyte molecules.\textsuperscript{50}

2.4.1.1. Electrospray Ionization

Electrospray ionization (ESI) was developed in 1968, but John Fenn’s application of ESI to analyzing macromolecules in 1984 kickstarted the recent uptick in mass spectrometry usage and advancements. ESI is now widely used for many types of analytes, from large proteins to small polar molecules.\textsuperscript{50}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{esi.png}
\caption{Schematic of ion production within an ESI source, adapted from Ref. 51.}
\end{figure}

A schematic of the ESI process is given in Figure 4. ESI ionizes analyte molecules using a strong electric field at atmospheric pressure. First, a liquid sample is introduced to the system at a low flow rate (1-10 μL/min) and enters a spraying nozzle. A potential difference of 2-5 kV is applied between this nozzle and a counter-electrode which is 0.3-2 cm away. This arrangement produces an electric field on the order of 10\textsuperscript{6} V/m. As a result of the potential difference, charges
within the liquid accumulate at the end of the spraying nozzle. Under low voltages, the liquid forms a rounded shape at the end of the nozzle. As the voltage increases, more charges accumulate and the liquid becomes more pointed, assuming a shape known as a Taylor cone. The liquid breaks away to produce charged parent droplets. Heated inert gas fills the ionization chamber to limit droplet dispersion. As solvent evaporates from a charged parent droplet, the density of charges on the droplet’s surface increases and approaches the Rayleigh limit, given below.

\[ q_r = \sqrt{\frac{64\pi^2 \varepsilon_0 \gamma r^3}{8}} \]  

\( q_r \) is the charge possessed by the droplet, \( \varepsilon_0 \) is the permittivity of free space, \( \gamma \) is the droplet’s surface tension, and \( r \) is the droplet’s radius. The Rayleigh limit is the maximum amount of charge per unit volume that a droplet can possess. At this point, the droplet undergoes Coulomb fission, bursting into numerous smaller charged progeny droplets. This is the “spray.” Solvent further evaporates to produce analyte ions, which are collected by the counter-electrode and delivered to the mass analyzer. ESI can produce singly- and multiply-charged ions, depending on the number of ionizable sites on the analyte molecules. Since compounds are detected by their \( m/z \) ratios, multiple charges allow for the detection of large compounds, even with low-mass range instruments. However, the quality of a mass spectrum is not solely determined by the ionization source. Another key factor is the mass analyzer.

2.4.2. Mass Analyzers

Mass analyzers separate ions by their \( m/z \) ratios. All mass analyzers use one or both of electric and magnetic fields to manipulate ions. The two main types of mass analyzers are scanning analyzers which deliver ions to the detector over time, and those which transmit all ions to the detector simultaneously. Scanning analyzers can be quadrupoles or flight tubes within a magnetic field. Simultaneous transmission analyzers may be time-of-flight (TOF), dispersive magnetic analyzers, or trapped-ion analyzers, which include ion traps, Orbitraps, and ion cyclotron resonance.

Mass analyzer technology is continually in development to increase ion transmission, analysis speed, mass range, mass accuracy, resolution, and resolving power. A mass analyzer’s ion transmission is the ratio of the number of ions that enter the analyzer from the ionization source to the number of ions that reach the detector from the analyzer. Increasing the ion transmission and duty cycle is of interest because they increase a mass spectrometer’s sensitivity.
Analysis speed is how quickly an analyzer can scan a given mass range. Higher analysis speed allows for shorter run times, increasing the number of samples that can be analyzed. Mass range is the \( m/z \) range for which a mass analyzer effectively measures ions. It is necessary to select a mass analyzer which is appropriate for the masses of the analyte ions. Resolution is the level of distinction between the signals for two ions with a small \( m/z \) difference. High resolution mass analyzers effectively separate two such ions into distinct peaks on a mass spectrum. With lower resolution mass analyzers, the peaks may be indistinguishable from one another. Peak resolution criteria vary for different analyzers. For magnetic or ion cyclotron resonance analyzers, a valley between two peaks must have an intensity no more than 10\% that of the weaker peak in order for the peaks to be considered resolved. Quadrupole, ion trap, and TOF analyzers must achieve a 50\% valley intensity relative to the weaker peak to be considered resolved. The limit of an instrument’s resolution is \( \Delta m \), which is the smallest \( m/z \) difference between two peaks for which those peaks are still resolved. Resolving power describes an instrument’s resolution at a specific \( m/z \) value, \( m \). Resolving power, \( R \), is calculated as:

\[
R = \frac{m}{\Delta m}
\]  

(9)

\( R \) can also be determined by taking an individual peak’s width at a certain intensity value as \( \Delta m \), and the corresponding \( m/z \) value of the peak as \( m \). Each of the above parameters of mass analyzer performance influence the mass spectra obtained from a sample. Mass analyzers can be coupled to one another to achieve additional information about ion structures, by separating ions in the first mass analyzer and reacting or fragmenting them in the second. Examples include the coupling of a quadrupole to a TOF (Q-TOF) and ion trap-Fourier transform ion cyclotron resonance. Using multiple analyzers allows for tandem mass spectrometry, also known as MS/MS or MS\( ^n \), to be performed. In this methodology, multiple rounds of mass analysis and detection are performed on selected ions in order to elucidate further information about their chemical structures.\(^{50} \)

2.4.2.1 Quadrupoles

Quadrupole mass analyzers use four rods to which potentials are applied, producing oscillating electric fields which separate ions. Figure 5 gives a visualization of a quadrupole.
Figure 5. Schematic of a quadrupole with the same potential applied to directionally opposite rods, taken from Ref. 52.

All rods have the same magnitude potential applied to them, and rods which are directionally opposite one another are given the same sign potential:

\[ \pm \Phi_0 = \pm (U - V\cos\omega t) \]  \hspace{1cm} (10)

\( \Phi_0 \) is the applied potential, \( U \) is the direct potential, \( V \) is the amplitude of the electromagnetic field voltage, and \( \omega \) is the angular frequency in radians per unit time based upon the electromagnetic field frequency. The potentials applied to the rods influence the electric field forces, which control the motion of ions within a quadrupole. The forces in the \( x \) and \( y \) directions, orthogonal to the length of the rods, are:

\[ F_x = m \frac{d^2x}{dt^2} = -ze \frac{\partial \Phi}{\partial x} \]  \hspace{1cm} (11)

\[ F_y = m \frac{d^2y}{dt^2} = -ze \frac{\partial \Phi}{\partial y} \]  \hspace{1cm} (12)

These relationships are differentiated to give the equations of the ion motion:

\[ \frac{d^2x}{dt^2} + \frac{2ze}{m\rho^2} (U - V\cos\omega t)x = 0 \]  \hspace{1cm} (13)

\[ \frac{d^2y}{dt^2} - \frac{2ze}{m\rho^2} (U - V\cos\omega t)y = 0 \]  \hspace{1cm} (14)

Ideally, all ions which enter a quadrupole would exit it to reach the detector. In reality, ions travel in waves. They can make contact with the quadrupole rods and become discharged.
These neutral species are no longer able to be manipulated by the electric field. Equations 13 and 14 demonstrate the time-dependence of ion position, with the origin of \( x \) and \( y \) at the center \( z \)-axis between the four rods. So long as \( x \) and \( y \) are less than the orthogonal distance from the \( z \)-axis to a rod \( (r_0) \), a given ion will successfully travel through the quadrupole without being discharged. Since the rod positions and the angular frequency \( \omega \) are fixed, \( x \) and \( y \) are determined by \( U \) and \( V \). Thus, ion separation is controlled by the direct potential and electromagnetic field voltage applied to the rods in a quadrupole.

### 2.4.2.2. Ion Traps

Ion trap mass analyzers operate using a similar principle to a quadrupole, even employing quadrupoles as trapping devices in some cases. In an ion trap, an oscillating electric field is used to hold ions within the analyzer and selectively eject them for analysis. Ion traps can be two-dimensional or three-dimensional, depending on their architecture. 2D ion traps, also called linear ion traps (LITs), consist of a quadrupole which has reflecting lenses at both ends that operate at a potential of the same sign as the ionic charges. Ions are confined to travel along the quadrupole axis. 3D ion traps contain a circular electrode which is capped on the top and bottom to produce a 3D quadrupolar field. Compared to 3D ion traps, LITs offer better trapping efficiency and focus, resulting in higher sensitivity, resolution, and dynamic range. All ion traps provide the potential to perform MS\(^n\) analyses. This is done by optimizing the values of \( U \) and \( V \) in order to eject from the trap all ions except for those of the desired \( m/z \). The remaining ions are collided with helium gas molecules inside the trap to produce fragments, which can be analyzed. Further fragmentation can be performed to obtain additional steps along fragmentation pathways and elucidate corresponding molecular structures.

### 2.4.2.3. Time-of-Flight

Time-of-flight (TOF) is a pulsed mass analyzer which separates ions based on how long they take to travel a fixed distance after they are accelerated to a specific kinetic energy. An electric field provides the initial acceleration, then the ions travel through a flight tube to eventually reach a detector. The acceleration potential \( (V_s) \) and velocity \( (v) \) of an ion are related by equating the imparted electric potential energy \( (E_{el}) \) and the kinetic energy \( (E_k) \) throughout the flight tube length \( (L) \):

$$E_k = \frac{1}{2}mv^2 = qV_s = zeV_s = E_{el}$$

(15)
\[ v = \sqrt{\frac{2zeV_s}{m}} \]  \hspace{1cm} (16)

\[ t = \frac{L}{v} \]  \hspace{1cm} (17)

\[ t^2 = \frac{m}{z} \left( \frac{L^2}{2eV_s} \right) \]  \hspace{1cm} (18)

Equation 18 specifies that ions with lower \( m/z \) ratios have shorter flight times. The resolution (\( R \)) of a TOF mass analyzer can be determined from the \( m/z \)-time relationship:\(^50\)

\[ \frac{m}{z} = \left( \frac{2eV_s}{L^2} \right) t^2 \]  \hspace{1cm} (19)

\[ \frac{1}{z} dm = \left( \frac{2eV_s}{L^2} \right) 2t \, dt \]  \hspace{1cm} (20)

\[ \frac{m}{dm} = \frac{t}{2dt} \]  \hspace{1cm} (21)

\[ R = \frac{m}{\Delta m} = \frac{t}{2\Delta t} \approx \frac{L}{2\Delta x} \]  \hspace{1cm} (22)

Since \( m/z \) is relative to flight time, TOF mass analyzers theoretically have no upper mass limit and can analyze a wide range of compounds. TOF has several other advantages, including simple two-point mass calibration, fast analysis, and high sensitivity due to high ion transmission. The main disadvantage of TOF arises from the direct relationship between resolution and flight time. TOF mass analyzers have low resolution which can be improved by providing a longer flight path. This can result in a larger instrument footprint, which is undesirable. Instead, the flight path can be made helical to increase the distance traveled by the ions in a shorter tube. Another option is to use a reflectron, which produces an electrostatic field that reflects ions back within the flight tube. Reflectrons increase the flight path length and eliminate error in the kinetic energy dispersion of ions, so that ions with the same \( m/z \) leave the reflectron with the same kinetic energy. These solutions are inadequate, however, because increasing the flight path length allows for more ion scattering, which leads to lower transmission and decreased sensitivity. An alternative way to increase TOF resolution is to lower the voltage by which the ions are accelerated, but this also decreases the sensitivity. There is no ideal solution to the low resolution problem with TOF mass analyzers. Instrument manufacturers must find a balance between flight tube length and acceleration voltage that optimizes resolution.
and sensitivity. Generally, this is a 1 to 2 meter long flight tube and an acceleration voltage of at least 20 kV.\textsuperscript{50}

2.4.3. Detectors

After the ions have been separated by a mass analyzer, they must be detected. The first type of detector for MS, the photographic plate, did not use a computer. Instead, separated ions collided with a plate, and ions with the same \textit{m/z} hit the same location. An ion’s abundance was determined by how dark its corresponding spot was. Modern detectors measure electric current to produce a signal readable by a computer. A higher abundance of ions at a specific \textit{m/z} results in a larger electric current, which is converted by the detector and reflected in the mass spectrum. In all cases, the number of ions transmitted from the mass analyzer to the detector is small, so the signal is amplified.\textsuperscript{50}

There are two groups of ion detectors: point ion collectors and array collectors. Point ion collectors detect ions of differing masses at different times. Array collectors are capable of simultaneous detection, meaning that the can detect all ions at the same time. Both point ion and array collectors have the main drawback of decreasing efficiency with increasing ion mass. New types of detectors seek to solve this problem by providing the same efficiency across the entire mass range. These include charge or inductive detectors, which directly detect the current induced by an ion on a plate, and cryogenic detectors, which detect ion kinetic energy through calorimetry. These detectors are still not as widely used as point ion or array collectors, and developments into consistent efficiency at high \textit{m/z}’s is ongoing.\textsuperscript{50}

There is a wide range of commercially available detectors, each of which uses a different detection method. Most are inherently destructive, as they require the ions to collide with a surface. For example, in a Faraday cup, ions collide with a surface and are neutralized. This process produces a current in a connected resistor, which is discharged, amplified, and detected. In electron multipliers and electro-optical ion detectors, ions collide with a surface to produce secondary electrons, which are amplified and measured as current. Image current detectors are non-destructive. They consist of metal plates in a circuit, which detect the ions and produce an image current. These are specific to Fourier transform ion cyclotron resonance and Orbitrap mass spectrometers.\textsuperscript{50}
2.4.3.1. Electron Multipliers

The electron multiplier is the most common ion detector. It uses a type of electrode called a dynode to multiply incident charges to detect single ions. Ions hit a conversion dynode, emitting secondary particles which are converted to electrons. These electrons are amplified by either multiple discrete dynodes or a single continuous dynode. The current is measured and is proportional to ion abundance. The gain of an electron multiplier can be modified to produce a larger signal proportional to the input signal. Theoretically, infinite gain is possible, but this reduces the lifetime of the detector.50

2.4.3.2. Microchannel Plates

A microchannel plate (MCP) detector is a type of continuous dynode electron multiplier. It consists of a plate with many channels drilled into it, the interiors of which are coated with a semiconducting material. A potential differential is applied so that the input side of the plate has a voltage approximately 1 kV lower than the output side. The plate is just millimeters thick, with channel diameters on the micrometer scale. Despite its thinness, an MCP can amplify signal to the order of $10^5$ by producing secondary electrons from the collision of ions with the walls of the channels. A single, large metal anode behind the plate, or a small one behind each channel, collects the secondary electrons and detects the current.50

MCP detectors give fast response times since secondary electrons travel only a short distance through the channels. This makes them useful for TOF analyzers, since they give precise arrival times and narrow ion pulses. Additionally, the large area of the MCP allows for the detection of large ion beams without focusing. MCPs are not without their drawbacks, however. They are expensive, fragile, and particularly unstable to air. Another type of detector, the microsphere plate (MSP), was designed to address these problems. Instead of drilling holes in a continuous surface, sintered glass beads are coated with an electron emissive material. Similar to MCPs, a potential difference is applied to the conductive sides of the plate. The collision of ions with the bead surfaces produces secondary electrons which travel through the plate, are multiplied, and ultimately detected. MSPs are less expensive than MCPs, and they have higher gain because more secondary electrons are produced by their increased collision surface area.50

2.4.4. High Performance Liquid Chromatography-Mass Spectrometry

When a sample is complex, separation is often performed before MS analysis. Two common separation methods are gas chromatography (GC) and liquid chromatography (LC). GC
requires samples to be volatile so that they can be separated in their gaseous form, while LC is used for nonvolatile samples. LC and GC both use a stationary phase within a column and a mobile phase which carries the sample through the column. The stationary phase can be coated on the column walls or packed into the column as particles. The stationary phase can either be more polar than the mobile phase, in the case of normal-phase chromatography, or less polar than the mobile phase for reversed-phase chromatography. As the mobile phase flows through the column carrying the sample, compounds within the sample interact with the stationary phase. The differential interaction of these compounds with the stationary phase causes them to travel through the column at different times. Compounds which have a higher affinity for the stationary phase have a longer elution time. High performance LC (HPLC), in which pressure is used to push the mobile phase through the column, is commonly used to decrease runtime. The composition of the mobile phase can be modified throughout a run by a gradient method, which can optimize separation. Performing a chromatographic separation before MS analysis can allow for more detailed detection of compounds from complex mixtures.
CHAPTER 3. ELUCIDATING THE PHOTODEGRADATION PATHWAYS OF DISPERSE RED 1 USING A COMBINED APPROACH OF EXPERIMENTS AND MOLECULAR MODELING

Ciera E. Cipriani, Erol Yildirim, Cody P. Zane, Stephanie E. Atkinson, Nelson R. Vinueza, Melissa A. Pasquinelli

Department of Textile Engineering, Chemistry and Science, North Carolina State University, Raleigh, NC 27606

3.1. Abstract

Azobenzene compounds are commonly used in the textile industry to dye synthetic fibers. When exposed to light, azobenzene derivatives can isomerize quickly and reversibly in the process of photoswitching. Light can also break down the molecules by photodegradation, in which the azo bond is generally reported to be cleaved by a photoreductive pathway, producing toxic, mutagenic, and carcinogenic aromatic amines. However, other analyses of C.I. Disperse Red 1 (DR1) have revealed that the dye molecule degrades at either its hydroxyethyl group or at the azo linkage. In this study, we investigated these photodegradation mechanisms using density functional theory (DFT) calculations, high resolution tandem MS (MS/MS), MS^n, and high performance liquid chromatography-MS (HPLC-MS). Hydrogenation of the azo bond induced a conformational change in DR1 when the molecular geometry was optimized. Additional DFT calculations suggest that isomerization redistributed the reactivity from the 2-(ethylamino)ethan-1-ol substituent to incorporate the regions surrounding the azo bond. The most reactive region of the DR1 molecule in most cases, however, was still within the 2-(ethylamino)ethan-1-ol substituent. Fragmentation and photodegradation product compounds detected by MS corresponded to breakdown of the 2-(ethylamino)ethan-1-ol substituent group, rather than the azo bond. The photodegradation pathway suggested by these results is preferred to azo bond cleavage because the bond conjugation of the dye survives the photodegradation process, improving color retention. Furthermore, this pathway does not produce harmful aromatic amines. Though previous literature has reported cleavage of the azo bond when azo dyes are exposed to light, the present results demonstrate that it is possible to inhibit the degradation of the azo bond. These results illustrate that the location and mechanism of photodegradation within DR1 can be controlled by altering its molecular structure.
3.2. Introduction

As elaborated in Section 2.2., the photochemical properties of azobenzene derivatives are unique in that they impart color, as well as the ability to reversibly change their molecular conformation with the application of light. However, a key problem lies in the photodegradation of these compounds – cleavage of the azo bond. The azo bond is generally reported as the main location for photodegradation.\textsuperscript{21,33,34,38,39,54-56} This reaction destroys the chromophore and interrupts bond conjugation, thus eliminating the color of azobenzene derivatives. Furthermore, this mechanism causes azobenzene derivatives to lose their photoswitching capability. More concerning is the potential to form toxic, carcinogenic, and mutagenic aromatic amines as a result of this mechanism. These compounds can wash out of fabrics during processing and laundering, enter waterways, and spread across the globe.\textsuperscript{18}

Since 1949, photoreduction and subsequent cleavage of the azo bond has been reported as the primary mechanism of the photodegradation of azobenzene derivatives.\textsuperscript{33,34,38,39,54-56} However, other photodegradation pathways are possible. When studying the photodegradation mechanisms of the azo dyes DR1 and Disperse Red 17 using gas chromatography MS and thin layer chromatography, Freeman and Hsu identified two photodegradation pathways for these compounds. One involved the expected reduction and cleavage of the azo bond, presented for DR1 as pathway I in Figure 6. Another involved degradation within the respective arylamine substituents of the dye molecules (pathway II). In this pathway, they proposed that the 2-(ethylamino)ethan-1-ol substituent is dehydrated to 2-(ethylamino)ethene, then rehydrated to form 2-(ethylamino)ethan-2-ol. Ethanal dissociates from the DR1 molecule, while the azo bond remains intact.\textsuperscript{34} These findings indicate that DR1 can undergo multiple photodegradation pathways, but the factors contributing to which pathway is followed are not yet known.
Figure 6. Photodegradation pathways of DR1 proposed by Freeman and Hsu, adapted from Ref. 34.

Chromatographic separation methods coupled to tandem mass spectrometry are commonly used for the identification of degradation product compounds and thus degradation mechanisms. These instruments are highly selective, offering the potential to isolate individual compounds for detailed analysis. They also have high sensitivity, requiring samples only on the milligram scale or even smaller. Despite these advantages, isolation of isomers is only possible with specialized techniques such as ion mobility MS. Furthermore, monitoring the multiple changes that light can cause azobenzene derivatives to undergo, such as photoisomerization, photoreduction, and photodegradation is highly complex. This difficulty is compounded by the potential for compounds to fragment as a result of ionization, which is a required step of MS detection and analysis. In fact, similar fragments to those in Figure 6 were detected in an MS study of undegraded 4-amino-4’-nitroazobenzene derivatives, whereby cleavage occurred in the arylamine substituent, as well as between the nitrogen atoms of the azo linkage and their adjacent carbons. An LC-MS/MS analysis of disperse dye wastewater found that fragmentation occurred at the azo linkage due to ionization by thermospray. The disagreement about the mechanism of photodegradation and fragmentation pathways of azobenzene derivatives is fueled by the variety of complicating factors in their experimental analysis.
In order to more deeply understand the photochemistry of azobenzene derivatives without the drawbacks of experimental methods, DFT is increasingly being used to visualize and predict conformation changes and reactions. DFT takes into account the electron distribution across a molecule, which is particularly useful when predicting reaction mechanisms. Many different molecular properties can be predicted quickly by computation. These can expedite the development of experimental procedures, provide explanation for experimental results, or even give information that cannot be measured experimentally. The energetic pathways of azobenzene derivatives as they isomerize are well-documented, where the trans isomer is more energetically favored than the cis isomer and the transition state is calculated to find the activation energy of isomerization.\textsuperscript{62-64} Through DFT calculations, isomerization to the cis form has been found to reduce the length of the azo bond and increase the lengths of bonds to adjacent carbon atoms.\textsuperscript{64} This finding aligns with experimentally determined fragmentation pathways at both of these sites.\textsuperscript{35,40} Many DFT studies have been performed which successfully predict the oxidative degradation of azobenzene derivatives.\textsuperscript{65-67} There is little research on the photoreductive degradation pathway. Bandara and coauthors reported the ability to prevent trans-to-cis isomerization in azobenzene by forming intramolecular hydrogen bonds between the nitrogen atoms of the azo bond and hydrogen atoms within bulky substituent groups.\textsuperscript{68} Hydrogenation plays a factor in the ability of azobenzene derivatives to isomerize, so the relationship between the photoreductive degradation pathway and isomerization is of great importance. However, research relating these two factors is lacking.

For these reasons, in the present study, we explored how isomerization and azo bond hydrogenation influence each other and the photodegradation of azobenzene derivatives. We focused on the commonly studied compound DR1, the isomeric forms of which are given in Figure 7. We sought to determine what conditions contribute to cleavage of the azo bond versus the 2-(ethylamino)ethanol group by examining the electronic properties and energetics of DR1 using DFT, and the photodegradation products of DR1 using HPLC-MS. A better understanding of the photodegradation mechanism of DR1 will lead to the reduction of photofading and decreased production of harmful aromatic amines. Furthermore, more effective optical devices, microelectronics, nanomachines, and functional materials can be designed with improved knowledge of DR1 photoreduction and photoswitching.
Figure 7. Molecular structures of trans-DR1 (left) and cis-DR1 (right). Red Arabic numeral labels indicate covalent bonds which were cleaved as a result of photodegradation in the study by Freeman and Hsu. Green lowercase Roman numerals specify the labeling convention for hydrogen locations. Red lowercase letter labels indicate bonds between which the torsional angle was calculated, with the azo bond (bond 1) as the torsion axis.

3.3. Methods

3.3.1. DFT Calculations

Molecular modeling computations were carried out on the High Performance Computing Center at North Carolina State University. DFT calculations were performed using the Gaussian 16 software package with the Becke three-parameter Lee-Yang-Parr (B3LYP) exchange–correlation functional and 6-31+G(d,p) basis set. An overview of DFT is provided in Section 2.3. To match the procedure of previous experimental work, ethyl acetate solvent effects were evaluated using the integral equation formulation variant of the polarizable continuum model (IEFPCM). Starting from 10 different initial structures, the trans isomer of DR1 was optimized to its lowest energy geometry at a gradient threshold of $4.5 \times 10^{-4}$ Hartree/Bohr. From this structure, the torsional angle between bonds (a) and (b) (see Figure 7) about the azo bond was perturbed, and the same optimization process was used to determine the optimized cis-DR1 structure. A 40-step isomerization process was computed with the VAMP module in BIOVIA Materials Studio 5.5 using the optimized ground state trans- and cis-DR1 geometries as the reactant and product, respectively. From the optimized trans- and cis-DR1 geometries, the nitrogen atoms comprising the azo bond were hydrogenated, and the resulting structures were optimized using the above method. Covalent bond lengths were determined from the optimized molecular geometries. Covalent bond orders were computed using the Natural Bond Orbital (NBO) analysis. Atomic Fukui indices, known as condensed-to-atom Fukui functions, were
calculated using atomic charges calculated by the Charges from Electrostatic Potentials Using a Grid-based Method\textsuperscript{75} (CHELPG) scheme and a finite difference approximation. The nucleophilic ($f_k^+$), electrophilic ($f_k^-$), and free radical ($f_k^r$) Fukui functions are:

\[
\begin{align*}
    f_k^+ &= q_k(N + 1) - q_k(N) \\
    f_k^- &= q_k(N) - q_k(N - 1) \\
    f_k^r &= \frac{1}{2}[q_k(N + 1) - q_k(N - 1)]
\end{align*}
\]

where the terms $q_k(N + 1)$, $q_k(N)$ and $q_k(N - 1)$ are the charges of atom $k$ with $N + 1$, $N$ and $N - 1$ electrons, respectively.\textsuperscript{76}

### 3.3.2. Experimental Methods

A $2.15 \times 10^{-3}$ M solution of DR1 in methanol was prepared, then directly injected and ionized via electrospray ionization (ESI) in negative mode in an Agilent Technologies Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer (MS) 6520 to obtain exact mass measurements. Ions corresponding to peak mass-to-charge ratios were isolated using a narrow isolation width of 1.3 Da. Isolated ions were then fragmented by collision induced dissociation (CID) using the same instrument to obtain high resolution MS/MS results. The DR1-MeOH solution was directly injected and ionized via ESI in negative mode in a Thermo Scientific™ Velos Pro Dual-pressure Linear Ion Trap (LIT) Mass Spectrometer. Ions corresponding to peak mass-to-charge ratios were isolated using an isolation width of 1.2 Da. Isolated ions were then fragmented by collision induced dissociation (CID) using the same instrument. This process was repeated to obtain MS\textsuperscript{n} results.

3.05 g of 100% poly(ethylene terephthalate) (PET) fabric was dyed with DR1 at 1% dye on the weight of the fabric (owf), with 150 mL deionized water as the dyeing medium and 1.3 mL acetic acid added to the dyebath to create an acidic environment. Dyeing was performed in a Datacolor AHIBA IR\textsuperscript{®} Pro starting from room temperature (23 °C), ramped by 4 °C per minute to 130 °C, and then held at 130 °C for 25 minutes. The dyebath beaker was then placed in ice bath to cool. The fabric was dried. The fabric was degraded for 48 hours in an Atlas Ci3000+ Xenon Fade-Ometer with two borosilicate filters to simulate outdoor daylight and in Arizona conditions. The fabric was agitated in a 90:10 dichloromethane:MeOH solution and filtered through polyvinylidene fluoride filters with 0.2 µm diameter pores.
The extract was separated and analyzed using an Agilent Technologies HPLC 1200 Series coupled to the aforementioned Q-TOF MS. The HPLC separation was performed using an Agilent ZORBAX StableBond-Aq column with 3.5 µm particle diameter, 2.1 mm internal diameter, and 150 mm column length. A gradient method was used for separation. The solvent composition was ramped from 20% water:80% acetonitrile to 10% water:90% acetonitrile in 3 minutes, then ramped back to 20% water:80% acetonitrile in 1 minute, for a total runtime of 4 minutes. The HPLC output was then ionized by ESI in positive and negative modes and analyzed by Q-TOF using the MassHunter LC/MS Data Acquisition Workstation Software Version B.05.01 interface to obtain exact mass measurements. Spectra were analyzed in profile mode with the MassHunter Qualitative Analysis Workstation Software Version B.06.00 interface.

3.4. Results and Discussion

3.4.1. Activation Energy of Isomerization

The plot in Figure 8 provides the calculated molecular energy values versus the torsional angle between bonds (a) and (b) (see Figure 7) throughout the isomerization process of DR1. The energy maximum corresponds to the transition state (TS) structure. The TS is a first-order saddle point and should, therefore, correspond to a single negative frequency, and the proposed TS structure in Figure 8 fulfills this requirement. Therefore, our proposed TS structure for the isomerization of DR1 is reasonable. The activation energy for isomerization, ΔE, was calculated as ΔE = ETs − Etrans to be 22.1 kcal/mol (92.4 kJ/mol). Previous studies of the isomerization of the azobenzene molecule reported ΔE to be approximately 200 kJ/mol. A potential explanation for DR1 having a lower ΔE than azobenzene is that substituent groups disrupt the conjugation of the aromatic rings, thus facilitating isomerization. By modeling the trans-to-cis isomerization process, we determined that it requires a relatively low activation energy.3
Figure 8. Energy of DR1 plotted against the torsional angle between bonds (a) and (b) during the trans-to-cis isomerization process. The initial trans isomer is on the left, proposed transition state structure is in the middle, and the product cis isomer is on the right. The activation energy of the process is 22.1 kcal/mol.

3.4.2. Isomerization and Hydrogenation Influence Bond Strength

Bond lengths and orders were calculated for covalent bonds of interest that are labeled in Figure 7, specifically the azo bond (1), and also N-C bonds (2) and (3) in the arylamine substituent. Torsional angles were calculated between bonds (a) and (b) along the azo bond. Table 2 includes the bond lengths, orders, and torsional angles that were computed for trans- and cis-DR1 with no additional hydrogen atoms, a single hydrogen at either position i or ii, and hydrogens bonded to both sites i and ii. As expected, bond orders scaled inversely with bond lengths. The azo bond is shorter and of higher order than bonds 2 and 3 in all structures, since it is part of the conjugated aromatic system.

Generally, hydrogenation leads to increases in the length of bond 1 and decreases in the bond order, while the lengths of bonds 2 and 3 decrease and orders increase. Hydrogenation weakens bond 1 and strengthens bonds 2 and 3. This finding corroborates the proposed photodegradation mechanism of Freeman and Hsu, whereby the azo bond was cleaved after
photoreduction. Hydrogenation in the ii position causes a greater destabilizing effect to bond 1 than hydrogenation in the i position. The structure with the least stable bond 1 is the cis isomer with hydrogenation in both positions, but there is not a significant difference between it and its corresponding trans form. As displayed in Figure 9, the optimized structure of reduced trans-DR1 is nearly the same as that for reduced cis-DR1, hence their nearly identical bond lengths, bond orders, and torsional angles. Thus, photoreduction of the azo bond of DR1 likely induces a conformational change as a key step of the photodegradation process which, to our knowledge, has not been reported before.

Table 2. As a function of where DR1 is hydrogenated, the bond lengths and bond orders of relevant covalent bonds, and torsional angles between bonds (a) and (b) along the azo bond. The respective hydrogen location and bond labels are indicated in Figure 7.

<table>
<thead>
<tr>
<th>Hydrogen Location(s)</th>
<th>Isomer</th>
<th>Bond Length (Å)</th>
<th>Bond Order</th>
<th>Torsional Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>None</td>
<td>trans</td>
<td>1.274</td>
<td>1.463</td>
<td>1.470</td>
</tr>
<tr>
<td></td>
<td>cis</td>
<td>1.261</td>
<td>1.462</td>
<td>1.470</td>
</tr>
<tr>
<td>i</td>
<td>trans</td>
<td>1.323</td>
<td>1.460</td>
<td>1.467</td>
</tr>
<tr>
<td></td>
<td>cis</td>
<td>1.337</td>
<td>1.460</td>
<td>1.467</td>
</tr>
<tr>
<td>ii</td>
<td>trans</td>
<td>1.342</td>
<td>1.461</td>
<td>1.468</td>
</tr>
<tr>
<td></td>
<td>cis</td>
<td>1.389</td>
<td>1.462</td>
<td>1.469</td>
</tr>
<tr>
<td>i &amp; ii</td>
<td>trans</td>
<td>1.391</td>
<td>1.457</td>
<td>1.463</td>
</tr>
<tr>
<td></td>
<td>cis</td>
<td>1.392</td>
<td>1.457</td>
<td>1.463</td>
</tr>
</tbody>
</table>
**Figure 9.** Molecular conformations of the input structures for the trans (top) and cis (bottom) isomers of DR1 hydrogenated at locations i and ii (left) and their conformations after optimization (right). Despite having very different input structures, the optimized structures adopt similar conformations. Atom numbers correspond to those in Figure 10.

### 3.4.3. Effect of Isomerization and Hydrogenation on Reactivity

Figure 10 provides the condensed-to-atom Fukui reactivity indices in order to investigate the effects of isomerization and azo bond hydrogenation on the photodegradation pathway for DR1. These values were calculated after optimization of the DR1 molecule in both the trans and cis conformations, and with the azo bond hydrogenated at location i, location ii, and both locations i and ii (positions and atom numbers indicated in Figure 9). We will discuss each hydrogenation scenario separately.

#### 3.4.3.1. No Azo Group Hydrogenation

For trans-DR1 with no hydrogenation of the azo group, C5 has the highest Fukui reactivity indices out of all of the atoms for all three forms of attack (nucleophilic, electrophilic, and radical), followed by C6. These two atoms are within the arylamine substituent of DR1 and are directly bonded to the nitrogen of this substituent, N2. In other words, C5 and C6 are the most reactive sites in trans-DR1. For nucleophilic attack of the trans isomer, C11 is the third most susceptible site; C11 is covalently bonded to N13, one of the nitrogen atoms comprising the azo chromophore. Thus, isomerization to the cis form causes C11 to become the most susceptible...
site, followed by C5 and C6. C15 is covalently bonded to the other azo N, N14. In the trans isomer, C15 is not susceptible to nucleophilic attack, but it is susceptible in the cis isomer. For electrophilic attack of the trans isomer, C5 and C6 are the most reactive sites. Isomerization to the cis isomer increases the susceptibility of C15 to electrophilic attack, thus the reactivity, in decreasing order, is: C5, C15, and C6. In this scenario, the C15 atom is at its most reactive out of all of the systems examined. C11 is calculated to not be susceptible to electrophilic attack in either isomeric form. In decreasing order, C5, C6, and C29 are most susceptible to radical attack of trans-DR1. Isomerization to the cis conformation increases the radical reactivity of C15 and decreases that of C5 and C6, resulting in the following decreasing order of radical reactivity: C5, C15, C29, and C6. Isomerization also causes C11 to become reactive to radical attack; C11 is unreactive to radical attack in trans-DR1.

These results indicate that for DR1 without additional hydrogenation, isomerization generally redirects the regions of nucleophilic susceptibility from within the arylamine substituent (C5 and C6) to include the azo region (C11 and C15). The sole exception was that C11 is unreactive to electrophilic attack in both the trans and cis isomers. This observation is likely because of the electron withdrawing N atoms bonded to the aromatic ring drawing electron density away from C11. This claim is supported by the susceptibility of C11 to nucleophilic attack.

3.4.3.2. Azo Group Hydrogenated at Location i

Trans-DR1 with hydrogenation at site i exhibits a trend in the change of the reactivity of its most reactive atoms as a result of isomerization. In the trans form, C11 is the site most reactive to nucleophilic attack, followed by C5, then C6. All of these sites are more reactive to nucleophilic attack than they are in trans-DR1 without hydrogenation. Isomerization to the cis form leads to a reduction in the nucleophilic susceptibility of C5, C15, and C29 to increase and C6 and C11. C15 is the most reactive site, followed by C5 and C6. For C20 that is bonded to the nitro group, it is unreactive to nucleophilic attack in the trans and cis forms of DR1 hydrogenated at site i, but it is reactive to nucleophilic attack in all other structures. Hydrogenation at site i increases the susceptibility of C15 to electrophilic attack as compared to nonhydrogenated trans-DR1. With hydrogenation, C15 becomes the most reactive and C5 the second most reactive site to electrophilic attack for both isomers. As in the nucleophilic case, isomerization to the cis form increases the electrophilic reactivity of C15, C5, and C29, and decreases the reactivity of C6 and
C11. The trans isomer is most reactive to radical attack at C5, C15, and C6, in decreasing order. For the cis isomer, the sites susceptible to radical attack are the same as those susceptible to electrophilic attack.

Isomerization increases the reactivity of C5, C15, and C29 and decreases the reactivity of C6 and C11 to all forms of attack. Hydrogenation of site i generally increases the reactivity of C15 and C11, increasing the reactivity of the region surrounding the azo chromophore. Isomerization further increases the reactivity of C15 but stabilizes C11. This change occurs because C11 is covalently bonded to the side of hydrogenation, N13, and isomerization relieves the steric strain of hydrogenation by allowing the structure to change from its planar trans conformation, as also illustrated by the changes in torsional angles and bond lengths in Table 2.

3.4.3.3. Azo Group Hydrogenated at Location ii

The order of susceptibility to nucleophilic attack differs when location ii is hydrogenated compared to when location i is hydrogenated. In both isomers of DR1 with location ii hydrogenated, C5 is the most reactive site to nucleophilic attack, followed by C11 and C6. Isomerization increases the susceptibility of C5 to nucleophilic attack and decreases the susceptibility of C6, but the order of the most reactive sites is unchanged. N14 is observed to only be reactive for nucleophilic attack in the cis isomer of DR1 with hydrogenation at location ii, which is N14. Otherwise, N14 is not susceptible to any of the other forms of attack in the singly hydrogenated and nonhydrogenated structures. In the trans isomer, C15 is the most susceptible site to electrophilic attack, followed by C5 and C6. Isomerization increases the reactivity of C5 and decreases that of C6 and C15, causing C5 to become the most reactive site, followed by C15 and C6. The electrophilic reactivity of C11 is higher for both the trans and cis isomers of the DR1 molecule hydrogenated at location ii than when it is hydrogenated at location i. C5 is the most reactive site to radical attack followed by C6 for both isomers. Isomerization increases the radical reactivity of C5 and C29 while decreasing that of C6 and C15.

Following the same trend as DR1 hydrogenated at location i, isomerization of DR1 hydrogenated at location ii increases the reactivity of C5 and decreases that of C6 for all forms of attack. The presence of a hydrogen atom bonded to either N13 (i) or N14 (ii) disrupts the electron flow throughout the system, which destabilizes the hydroxyethyl group and increases the C5 reactivity. In the case of hydrogenation at location ii, C5 is generally the most reactive.
site, so the hydroxyethyl group within the arylamine substituent would be the most likely region for degradation in this scenario.

### 3.4.3.4. Azo Group Hydrogenated at Locations i and ii

The most striking impact on reactivity is observed when the azo bond is fully reduced because locations i and ii are both hydrogenated. In the trans isomer, C20 and N13 are the most susceptible sites to nucleophilic attack, followed by C6 and C5. The electron withdrawing nature of the reduced azo bond, along with the nitro group to which C20 is bonded, leads to the previously unobserved behavior for this atom. In all other structures and for all other types of attack, C5 is calculated to be more reactive than C6. This observation suggests a destabilizing of the nitro side and stabilizing of the arylamine side of the DR1 molecule as a result of reducing the azo bond. N13 is unreactive to any form of attack for any structure, except for nucleophilic attack of the trans isomer with locations i and ii hydrogenated. For the cis isomer, C17 is the most reactive location to nucleophilic attack, followed by C5, then C11. The C6 is still reactive to nucleophilic attack, but it is not one of the most reactive sites. The electrophilic attack susceptibility also has some surprising results. In the trans isomer, C5 and C6 are very reactive to electrophilic attack, distantly followed by C29. Isomerization decreases the C5 and C6 electrophilic reactivity and causes C15 to no longer be reactive. In the cis isomer, C6 is the most reactive site to electrophilic attack, followed by C16, then C5. This structure and type of attack is the only one for which C16 is predicted to be reactive. The newly observed reactivity of C16 to electrophilic attack and C17 to nucleophilic attack indicates that the combination of reduction with isomerization destabilized the aromatic ring bonded to the nitro group.

The differences in reactivity for DR1 with a reduced azo bond counteracts each other when averaged to determine the radical reactivity. In both isomers, C5 is most reactive to radical attack and C6 is the second most reactive. Isomerization decreases the radical reactivity of both of these sites. The same sites exhibit radical reactivity in both isomers, a phenomenon not observed for any other pair of isomers or any other reaction type. As previously discussed, the trans structure with locations i and ii hydrogenated occupies a highly similar molecular geometry to that of the cis isomer when optimized. This factor explains the similarity in the reactivity indices for these two structures.
| Atom # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |
|--------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Element| C | N | C | C | C | C | C | C | C | C | C | C | C | N | C | C | C | C | C | C | C | C | C | C | C | O | O | H | H | H | H | H | C | H | H | H | H | H | H | H | H | H | H | H | H | H |

**Figure 10.** Condensed-to-atom Fukui reactivity indices of DR1 with no hydrogenation of the azo bond, single hydrogenation at location i, single hydrogenation at location ii, and hydrogenation of both locations i and ii, in the trans and cis conformations. See Figure 7 for the hydrogenation location labels and Figure 9 for the atom number labels. A red cell indicates a higher reactivity index, and green indicates a lower reactivity index. Cells boxed in gray indicate the location of hydrogenation and corresponding added hydrogen atoms.
3.4.4. Isolation Induces DR1 Fragmentation

Figure 11 gives the full spectrum for a solution of DR1 in methanol, directly injected and analyzed by Q-TOF MS in negative ion mode. A peak with $m/z=313.1303$ Da corresponding to the dye ion (mass error=-0.96 ppm) is clear, indicating the presence of DR1 in the system. As indicated by the relatively high abundance of many other peaks, there were many other compounds present in the system. Figure 12 gives the product ion spectrum from the isolated DR1 ion with no collision energy (CE) applied. The presence of fragment ions indicates that isolation itself imparts enough energy to the system to fragment DR1. Fragments with $m/z=283, 268, 227,$ and $197$ Da were detected.

![Figure 11](image_url)

**Figure 11.** Full spectrum for the DR1:MeOH solution, directly injected and ionized by ESI in negative ion mode. The DR1 anion was observed at $m/z=313.1303$ Da, along with many other ions.
Figure 12. Product ion scan spectrum resulting from the isolation of m/z=313 Da (DR1 anion) from the DR1:MeOH solution, producing fragment ions at m/z=283, 268, 227, and 197 Da.

When the DR1 ion was isolated and CID was performed at a CE of 10 eV, the same fragments were detected as in the isolation only system but at higher abundances (Figure 13). Since the compound with m/z=283 Da was detected in the full scan (Figure 11), isolation and CID of this ion was performed, and the resulting spectrum appears in Figure 14. With a CE of 10 eV, fragments were detected with m/z=227.06 and 197.06 Da. The fragment ion with m/z=268 Da was not detected, indicating that this compound was formed as a result of a separate fragmentation pathway of the DR1 anion.
Figure 13. Product ion scan spectrum resulting from the CID (CE=10 eV) of $m/z=313$ Da (DR1 anion) from the DR1:MeOH solution, producing fragment ions at $m/z=283$, 268, 227, and 197 Da, and corresponding mass differences.

Figure 14. Product ion scan spectrum resulting from the CID (CE=10 eV) of the compound with $m/z=283$, producing fragment ions at $m/z=227$ and 197 Da.
3.4.5. Elucidating Two Fragmentation Pathways

MS<sup>n</sup> results from the LIT corroborated the Q-TOF MS/MS findings and allowed for further steps along the multiple fragmentation pathways to be elucidated. Figure 15 gives the precursor ion, CE, and spectra resulting from each step of the MS<sup>4</sup> analysis for the two pathways: 

- $m/z$ 313 $\rightarrow$ 283 $\rightarrow$ 227 $\rightarrow$ 197 $\rightarrow$ 169 and 92 Da, and
- $m/z$ 313 $\rightarrow$ 268 $\rightarrow$ 238 $\rightarrow$ 210 and 192 Da.

MS<sup>n</sup> allowed for the isolation of each of the two fragmentation pathways of DR1, which were combined with the Q-TOF MS/MS results to elucidate the proposed molecular structures along these pathways in Figure 16. Both of the proposed fragmentation pathways involve breakdown within the 2-(ethylamino)ethan-1-ol substituent and not breakage of the azo bond.
Figure 15. Product ion scan spectra resulting from MS^4 analyses of the DR1 anion, demonstrating two fragmentation pathways: m/z=313 → 283 → 227 → 197 → 169 and 92 Da (left) and m/z=313 → 268 → 238 → 210 and 192 Da (right). Each spectrum was produced by CID of the specified precursor ions (circled) at corresponding CEs (boxed), relative to the maximum of 5 V based upon the Thermo Fisher Normalized Collision Energy™ Technology.
Figure 16. Proposed fragmentation pathways of DR1. Two pathways are possible which are independent of one another. Both result in breakdown of the 2-(ethylamino)ethan-1-ol substituent.

3.4.6. Isolating Photodegradation from Fragmentation

ESI was the softest ionization method available, but it caused DR1 to fragment, making it impossible to isolate products of photodegradation from ionization fragments using MS alone. HPLC-MS was employed to separate the products of these processes and evaluate the effects of photodegradation on PET fabric dyed with DR1. After 48 hours of photodegradation, the fabric exhibited a visible color change from its original red to an orange hue (Figure 17). By comparing the compounds present in these two fabrics and expanding the analysis to include positive ionization, three photodegradation products were identified and are presented in Table 3. In negative ion mode, a photodegradation product was detected with \( m/z = 285 \) Da, which we propose was formed by cleavage of the C-N bond of the ethyl group in the 2-(ethylamino)ethan-1-ol substituent. In positive ion mode, photodegradation products were detected with \( m/z = 271 \) and 255 Da. The proposed structures for these two ions both require cleavage of the C-N bond of the ethanol group, and the mechanism to produce the ion at 255 Da involves further breakdown within the ethyl group. There were no ions detected corresponding to cleavage of the azo bond.
Figure 17. Undegraded PET dyed with DR1 (left) is a vibrant red color and changes to a muted orange tint when irradiated for 48 hours with a xenon arc fade lamp to simulate daylight (right).

Table 3. \( m/z \) ratios of ions which were unique to the photodegraded sample of PET dyed with DR1 and their proposed molecular structures.

<table>
<thead>
<tr>
<th>Detected ( m/z ) (Da)</th>
<th>Proposed Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>285</td>
<td><img src="image1" alt="Proposed Structure 1" /></td>
</tr>
<tr>
<td>271</td>
<td><img src="image2" alt="Proposed Structure 2" /></td>
</tr>
<tr>
<td>255</td>
<td><img src="image3" alt="Proposed Structure 3" /></td>
</tr>
</tbody>
</table>

3.5. Conclusions

This study explored the relationship between isomerization, azo bond hydrogenation, fragmentation, and photodegradation of the azo dye Disperse Red 1. Results aligned with the two main pathways of photodegradation of DR1 presented by Freeman and Hsu. Contrary to previous degradation studies, however, we determined that cleavage of the azo bond was unlikely in most cases. Isomerization and hydrogenation were found to play a key role in the photodegradation of
DR1. Trans-to-cis isomerization of DR1 was calculated to require relatively low activation energy, even lower than that of the azobenzene molecule. It is widely known that azobenzene derivatives have the ability to photoswitch in mild conditions, making conformation a necessary consideration in the process of photodegradation. Through DFT calculations, we determined that reduction of the azo bond of DR1 instigated isomerization, which to our knowledge has not been previously reported. Furthermore, isomerization and hydrogenation influenced the reactivity of the DR1 molecule. Isomerization to the cis form generally redirected the reactive regions from being concentrated within the 2-(ethylamino)ethan-1-ol substituent to include areas surrounding the azo bond. Despite this change, the most reactive region of the DR1 molecule, was within the 2-(ethylamino)ethan-1-ol substituent in most cases. Two fragmentation pathways for DR1 were elucidated by MS, and photodegradation products were identified using HPLC-MS. All of these cases involved breakage of bonds within the 2-(ethylamino)ethan-1-ol substituent and not the azo bond. These findings corroborate our DFT results. Since isomerization and hydrogenation are integral factors in DR1 photodegradation, controlling its molecular structure may allow for control of the location and mechanism of photodegradation. This control could have profound impacts on the efficiency of photoswitching devices and the reduced formation of toxic, carcinogenic, and mutagenic aromatic amines from the photodegradation of azobenzene derivatives. Further work is necessary to determine whether the photodegradation behavior exhibited by DR1 is common throughout azobenzene derivatives.
CHAPTER 4. SUMMARY AND FUTURE WORK

In this thesis, I revealed new information about the connection between isomerization, hydrogenation, and photodegradation of the azobenzene derivative dye Disperse Red 1. The most reactive regions of the DR1 molecule were calculated to be within its 2-(ethylamino)ethan-1-ol substituent. Experimentally, I identified products of fragmentation and photodegradation which were formed by breakdown of this substituent. Neither of these processes cleaved the azo bond, contrary to previously reported fragmentation and photodegradation pathways of azobenzene derivatives. I also determined that isomerization and hydrogenation play a key role in the photodegradation of DR1. The photoreductive pathway of DR1 degradation must include trans-to-cis isomerization. Relating the light-induced processes of photodegradation and photoisomerization within these widely-used compounds illuminates the potential to inhibit the formation of harmful aromatic amines and improve the performance of molecular photoswitches.

There are many directions for future work on this topic. One next step is to perform a more detailed study of the photodegradation products of DR1 to further develop knowledge of these pathways. Substrate and solvent effects could be evaluated to simulate the multiple environments to which dyed textiles are exposed throughout their life cycle. The potential to control or prevent photoisomerization is particularly interesting. By reducing the azo bond, DR1 occupies a rigid conformation between the trans and cis forms and loses its photoswitching ability. Such compounds could be used as reversible photoswitches and then locked into a rigid state by selectively timed reduction to add functionality. Alternatively, encapsulating azobenzene derivatives or bonding them to a substrate could combat photoisomerization and photoreduction.

In the near future, my study will be applied to a wider selection of dyes available in the Max Weaver Dye Library (MWDL). The MWDL contains approximately 98,000 synthetic dyes, many of which possess the azobenzene moiety. Using this collection, it can be determined if the degradation-isomerization relationship is a trend throughout azo dyes. Based upon my work, standardized set of DFT calculations and MS experiments will be performed on each dye candidate. Dyes which are unlikely to cleave at or near their azo bonds can be found. Previously unknown photoswitching candidates can also be identified. I have trained Stephanie Atkinson, coauthor of Chapter 3, to carry on this experiment. By combining my work with the resources of the MWDL, we have the potential to improve textile colorants, decrease their environmental impact, and develop molecular-scale devices with numerous cutting-edge applications.
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