Designing Molecular and Nanoscale Materials for Environmental Chemistry Processes

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DESIGNING MOLECULAR AND NANO SCALE MATERIALS FOR ENVIRONMENTAL CHEMISTRY PROCESSES

by

Wen Guo

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Doctor of Philosophy
Department of Chemistry
Advisor: Sherine Obare, Ph.D.

Western Michigan University
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This dissertation focuses on fundamental studies to identify materials that detect and degrade common organic environmental pollutants. Chapter 1 represents the overview of two widespread ground water contaminants: organohalides and organophosphorus compounds. Due to continuous usage of these compounds as well as their toxicity, reliable and sensitive methods for their detection and degradation are urgently needed. In Chapter 2 a description of molecular sensors designed with high sensitivity and selectivity to detect and distinguish between three organophosphorus (OP) pesticides are described. These sensors provide dual optical and electrochemical signals for detection, which minimizes false-positives. The signal transduction occurs in real time with detection limits in the ppm range. Chapter 3 reports an organic molecule 9,11,20,22-tetraaza-tetrapyridopentacene (TATPP), capable of storing and shuttling multiple electrons, which are desirable for potential applications including remediation of environmental pollutants. We demonstrate the ability to photochemically modulate the reduction of TATPP and we investigate its reactivity. Chapter 4 describes the degradation of the chlorinated ethylenes: cis-1,2-dichloroethylene (cis-DCE), trichloroethylene (TCE) and tetrachloroethylene (PCE). Flavin mononucleotide (FMN) was used as a catalyst to aid in the degradation process. FMNH$_2$ was produced in methanol solvent by the photoreduction of FMN. In aqueous solution, FMN was not fully reduced to FMNH$_2$ but instead yielded the
semiquinone radical FMNH\textsuperscript{•}. However, when FMN was anchored to nanocrystalline TiO\textsubscript{2}, band gap irradiation resulted in electron transfer from the TiO\textsubscript{2} conduction band to FMN, thus yielding FMNH\textsubscript{2}. The FMNH\textsubscript{2} generated in aqueous solution on the TiO\textsubscript{2} surface was a stronger reductant toward chlorinated ethylenes, relative to FMNH\textsubscript{2} in solution. By combining the reactivity of the TiO\textsubscript{2} conduction band electrons (TiO\textsubscript{2}(e\textsuperscript{−}\textsubscript{CB})) with FMNH\textsubscript{2}, the reduction rate constants for the chlorinated ethylenes increased by two orders of magnitude relative to FMNH\textsubscript{2} alone. In Chapter 5, we report that the FMN/TiO\textsubscript{2} hybrid catalyst is effective toward the reduction of three organophosphorus compounds: fenthion, ethion and diethyl chlorophosphate. The reactivity of the catalyst with the organophosphorus compounds occurs at mild conditions in both aqueous solutions and in organic solvents.
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DETECTION AND DEGRADATION OF ORGANIC POLLUTANTS IN THE ENVIRONMENT

The chemical industry has provided society with enormous advances spanning all sectors of modern life. But accompanied by several major advancements, the chemical industry has also been heavily responsible, in part, for damaging the environment through unintentional contamination.\[1\] Pollution can take many forms, such as the water, the atmosphere and the soil pollutants, which all contribute to health problems.\[1\] For instance, the World Health Organization (WHO) reported that about 800,000 people per year die prematurely from the effects of air pollution in 2005.\[2\] This represents about 1.2 percent of total annual global deaths. Preventing pollution and conserving the environment are urgent and important for society and future generations. Governments worldwide, on every level are investing millions of dollars to clean the environment.

This dissertation describes studies to identify materials that either detect or degrade common classes of environmental pollutants, namely organohalide and organophosphorus pollutants. These two classes of compounds are common ground water contaminants. Although more than three quarters of the earth's surface is made up of water, only 2.8 percent of the Earth's water is available for human consumption.\[3\] Water pollution is contamination of water by foreign matter that deteriorates the quality of the water. The foreign matter includes toxic substances released to the environment, pathogens, oxygen-depleting substances, thermal
pollutants and radioactive substances. Water quality has been heavily impacted worldwide by increased consumption and production of pesticides, fertilizers or hazardous industrial byproducts.[3,4] When our water supply is contaminated by such pollutants, it threatens ecological systems. Toxicological studies have linked several synthetic compounds to adverse human health effects, for example the synthetic pesticide DDT is deposited and stored in the fatty tissues, suspected to cause cancer and diabetes.[5,6]

Contaminants can seep into water and consequently migrate into the food chain. Providing clean drinking water to the population becomes a challenge with depletion of water sources and pollution of water bodies. In 2008, EPA agents estimated that over $200 billion would be needed to clean wastewater pollution nationwide for up to a twenty year period.[7]

The goal of the research reported in this dissertation is to identify materials that can detect environmental pollutants commonly found in ground water, design catalysts that degrade such pollutants, and understand the processes and interactions between materials and the environmental pollutants.

1.1 Ground Water Contaminations

Ground water constitutes ninety-five percent of the earth’s fresh water, making it one of the most valuable drinking water resources.[8] Unfortunately, various contaminants including organic pollutants have severely impacted water quality, and presence of such pollutants renders the water unsuitable for consumption. A variety of organic compounds have gained widespread; solvents, volatile organic compounds (VOC), persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), heavy metals and arsenic compounds. Compounds that have posed
significant hazards include vinyl chloride (VC), dichloroethylene (DCE), trichloroethylene (TCE), tetrachloroethylene (PCE), carbon tetrachloride (CCl₄), methylene chloride (CH₂Cl₂) and organic-based pesticides, due to their impacts on human health.[9-12] High production rates and usage patterns of these compounds lead to significant environmental release, resulting in their widespread occurrence in ground water. Several other organic compounds continue to be introduced into the environment in the form of pesticides. These toxic compounds are not rapidly degraded in natural waters.

1.1.1 The Nature of Organophosphorus Compounds

Organophosphorus (OP) compounds are chemical compounds containing carbon-phosphorus bonds, derivatives of phosphoric, phosphonic or phosphinic acids. Various forms of OP compounds are classified as amides, esters and thioesters. In general, the typical structure of OP compounds is shown in Scheme 1.1. Phosphorus is the central atom, and is bonded to oxygen or sulfur atoms.

(S)
O
R₂ ─ P ─ R₁
X

Scheme 1.1 General structures of organophosphorus compounds.

Organophosphorus compounds were first synthesized in the early 1800s when Lassaigne reacted alcohol with phosphoric acid.[13] However, their widespread use began in Germany in the 1930s, when these compounds were first synthesized as
insecticides.[14] OP pesticides are structurally related to compounds used as chemical warfare agents, such as sarin, soman, and VX (as shown in Scheme 1.2), which affect the nervous system by covalently inhibiting acetylcholinesterase.[15,16] In World War II, OP compounds were developed for use as chemical warfare agents due to their highly neurotoxic properties.

Scheme 1.2 Structures of the common chemical warfare agents.

Scheme 1.3 Structures of the common organophosphorus (OP) pesticides.
Scheme 1.4 The mechanism of inhibition of organophosphorus compounds to carboxyl ester hydrolases.[17,18]

When used for agricultural purposes as herbicides or insecticides, OP compounds are required to be stable and easy to handle, effective for insects, and to have low bioaccumulation effect. Common examples of organophosphorus (OP) pesticides are shown in Scheme 1.3.

The primary mechanism of action of organophosphate pesticides is inhibition of carboxyl ester hydrolases, particularly acetylcholinesterase (AChE). AChE is an enzyme that degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid. ACh is found in the central and peripheral nervous system, neuromuscular junctions, and red blood cells (RBCs). Organophosphates irreversibly inactivate AChE by phosphorylating the serine hydroxyl group located at the active site of AChE. The phosphorylation occurs by loss of an organophosphate leaving group and establishment of a covalent bond with AChE (as shown in Scheme 1.4). Once AChE has been inactivated, ACh accumulates throughout the nervous system, resulting in overstimulation of muscarinic and nicotinic receptors.
Table 1.1 Common OP pesticides classified by oxon/thion structure and LD$_{50}$ toxicities.[1,19,20]

<table>
<thead>
<tr>
<th>No</th>
<th>OP Name</th>
<th>Structure (Thions: 1 – 17; Oxons: 18 – 29)</th>
<th>LD$_{50}$, mg/kg *</th>
<th>WHO Acute Hazard$^b$</th>
<th>IARC Carcinogens$^c$</th>
<th>U.S. EPA Carcinogens$^d$</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Oral</td>
<td>Dermal</td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>Parathion</td>
<td><img src="image1" alt="Parathion Structure" /></td>
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<td>21</td>
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<tr>
<td>2</td>
<td>Fonofos</td>
<td><img src="image2" alt="Fonofos Structure" /></td>
<td>8-17</td>
<td>147</td>
<td>Ia</td>
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</tr>
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<td>3</td>
<td>Azinphos-methyl</td>
<td><img src="image3" alt="Azinphos-methyl Structure" /></td>
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<td>220</td>
<td>Ib</td>
<td>N/A</td>
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<tr>
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<td>Coumaphos</td>
<td><img src="image4" alt="Coumaphos Structure" /></td>
<td>16-41</td>
<td>1000</td>
<td>Ib</td>
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<tr>
<td></td>
<td>Compound</td>
<td>Molecular Structure</td>
<td>LC50 (ppm)</td>
<td>LD50 (mg/kg)</td>
<td>Developmental Class</td>
<td>Teratogenic Potential</td>
</tr>
<tr>
<td>---</td>
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<td>------------</td>
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</tr>
<tr>
<td>5</td>
<td>Methidathion</td>
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<td>25-48</td>
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<tr>
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<td>&gt;800</td>
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<td>2300</td>
<td>Ib</td>
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<td>Carbophenothion</td>
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<td>&gt;1500</td>
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<td>Suggestive</td>
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<td></td>
<td>Chlorpyrifos</td>
<td>Fenthion</td>
<td>Fenitrothion</td>
<td>Dichlofenthion</td>
<td>Dicapthon</td>
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<td>--------------</td>
<td>----------------</td>
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</tr>
<tr>
<td>10</td>
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<td>E, unlikely</td>
<td>E, unlikely</td>
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<td>N/A</td>
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<td>N/A</td>
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<td>II</td>
<td>II</td>
<td>II</td>
<td>II</td>
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<td>12</td>
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<td>2000</td>
<td>330</td>
<td>&gt;3000</td>
<td>790-1250</td>
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<td>13</td>
<td>214-245</td>
<td>250</td>
<td>270</td>
<td>6000</td>
<td>330-400</td>
<td></td>
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<tr>
<td>14</td>
<td>250</td>
<td>270</td>
<td>6000</td>
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<td>N/A</td>
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<tr>
<td></td>
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<td>2150</td>
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<tr>
<td>15</td>
<td>Diazinon</td>
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<td>2150</td>
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<td>2.4</td>
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<td>19</td>
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<td></td>
<td>3.7-6.1</td>
<td>4.2-2.7</td>
<td>Ia</td>
<td>N/A</td>
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<td>#</td>
<td>Insecticide</td>
<td>Molecular Structure</td>
<td>LD50</td>
<td>LD95</td>
<td>Class</td>
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<tr>
<td>20</td>
<td>Schradan</td>
<td><img src="image1.png" alt="Schradan Molecule" /></td>
<td>10</td>
<td>15</td>
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<td>N/A</td>
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<tr>
<td>21</td>
<td>Monocrotophos</td>
<td><img src="image2.png" alt="Monocrotophos Molecule" /></td>
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<td>112-126</td>
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<td>22</td>
<td>Phosphamidon</td>
<td><img src="image3.png" alt="Phosphamidon Molecule" /></td>
<td>24</td>
<td>107-143</td>
<td>Ia</td>
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<tr>
<td>23</td>
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<td><img src="image4.png" alt="Oxydemeton methyl Molecule" /></td>
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<td>158-173</td>
<td>Ib</td>
<td>N/A</td>
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<tr>
<td>24</td>
<td>Ethoprophos</td>
<td><img src="image5.png" alt="Ethoprophos Molecule" /></td>
<td>61</td>
<td>26</td>
<td>Ia</td>
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Table 1.1 — Continued

<p>| | | | | |</p>
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<tbody>
<tr>
<td>25</td>
<td>Dichlorvos</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>56-80</td>
<td>75-107</td>
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<td>26</td>
<td>Crotoxyphos</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>74-110</td>
<td>202-375</td>
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<td>27</td>
<td>Naled</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>250</td>
<td>800</td>
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<td>28</td>
<td>Tribufos</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>560-630</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>29</td>
<td>Trichlorfon</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>400-800</td>
<td>&gt;2000</td>
</tr>
</tbody>
</table>
Toxic interactions of organophosphorus compounds with any given biological system are dose-related. Their toxicity is expressed in terms of the lethal dose (LD) which will kill 50% of the animal species (LD₅₀). LD₅₀ values are generally expressed as amount per unit weight (e.g. mg·kg⁻¹).

WHO = World Health Organization, acute hazard classify: Ia = extremely hazardous to human health; Ib = highly hazardous; II = moderately hazardous; III = slightly hazardous.

IARC = International Agency for Research on Cancer.

EPA = Environmental Protection Agency.

Besides their inhibitory effects on enzymes, there is increasing evidence that OP compounds may induce oxidative stress through generation of free oxygen radicals, leading to lipid peroxidation and DNA damage.[21,22] However, there is still no clear indication that oxidative stress has any pathogenic role in OP compounds poisoning. There are great numbers of OP pesticides, the common ones are shown in Table 1.1 with their toxicity information.

Even though organophosphorus compounds are generally considered safer than the organochlorides, OP compounds are highly toxic to human health. While the toxicity varies depending on the type of OP compounds, generally, excess exposure leads to symptoms that include negative effects on the visual system, sensory function, cognitive function, and nervous system.[23-25] The major effect of OP compounds is on nervous system, since they disrupt the cholinesterase enzyme that regulates acetylcholine,[26,27] a neurotransmitter needed for proper nervous system function. Exposure to OP compounds has been shown to cause headache, dizziness, profuse sweating, blurred vision, nausea, vomiting, reduced heart beat, diarrhea, loss of coordination, slow and weak breathing, fever, coma, and death.[28] The symptoms range widely in severity based on the degree of acetylcholinesterase inhibition. Excess exposure of OP compounds ultimately leads to death.
According to 2004 Annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System, 102,754 persons were exposed to pesticides, and some OP pesticide exposure cases cause death.[29] Infants and children may be especially sensitive to health risks posed by pesticides: an estimated 74,000 children were involved in common household pesticide-related poisonings or exposures in the United States in 1994.[30] Due to the adverse effects of OP pesticides, serious efforts are required in monitoring and degrading them, for the safety of public and the environment.

1.1.2 The Nature of Organohalide Compounds

Organohalide compounds are halogen-substituted hydrocarbons, each of which contains at least one atom of F, Cl, Br or I. They are produced in large quantities as solvents, heat transfer fluids, chemical intermediates, and for other applications. Most organohalide compounds are chlorides (chlorocarbons and chlorohydrocarbons), but they also include compounds of fluorine, bromine, and iodine, as well as mixed halides, such as the chloro-fluorocarbons. Organohalide compounds can be saturated (alkyl halides), unsaturated (alkenyl halides), or aromatic (aryl halides).[31]

Organohalides are used in various fields of industry,[32] as well as in agriculture as pesticides.[33] The wide usage of organohalides causes their discharge to the environment. The most problematic compounds for ground water are the chlorinated solvents, which are mainly introduced to water resources by industry. Such chlorinated compounds tend to migrate downward though soil and sediments, and contaminate ground water.

Chlorinated chemicals, specially chlorinated ethylenes are toxic substances
used for dry cleaning and metal degreasing operations. They have become the main contaminants in ground water, and are persistent in the subsurface environment.[34,35] Chlorinated ethylenes have become drinking water pollutants that are toxic to the kidneys, liver, and nervous system, and also linked to cancer and birth defects in animals and humans.[36-40] For example, tetrachloroethylene (PCE) and trichloroethylene (TCE) are considered carcinogenic.[36,40,41] Table 1.2 shows several common chlorinated compounds and the information of their toxicity as provided by several organizations. Based on the toxicity of the compounds, it is clear that there is an urgent need for degradation of chlorinated compounds in the environment.

Table 1.2 Chlorinated solvents and related compounds: Toxicity assessments.[37]

| No | Compounds             | Composition | WHO* | EPA IRIS | US. NIOSH REL*
|----|-----------------------|-------------|-------|----------|------------------|
| 1  | Carbon tetrachloride  | CCl₄        | Possible CNS, Liver, Kidney toxin, carcinogen (ICSC 24)
| 2  | 1,1-dichloroethane    | C₂H₄Cl₂     | Possible CNS, liver, kidney toxin (ICSC 249) | B2 | 2 ppm; lowest feasible
| 3  | Vinyl chloride        | C₂H₃Cl      | Possible circulatory system, liver, spleen toxin, known carcinogen (ICSC 82) | C | 100 ppm; caution
| 4  | 1,1-dichloroethylene  | C₂H₂Cl₂     | Possible liver, kidney toxin (ICSC 83, CICAD 51) | C | Lowest feasible

*WHO: World Health Organization
**EPA IRIS: Environmental Protection Agency Integrated Risk Information System
***US. NIOSH REL: United States National Institute for Occupational Safety and Health Recommended Exposure Limits
Table 1.2 — Continued

<p>| | | | |</p>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>1,2-dichloroethylene</td>
<td>C₂H₂Cl₂</td>
<td>Possible liver toxin (ICSC 4236)</td>
</tr>
<tr>
<td>6</td>
<td>1,1,2-trichloroethylene</td>
<td>C₂HCl₃</td>
<td>Possible CNS, liver, kidney toxin, probable carcinogen (ICSC 81)</td>
</tr>
<tr>
<td>7</td>
<td>Tetrachloroethylene</td>
<td>C₂Cl₄</td>
<td>Possible CNS, liver, kidney toxic, probable carcinogen (ICSC 76)</td>
</tr>
</tbody>
</table>

<sup>a</sup> WHO = World Health Organization;  
<sup>b</sup> ICSC = International Chemical Safety Card;  
<sup>c</sup> CICAD = Concise International Chemical Assessment Document;  
<sup>d</sup> EPA = Environmental Protection Agency; IRIS = Integrated Risk Information System; weight of evidence characterization: A = human carcinogen; B₂ = probable human carcinogen based on sufficient evidence of carcinogenicity in animals; C = possible human carcinogen; D = not classifiable as to human carcinogenicity.  
<sup>e</sup> NIOSH = National Institute for Occupational Safety and Health; REL = Recommended Exposure Limits. [42]  
<sup>f</sup> Potential occupational carcinogen, NIOSH Pocket Guide Appendix A.[43]  
<sup>g</sup> All chloroethanes are given a “caution” rating because of their structural similarity to the four chloroethanes shown to be carcinogenic in animals. NIOSH Pocket Guide Appendix C.[44]

### 1.2 Detection of Environmental Contaminants

Since many pollutants are continuously used and dissipated in our environment, it is important to design reliable and sensitive sensors for the detection of widespread contaminants. Chemical sensors are defined by R. W. Catterall[45]:
“as reagents that interact with an analyte with high affinity and yield a measurable signal in response”. Expanded uses of chemical sensors occur with emerging technologies, such as optical sensing and nanotechnology.[46,47] An ideal chemical sensor offers in situ measurements, high specificity for analyte, and an affinity commensurate with the average concentration of the analyte solution.[48]

Optical sensing is based on the optical technique: absorption spectroscopy, fluorescence spectroscopy, surface plasmon spectroscopy and light scattering. The detection offers the visual output. In the dissertation, the sensor design is based on fluorescence technique for analysis, combining with alternative detection approaches. The dual signal transductions take advantage of avoiding the false positives.

1.2.1 Sensors for Organophosphorus Compounds

Currently applied methods for the determination of organophosphates in water are mainly based on gas, liquid or thin layer chromatographic techniques,[49-51] which generally have high sensitivity. However, these sampling based techniques are usually incapable of tracking these changes and therefore inappropriate.

Reports in the literature showed that many biosensors for detection of organophosphates are based on their interaction with enzymes.[9,47,52-58] OP pesticides bind to a number of enzymes, undergo a hydrolysis process, and produce a stable, phosphorylated and unreactive enzyme. The inhibited enzymes include acetylcholinesterase (AChE), butyryl cholinesterase etc (BChE).[52-54]

AChE based biosensors have been an active research area over the last decade.[47,59,60] Organophosphorus pesticides bind to acetylcholinesterase (AChE) enzyme, and block its active site. This irreversible process is the decisive factor for sensing response, in which the enzyme activity in the presence of OP pesticides is
determined. [55, 56, 61] AChE based biosensors present fast responses accompanied by inherent ability to bind for specific target molecules, and provide qualitative and quantitative information about the composition of a sample. Although these biosensors offer detection toward OP compounds, there still some limitations of such approach: the time for inhibition, low stability of enzymes.

Recently, nanomaterial-based AChE sensors were developed for OP pesticides, and this combination significantly promotes sensitivity of determination and the storage stability. [62] In some cases, the detection limits of OP pesticide can reach nM to pM range. [9, 47, 63, 64] In such sensors, enzymes are immobilized onto various nanomaterials, such as carbon nanotubes, [47, 65, 66] gold nanoparticles, [54, 64, 67] cadmium sulphide nano particles or quantum dots [60, 68]. The immobilisation conditions strongly affect the selectivity and sensitivity.

However, major drawbacks of most AChE biosensors are that (1) enzymes are usually difficult to purify, (2) the irreversible nature of cholinesterase enzyme inhibition prevents their use more than once, and (3) these biosensors are used for screening purposes and are unspecific for individual pesticides.

Sensors based on immunoassays have also been successfully used for the detection of OP pesticide. [69-71]. Immunosensors use antibodies (Ab) or antigens (Ag) as the specific sensing element, and provide concentration-dependent signals. The advantages of immunosensor are specificity and sensitivity due to the antibody-hapten reaction, and specific information about a particular pesticide. [72, 73] For instance, Hu and co-workers reported an electrochemical immunosensor based on gold nanoparticles loaded with paraoxon antibodies for detection of paraoxon. [74] Immunosensors used for pesticides have two classic types: labeled and label-free. When a transduction is achieved using labeled species, the information of target
analyte can be inferred from the amount of labels, this procedure is labeled format.[75-77] While the detection is based on the binding of pesticide and the antibodies without any labels, this procedure is label-free.[78-80] Immunosensor-based detection methods rely on electrochemical, optical, and piezoelectric transducers, which have the potential to achieve the low limits of detection required for effective monitoring. Despite their promise, immunosensors have several limitations: (1) extensive sample handling, such as complex washing steps etc; (2) a lack of validated protocols for a wide range of sample matrices for research; and (3) in some case, biomolecule deactivation or leaking and high diffusion resistance of the substrate or biocomponent.

\[
\begin{align*}
\text{R}_1 \text{O(S)} \text{|| OPH} & \quad \text{R}_2 \text{P—OH + HX} \\
\text{R}_1 \text{P—X} + \text{H}_2\text{O} & \quad \text{R}_1 \text{P—OH} + \text{HX}
\end{align*}
\]

Scheme 1.5 The hydrolysis mechanism of organophosphorus compounds, catalyzed by OPH. [57]

Organophosphorus hydrolase (OPH) has attracted much attention for detecting pesticides since it catalyzes the hydrolysis of a wide range of OP esters, such as parathion, acephate and the chemical warfare agent, Soman. [57,81-83] During the detection process, OPH acts as the catalyst for the hydrolysis of OP compounds, unlike the covalent binding process of AChE sensors. Upon the hydrolysis process of organophosphorus compounds, easily detectable nitrophenol (Example shown in Scheme 1.6), or fluoride or hydrogen ions are released, and the principle of detection is based on monitoring these products. Several types of OPH-
based biosensors have been introduced, including optical,[84-86] potentiometric,[87]
or amperometric.[88,89] For instance, detection of \( p \)-nitrophenyl-substituted
organophosphates using the fluorescence changes of coumarin 1 was reported by
Paliwal et al (as shown in Scheme 1.6).[90] Coumarin 1 (scheme 1.7) is a competitive
inhibitor of OPH. The fluorescence quenching with the addition of OPs is due to
fluorescence resonance energy transfer. The approach allows the selective and
effective detections of \( p \)-nitrophenyl-substituted organophosphates.

\[
\begin{align*}
& \text{R—P—O—NO}_2 + \text{H}_2\text{O} \\
& \text{OPH} \\
& \text{R—P—OH} + \text{OH—} + \text{OH—NO}_2 \\
& \text{nitrophenol}
\end{align*}
\]

**Scheme 1.6** Detection of \( p \)-nitrophenyl-substituted organophosphates by OPH
sensors. [90]

\[
\begin{align*}
& \text{CH}_3 \\
& \text{H}_2\text{C—N—} \\
& \text{H}_2\text{C}
\end{align*}
\]

**Scheme 1.7** Structure of the fluorophore Coumarin 1, that undergoes fluorescence
quenching with increasing \( p \)-nitrophenol concentration. [90]
OPH-based sensors provide selectivity for target organophosphorus compounds, reversibility and fast response time. However, OPH biosensors still suffer from drawbacks associated with relatively high operating potential (range from +0.85 to +0.9 V vs. Ag/AgCl),[91] which makes it difficult to avoid interference from other electro-active species in samples. Moreover, the detection limit of OPH biosensors is higher than that of inhibition methods.[57]

![Figure 1.1 Inclusion phenomena of guest molecule in the cavity of CDs sensor and cause the change in fluorescence.][92]

Delattre and co-workers reported a fluorescent sensor cyclodextrins (CDs) for the detection of pesticides in water.[92] \(D\)-glucopyranose units in CDs form truncated cone-shaped molecules with a hydrophobic cavity, which can induce the inclusion phenomena of a guest, as shown in Figure 1.1. The dipole of the macromolecular system varies with the entry of guest molecules and as the result, the fluorescence is affected. With the guest-induced studies of parathion or malathion, the fluorescence was quenched.
Organophosphorus (OP) pesticides methylparathion and monocrotophos were detected via fluorescence and oxidation of the indole group of the quartz/APES/AuNP/L- cysteine/ID film.[93] With the exposure to the pesticide, the indole group of the sensor on the modified film is oxidized to a fluorescent indoxyl group. The sensor is capable of detecting methylparathion and monocrotophos in the ppm and ppb range, respectively. However, the indol modified SAM is subject to interference at 20 equivalents of Fe$^{3+}$ ions. An advantage of the fluorescing quartz/APES/AuNP/L-Cys/ID film is that it can detect the OP pesticides in ionic and other environmental species. Additionally, the SAMs sensor exhibits electrochemistry and fluorescence which has the potential of dual-sensing capabilities. Furthermore, the limits of detection were in the ppm and ppb range. Limitation of this sensing material is the extremely lengthy preparation method. The sensing process is also affected by factors such as light, temperature, and pH.

Unfortunately, to date there have been no reports to demonstrate molecular sensors that differentiate selectively between OP pesticides. The broad range in toxicity levels of OP pesticides necessitates new methods to discriminate between various OP pesticides. In this regard, there is an urgent need for organophosphorus pesticide sensors that are sensitive, selective, and provide results in real time. Chapter II of this dissertation reports two molecular sensors provide dual fluorescent and electrochemical signal transductions which are effective in detection of organophosphorus (OP) pesticides. The signal transduction occurs in real time with detection limits in the ppm range. The developed sensors not only detect the OP pesticides, but also distinguish them. Sensors that provide dual modes of signal transduction enable the minimization of false-positives; an essential requirement for environmental sensing applications. This is the first report of sensors with dual signal...
transductions for OP pesticides and the capability of distinguishing between pesticides.

1.3 Remediation for Ground Water Contamination

The Clean Water Act passed in 1972 is the basis for water quality management for all states.[94] Federal and state governments in the United States have instituted environmental regulations to protect the quality of water recourse from various contaminants.[95,96] Scientists have conducted research focused on characterization, monitoring, and modeling of subsurface environments, with a particular emphasis on contaminant remediation and water resources.

1.3.1 Degradation of Organophosphorus Pesticides

Organophosphorus pesticides (OPs) are one of the most important groups of pesticides applied in agricultural areas.[97] However their presence as contaminants in environments may cause serious problems to human beings and other organisms. Because of their widespread use and high toxicity, the employment of an appropriate technique for remediation is required. In this regard a number of methods for OP compound degradation have been reported in the literature.

In nature, organophosphorus pesticides may undergo different physical, chemical and biological processes in the aquatic environment. Among these processes, sun light may trigger the degradations of OP pesticides, which depends on the molar absorption coefficient and on the efficiency of the photochemical transformation.[98-100] During the direct photolysis, OP pesticides exhibit weak absorption in the 280–320nm UV wavelength range. The solar spectral intensity in this region is sufficient to break down chemical bonds of the pesticide
molecules,[101] and this process is largely enhanced by the presence of other species like oxygen or humic substances acting as natural sensitizers (indirect photolysis).[99,102] The natural occurring photolysis degradation processes were exploited in research for the treatment of OP pesticides.

Photocatalysis has been extensively used in the remediation of organophosphorus pesticides.[103-107] TiO$_2$ was widely recognized as the most appropriate one for mediating the photocatalytic processes.[103] When the energy was greater than the band gap of TiO$_2$, electron-hole pairs were generated on the surface of TiO$_2$. The holes are either trapped by surface hydroxyl groups to yield hydroxyl radical or recombined with electrons to inhibit the photocatalytic reaction process.[108]

\[ TiO_2 + h\nu \rightarrow TiO_2(e_{CB}^- + h_{VB}^+) \]
\[ h_{VB}^- + H_2O \rightarrow OH^- + H^+ \]
\[ e_{CB}^- + h_{VB}^+ \rightarrow TiO_2 \]

In an oxygenated solution, oxygen may adsorb on the surface of TiO$_2$ to prevent the recombination process by trapping electrons resulting in the formation of superoxide radical and hydrogen peroxide. [103]

\[ O_2 + e_{CB}^- \rightarrow O_2^- \]
\[ O_2^- + H^+ \rightarrow HO_2^- \]
\[ HO_2^- + HO_2^- \rightarrow H_2O_2 + O_2 \]
\[ O_2^- + HO_2^- \rightarrow O_2 + HO_2^- \]
\[ HO_2^- + H^+ \rightarrow H_2O_2 \]

Recent research showed that the hydrogen peroxide is an intermediate during the photo-oxidation of organophosphorus compounds on TiO$_2$. The combination of TiO$_2$ with hydrogen peroxide presented the highly effective in the remediation of OP compounds in aqueous solution via UV radiation.[103] With the addition of oxidation
agents, such as H$_2$O$_2$ and Na$_2$S$_2$O$_8$ over TiO$_2$ catalyst, the reaction results showed higher degradation rate.\[103,109\] Photocatalytic oxidation works over wide range of pH, while the limitation is that complete degradation requires excess of oxidation agents.

In recent years, the Fenton process has been extensively used with success for the oxidation of many classes of organic compounds, including OP pesticides.\[110-112\] The Fenton processes generate hydroxyl radicals during decomposition of H$_2$O$_2$ by Fe$^{2+}$ in acidic medium.\[102\] The hydroxyl radical, a strong oxidizing agents (E$_{\text{Ox/red}}$ = 2.80 V vs. NHE, \[113\]) has been identified as an attractive option for the degradation of OP pesticides.\[113,114\] Although the Fenton process offers high efficiency, it requires low pH of solution, which means that reaction products require neutralization before discharge.\[115\]

Recently, scientists developed the electro-Fenton or photo-assisted Fenton process to produce hydroxyls radicals which react to OP pesticides, leading to their mineralization.\[116,117\] Evgenidou et. al \[117\] studied the advanced oxidation process for the degradation of dimethoate and methyl parathion via Fenton process. Diagne and co-worker \[116\] showed that the electro-Fenton process could be use for \textit{in situ} mineralization of methyl parathion in water. However, in these processes, the by-products are more toxic than the parent compound.

In addition to the Fenton's reagent, another oxidation process for the degradation of OP pesticides was developed by Collins's group.\[118\] Fe-TAML peroxide activators (TAML: tetraamidomacrocyclic ligands) were used as catalysts for the degradation of OP pesticides. It can function effectively in low concentration at room temperature over a wide pH range.

Fujisawa and co-workers \[119\] investigated iron porphyrins for the
degradation of OP pesticides. The process involved porphyrin-mediated oxidative reactions of pesticides, and the major products were the corresponding phenols. In addition, the authors found a phosphoxathiirane intermediate formed in the oxidation process might produce oxon formed of pesticides by oxidation at the P=S moiety similarly as reported for their mammalian metabolism by cytochrome P450 enzymes.

A great number of different types of surfactants have been used to accelerate the hydrolytic process of OP pesticides for degradations.[120-122] In general, surfactants form spherical micelles in aqueous solution above the critical micelle concentration (cmc). The rate of micellar-assisted hydrolysis reaction involved aqueous phase and micellar phase. Moreover, Han et al. [123] found that varying chain length of surfactants and the ionic strength presented the effect to the degradation of OP pesticides. Currently, inadequate chemical[124] and enzyme-mediated hydrolysis[125] are the most commonly employed detoxification methods; however, these often produce hydrolysates that have mild to acute toxicity.[126]

Metal complex catalyzed hydrolyses[124,127] can contribute their own additional toxicity to the final wastes.[128] The hydrolysis of OP compounds can be catalyzed by transition-metal complexes.[129-131] However, in many cases the hydrolytic reactions of phosphorus compounds promoted by transition-metal ions present problems. They are not truly catalytic, because the products of hydrolysis bind to the metal ion, thereby inhibiting further turnover. Moreover, at high pH values where the metal-containing complexes have activity, the insolvency of $M^+(\text{OH})_n$ is a problem which often necessitates the use of complexing ligands to ensure homogeneity.

Microorganisms can use a variety of xenobiotic compounds for their growth and in turn they can mineralize and detoxify them making microbial degradation an
effective method for remediation.[132] Several reports indicate that microorganisms are capable of catalyzing the degradation of organophosphorus pesticides.[133-135] Foster et al. [136] reported aerobic degradation of ethion by mesophilic bacteria, and identified Pseudomonas and Azospirillum species were active toward the degradation process. In their experiments, ethion was found to be a carbon source supporting microbial growth. Yang et al. [137] isolated Alcaligenes faecalis DSP3, and the strain DSP3 resulted in a rapid degradation for chlorpyrifos in soil. Three bacterial strains were screened and identified as Serratia marcescens and Pseudomonas sp by Cycoń and co-workers.[132] These strains were able to grow with OP insecticide diazinon as the carbon sources, and resulted in the biodegradation of toxic diazinon.

Microbial degradation is one of the widespread methods to clean up contaminated environments,[138,139] but in many cases biodegradation proceeds at a low rate.[140-142] Many conditions such as moisture, temperature, aeration, pH, and the amount of organic matter affect the rate of microbial degradation because of their direct influence on microbial growth and activity.

Immobilized enzymes,[143] represent an alternative approach for OP pesticide decontamination. Several literature reports show biotechnological methods based on the organophosphorous hydrolase (OPH) enzyme from Pseudomonas diminuta, can hydrolyze a range of OPs containing P–O, P–S, P–CN, and P–F bonds.[144-146]

An ideal decontamination process would be inexpensive, technically straightforward and flexible, would work rapidly under ambient conditions at environmental pHs, and would not leave toxic residues. Based on the studies of the degradation of OP compounds, common degradation pathways are followed by all the structures, apart from the nature of the substituents. Common pathways are
represented by the detachment of the OP moiety and by the oxidation of P=S to P=O. The formation of oxon derivative is of concern, since the oxon analogous of OP pesticides were found to be toxic compounds.[117,118,147,148] Very little research has been reported to develop materials that react with OP pesticides via a reduction pathway. The development of such pathway will avoid the possible toxic intermediates during the degradation processes.

The design of efficient and effective techniques to mitigate the environmental contaminations requires the knowledge of the behaviors of chemical contaminants in groundwater. The understanding of chemical, physical and biological reactions between pollutants and the environment will provide the reliable predictions of contaminants, and further offer controlling methods.

1.3.2 Degradation of Organohalide Compounds

Remediation of organohalide pollutants is important for maintenance of underground aquifers and groundwater resources. The traditional pump-and-treat strategy is both technically challenging and costly.[149] Currently, researchers studied more effective approaches. Both the oxidation and reduction methods of organohalide pollutants have been well documented.[33,150-153]

In wastewater treatment, ozone has been shown to be effective at removing of organic pollutants.[154-157] With the degradation processes of organohalides, the usage of ozone involves oxidative mechanism. Destruction of TCE by ozone has been reported by Glaze and Kang[158]; however, the low concentration of ozone and reactivity with other solutes of hydroxyl radicals is still a problem.

Fenton’s reagent is a Fe(II)/H₂O₂ mixture that has strong oxidizing properties against many organic compounds, such as phenol, nitrophenols, and
trichloroethylene.\textsuperscript{[159,160]} The active species in this system is commonly thought to be the hydroxyl radical. Recently Joo et al. \textsuperscript{[161]} investigated the Fenton-based reagent to undergo the oxidative mechanism about TCE degradation. Yang and his research group \textsuperscript{[162]} have successfully combined the electrokinetic (EK) process and the Fenton process for \textit{in situ} treatment of trichloroethylene (TCE) in soil. Lindsey and co-worker used cyclodextrins (CDs) with Fenton’s reagent for the degradation of organohalides. The combination raises the degradation rate.\textsuperscript{[163]}

The rate of organohalide degradation could be increased by irradiation of Fenton’s reagent with UV-light. In the photo-Fenton process, UV-light leads not only to the formation of additional hydroxyl radicals but also to recycling of ferrous catalyst by reduction of Fe(II). As the result, the concentration of Fe(II) increases and the overall reaction is accelerated.\textsuperscript{[164]} For instance, Esplugas’s group \textsuperscript{[165]} did a comparative study of the advanced oxidation of 2,4-dichlorophenol (DCP), and found that the photo-Fenton reaction showed the best efficiency in DCP elimination.

Reductive dehalogenation is a primary pathway for the degradation of organohalides. A common approach for organohalide remediation has been to use electron transfer reactions from semiconductors, metals, or transition metal compounds.\textsuperscript{[166-168]} The current technology used for treating ground water contaminated with organohalides, specially chlorinated ethylenes, is zero-valent iron (ZVI).\textsuperscript{[10,169-178]} In this system, the metal serves as an electron donor for the reduction of oxidized species. For example, the dehalogenation process of alkyl chlorides (R-Cl) occurs in the presence of Fe\textsuperscript{0}.\textsuperscript{[179]}

\[
\text{Fe}^0 + \text{R-Cl} + \text{H}^+ \rightarrow \text{Fe}^{2+} + \text{R-H} + \text{Cl}^-
\]

When zero valent particles are used for the degradation chlorinated ethylenes, the generated byproducts can be more toxic than parent compounds, e.g. vinyl
Arnold and Roberts [180] investigated the reactions of Fe$^0$ particles with chlorinated ethylene, and proposed the dehalogenation pathways (as shown in Figure 1.2). In the process, zero valent iron is oxidized to Fe(II), while chlorinated ethylene is dechlorinated.

Figure 1.2 A schematic of hypothesized reaction pathways for the chlorinated ethylenes and other intermediates during reduction by Fe$^0$. [180]
ZVI can active oxygen in the environment, which is generally assumed to lower the efficiency of the reduction process, and compete with the organohalides. This process involves a four-electron transfer step as shown in Figure 1.3.

\[
\begin{align*}
4 \text{ e}^- & \quad \text{Fe}^0 \quad \text{Fe}^{2+} \quad 2 \text{ H}^+ \quad 2 \text{ H}^+ + \text{Fe}^0 \quad \text{Fe}^{2+} \\
\text{O}_2 & \quad \text{O}_2^{2-} \quad \text{H}_2\text{O}_2 \quad \text{2 H}_2\text{O}
\end{align*}
\]

**Figure 1.3** Oxygen activation by zero valent iron (ZVI).

In order to enhance dechlorination reaction rates and minimize byproduct formation, bi-metal systems of zero valent iron were developed.[181] Bimetallic particles are those particles on which a thin layer of catalytic metal (e.g. Pd, Pt, which are not active in themselves) is doped onto the surface of the active (reducing) metal.[182] In most cases, rates of transformation by bimetallic combinations have been significantly faster than those observed for iron metal alone. When catalyzing the dechlorination reactions, palladium has been found to be the most active catalyst,[181] but other metals, such as platinum and nickel, have also shown effective results.[181,183] When bimetallic particles were used for the degradation of chlorinated ethylene, the products of the reaction yielded no toxic byproducts, e.g. VC and dichloroethene (DCE).[181] Another advantage of using bimetallic particles is that they stabilize the metal. ZVI particles undergo surface oxidation within a few hours, while palladium modified iron particles do not show an observable color change in air, indicating the stability of these particles.[181]
Nanoscale titanium dioxide is the commonly used environmental photocatalyst for the degradation of organic pollutants.[184,185] The photocatalyst occurs in the presence of a semiconductor catalyst (i.e., TiO₂) and a UV or near-UV light source. Undergoing the photolysis process, TiO₂ generates the conduction band electron (e\textsubscript{CB}⁻) and the valence band hole (h\textsubscript{VB}⁺). Conduction band electrons react with a variety of organohalides through reductive pathways.[186,187] The possible dehalogenation process is shown as follows:[186,187]

\[
\text{TiO}_2 + h\nu \rightarrow e\textsubscript{CB}^- + h\textsubscript{VB}^+ \\
RX + \text{TiO}_2(e\textsubscript{CB}^-) \rightarrow R^* + X^- \\
RX + 2 \text{TiO}_2(e\textsubscript{CB}^-) \rightarrow R^* + X^-
\]

Hung and co-workers[188] investigated the photocatalytic degradation of TCE vapor on TiO₂ films. Chlorinated organic intermediate/product formation was the result of various reactions involving chlorine and hydrogen atom extractions and additions.

Microorganisms are the major mediators for cycling the halogenated organic compounds in the environment. Consequently, efforts on the biodegradation methods for organohalides were made.[189-191] Due to the oxidation state of organohalides, the biodegradation processes are most likely to occur in a reducing environment. Biological degradation of organohalide under anaerobic conditions has been studied for a number of years.[192,193] The key factor affecting the reactivity of organohalides is the nature of the carbon-halogen bond, but other structural properties of the molecule also contribute to their reactivity. The advantage of anaerobic processes of degradation is a low concentration of OP pesticide in the environment does not limit the activity of microorganisms. However, the limitation of the biodegradation is its relatively slow rate of decontamination.[139] Furthermore, some
biodegradation processes produce the byproducts which are more toxic, such as vinyl chloride.

1.3.3 Electron Transfer Mechanism

Electron transfer (ET) is an essential process that occurs in nature, and plays an important role in chemistry and biology. There are a wealth of fundamental studies of energy and electron transfer and photoinduced charge separation that model natural photosynthesis for potential applications in molecular devices, photocatalysis, and energy conversion.

The minimal set for any electron transfer process includes two redox active molecular units, an electron donor (D) and an electron acceptor (A). Electron transfer events can be divided into thermally-induced ET, and photo-induced ET or potential-driven ET. Furthermore, ET reactions, between separate molecules (intermolecular) or between distinct regions within the molecule (intramolecular), are some of the most frequently encountered photochemical primary processes. In general, ET has two classic types: outer-sphere and inner-sphere mechanisms.

The first generally accepted theory of ET was developed by Rudolph A. Marcus to address outer-sphere electron transfer and was based on a transition-state theory approach.[194,195] In outer-sphere ET reactions, the participating redox centers are not linked via any bridge during the electron transfer process, and there is no intermediate formation of a chemical bond (Eq.1.1). The Frank-Condon principle is fundamental to the theory. This principle states that electron movement is much faster than nuclear motion; the position of the atoms and the interatomic distances remain unchanged during the process of ET. Therefore, it is assumed that on approaching the transition state, the bond lengths of the reactant will adjust to
approach those of the products. In general, if electron transfer is faster than ligand substitution, the reaction will follow the outer-sphere electron transfer.

\[
D + A \rightarrow D^+ + A^- \quad \text{(Eq. 1.1)}
\]

\textit{Here, } D = \text{donor}; A = \text{acceptor.}

Inner-sphere ET mechanism describes the transfer process involving a bridging ligand between donor and acceptor (Eq.1.2). This mechanism was first demonstrated by Taube and co-workers.\cite{196} In inner-sphere ET, the two redox centers are covalently linked during the ET. The covalent linkages between donor and acceptor units play the key role in the ET processes. This bridge can be permanent, in which case the electron transfer event is termed intramolecular electron transfer. More commonly, however, the covalent linkage is transitory, forming just prior to the ET and then disconnecting following the ET event. In such cases, the event is related to intermolecular electron transfer. This mechanism is inferred if the electron transfer is unusually fast and sensitive to the chemical nature of the bridging group.

\[
D^{\text{+m}} \text{ -B - A} \rightarrow D^{\text{+m+1}} \text{ - B} - A^{\text{+n-1}} \rightarrow \text{Products} \quad \text{(Eq. 1.2)}
\]

\textit{Here, } B = \text{bridging ligand.}

Electron transfer is amongst the most critical processes in chemistry. In the environmental remediation process, electron transfer events are indispensable. The process of efficiently and controllably moving electrons around is one of the primary steps. Understanding of electron transfer mechanisms allows to predict and to enhance the degradation of contaminants. Both inner and outer sphere pathways have been proposed for reduction of organohalides.\cite{197-202}

Castro et al. investigated the reaction mechanism of organohalides by use of Cr(H_2O)_6^{2+} as a model reductant, and found that the dehalogenation processes take
place by an inner-sphere ET.[203,204] Mansuy and coworkers [205] studied the reaction between iron porphyrins -tetraphenylporphyriniron(II) (TPP) with 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT), and isolated the vinylidene carbene complex of a metalloporphyrin. This provides the evidence for the inner-sphere electron transfer. Larson [206] examined the dechlorination reactivity of the iron(II) porphyrins toward a series of substituted trichloromethanes (CCl₃R). Iron porphyrin serves as an electron-transfer mediator in the system. Formation of chlorohemin as a terminal product of the reaction between iron porphyrin and CCl₃R suggests that electron transfer occurs via inner-sphere mechanisms.

In contrast with inner sphere mechanism, some evidence suggests an outer-sphere electron transfer pathway for the reduction of organohalides.[200,202,207] Follett et al. studied the outer sphere mechanism of the reduction of trichloroethylene (TCE) based on the product distribution. In the literature, stereochemical product ratios have been used to distinguish between outer-sphere and inner-sphere electron-transfer mechanisms.[208,209] Authors used a series of well-characterized outer-sphere electron-transfer agents to explore the reduction of TCE. With the presence of reducing agent, TCE undergoes the reduction process as shown in Figure 1.4.

![Figure 1.4 Reduction pathways of trichloroethylene (TCE).][200]
The inversion rates of the cis-dichlorovinyl radical (cDCE\(^*\)) and the trans-dichlorovinyl radical (tDCE\(^*\)) are competitive with further reduction to the stereochemically rigid vinyl anions. All reducing agents resulted in same products, and led the ratios of [cDCE]:[tDCE] less than 5:1. The product ratio can be used as a diagnostic for the mechanism of TCE reduction.

In ET, many aspects can affect the whole process. Single electron transfer processes influenced by activation energies, dynamical solvent effects, and tunneling pathways are among the best understood chemical reactions. While single electron transfer is critical in numerous biological and chemical systems, ET chains and electrodes often facilitate the delivery of numerous electrons. Multi-electron transfer (MET) processes avoid high-energy free radical intermediates and can yield desired reaction products under mild, environmentally relevant reaction conditions. So controlling multi-electron transfer is a challenge to scientists. Multi-electron processes are essential for server fields including biological processes, conversion of solar energy to chemical energy, splitting water to H\(_2\), small molecule activation, and environmental remediation.[153,210,211]

Organohalide and organophosphates are major ground water pollutants that could be easily degraded by multi-electron catalysts. Some two-electron transfer reaction products derived from organohalide pollutants have been observed. Stromberg and co-workers [212] investigated the dechlorination processes of 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT) and CCl\(_4\) with heme-functionalized nanocrystalline TiO\(_2\) (Figure 1.5).
Obare et al. [153] also investigated the degradation of organohalides with heme-functionalized nanocrystalline TiO$_2$. The multiple electron transfer process is more favorable for some organohalides, such as TCE.

In 2001, Totten and coworker [213] predicted that two-electron transfer reduction potentials of many organohalide compounds are more positive than the one-electron transfer reduction potentials based on theoretical calculations. This means that with two electron transfer pathways, the process will be more thermodynamically favored than one electron transfer ones. The results also indicated that reduction of organohalides occurs more easily with systems that provide multiple electrons.
Table 1.3 One- and two-electron reduction potentials of organohalide pollutants.* [213]

<table>
<thead>
<tr>
<th>Organohalides</th>
<th>1 e⁻ reduction potential</th>
<th>2 e⁻ reduction potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl₄</td>
<td>+0.085</td>
<td>+0.673</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>-0.145</td>
<td>+0.560</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>-0.428</td>
<td>+0.494</td>
</tr>
<tr>
<td>CH₂=CHCl</td>
<td>-1.141</td>
<td>+0.386</td>
</tr>
<tr>
<td>CHCl=CHCl</td>
<td>-1.012</td>
<td>+0.464</td>
</tr>
<tr>
<td>CH₂=CCl₂</td>
<td>-0.802</td>
<td>+0.497</td>
</tr>
<tr>
<td>CHCl=CCl₂ᵃ</td>
<td>-0.674</td>
<td>+0.537</td>
</tr>
<tr>
<td>CCl₂=CCl₂</td>
<td>-0.598</td>
<td>+0.598</td>
</tr>
</tbody>
</table>

* Here, the reduction potential in V vs. SHE. Condition: aqueous solution, 1mM halide ion activity, pH 7.

ᵃ CHCl=CCl₂ will form different products with the application of various reduction potentials. The reduction potentials values showed in Table 1.3 are for CHCl=CCl (Z) as one electron transfer product, and CHCl=CHCl (Z) as two-electron transfer product.

1.4 Research Objectives

The main objectives of the work to be undertaken in this dissertation are:

1. Develop a selective molecular sensor that targets specific analytes organophosphorus (OP) compounds, which are highly toxic compounds.

   Ensure dual optical and electrochemical signal transductions to minimize
false-positives.

2. Develop a molecular system capable of storing and shuttling multiple electrons, understand the mechanism of structural changes and charge storage.

3. Establish a catalyst system that reacts with chlorinated ethylenes via two-electron transfer, that promotes chlorinated ethylenes degradation in aqueous solution.

4. Develop a catalyst system that promotes degradation of organophosphorus (OP) pesticides via reduction processes; ensure the catalyst functions in aqueous solution.
1.5 References


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CHAPTER II

SELECTIVE DUAL OPTICAL AND ELECTROCHEMICAL DETECTION OF ORGANOPHOSPHORUS PESTICIDES

2.1 Introduction

Organophosphorous (OP) compounds with a thiophosphoryl (P=S) functional group constitute a broad class of widely used insecticides. According to EPA, about 70% of the insecticides in current use in the US are organophosphorus pesticides.[1] They are related to the more reactive phosphoryl (P=O) organophosphates, which include very lethal nerve agents and chemical warfare agents, such as, VX, Soman and Sarin. Unfortunately, frequent use of OP compounds in agricultural lands worldwide has resulted in their presence as residuals in crops, livestock, and poultry products and has further led to their migration into underground aquifers.[2-4] These compounds are highly toxic to human health and are powerful inhibitors of cholinesterase enzymes.[5]

Significant advances toward the development of detection methods for OP pesticides have been reported that include gas, liquid, and thin layer chromatography,[6,7] immunoassays,[8,9] and biosensors based on inhibition of cholinesterase activity.[10-13] While the inhibition-based biosensors[3,14-16] have shown the most promise for detection of OP pesticides, they suffer from three main drawbacks; (1) the enzymes are susceptible to losing activity due to changes in environmental conditions, for example temperature, pH, or due to improper handling
factors, therefore these enzymes may provide false positive signals,[17,18] (2) prior to analysis, the biosensors require baseline testing and thus lengthy incubation times to allow enzyme-analyte interaction, and (3) the irreversible nature of cholinesterase enzyme inhibition prevents their use more than once. From a practical viewpoint, the above approaches have limitations such as low sensitivity, lack of portability, limited selectivity, difficulties in real-time monitoring, and operational complexity.

Both soil and water are likely to contain OP pesticides due to heavy urban and rural use of these compounds.[19] Unfortunately, to date there have been no reports to demonstrate molecular sensors that differentiate selectively between OP pesticides. The broad range in toxicity levels of OP pesticides necessitates new methods to discriminate between various OP pesticides.

In this chapter, we report that highly conjugated phenanthroline derivatives benzodipyrido[3,2-a:2',3'-c]phenazine (BDPPZ) and 3,6-dimethylbenzodipyrido-[3,2-a:2',3'-c]phenazine (DM-BDPPZ) (Scheme 2.1) overcome the limitations of biosensors and further differentiate between the well-known OP pesticides.

Scheme 2.1 Structures of phenanthroline, BDPPZ and DM-BDPPZ and Tribenzo[a,c,i]phenazine.
Scheme 2.2 Structures of fenthion, ethion and malathion.

Table 2.1 Physicochemical properties of the selected organothiophosphorus pesticides. [20,21]

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Molecular Weight (g/mol)</th>
<th>Solubility in water (Avg, mg/L)</th>
<th>Vapor pressure (mmHg, at 20°C)</th>
<th>Hydrolysis Half-life (Avg, Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenthion</td>
<td>278.34</td>
<td>4</td>
<td>$4 \times 10^{-5}$</td>
<td>41</td>
</tr>
<tr>
<td>Malathion</td>
<td>330.36</td>
<td>125</td>
<td>$8 \times 10^{-6}$</td>
<td>6</td>
</tr>
<tr>
<td>Ethion</td>
<td>384.48</td>
<td>practically insoluble</td>
<td>$1.5 \times 10^{-6}$</td>
<td>25</td>
</tr>
</tbody>
</table>
2.2 Experimental Section

2.2.1 Materials and Instrumentation

1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline, potassium bromide, sulfuric acid, nitric acid, fenthion, ethion and malathion, were obtained from Sigma-Aldrich. 2,3-diaminonaphthalene was obtained from Alfa Aesar. Tetrabutylammonium hexafluorophosphate (TBAPF$_6$) was obtained from Fluka. The solvents ethanol, dichloromethane and acetonitrile were obtained from Sigma-Aldrich. All solvents were of HPLC grade or better and were dried prior to usage. UV-visible absorbance spectra were acquired using a Varian Cary 50 spectrophotometer. Emission spectra were acquired using a Varian Eclipse spectrofluorometer. $^1$H NMR was recorded on a 400 MHz JEOL spectrometer at room temperature. Differential pulse voltammetric (DPV) measurements were carried out through a three-electrode arrangement using a BAS Epsilon electrochemical analyzer. A glassy carbon electrode was chosen as the working electrode, the counter-electrode was a platinum wire and the reference electrode was Ag/AgCl (3 M KCl). The supporting electrolyte solution was 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF$_6$) in acetonitrile. A N$_2$ atmosphere was kept throughout the measurements.

2.2.2 Synthesis Procedures

BDPPZ and DM-BDPPZ were synthesized following a modified literature procedure as shown in Scheme 2.3.[22-24] Briefly, phenanthroline was oxidized to the corresponding dione, using $\text{H}_2\text{SO}_4/\text{HNO}_3$ in the presence of potassium bromide (KBr). Each of the diones was coupled with 2,3-diaminonaphthalene in a condensation reaction to provide BDPPZ or DM-BDPPZ.
Scheme 2.3 Synthesis of benzodipyrido[3,2-a:2'3'-c]phenazine BDDPZ (where R = H), and 3,6-dimethyl-benzodipyrido[3,2-a:2'3'-c]phenazine DM-BDDPZ (where R = CH₃).

2.2.2.1 Synthesis of BDPPZ

(a) 1,10-Phenanthroline-5,6-Dione

20 mL of concentrated sulfuric acid (H₂SO₄) and 10 mL of nitric acid (HNO₃) were added dropwise to the mixture of 1,10-phenanthroline (1.00 g, 5.56 mmol) and KBr (5.95 g, 50 mmol) at 0 °C. The mixture was refluxed at 80 °C for 2 hours, then cooled to room temperature. The content of the reaction flask was diluted
with deionized water (400 mL), and neutralized with sodium bicarbonate (NaHCO₃). The product was extracted with methylene chloride, and dried over anhydrous MgSO₄. After all solvents were removed on a rotary evaporator, the product was concentrated in vacuum, resulting in a yellow solid. The compound was purified by recrystallization from methanol. The average yield for this reaction was 95% (1.11 g, 5.31 mmol). ^H NMR (CDCl₃, 400 MHz) δ: 9.12 - 9.10 (t, 2H, J = 2.85 Hz), 8.5 - 8.48 (d, 2H, J = 1.83 Hz), 7.60 - 7.55 (m, 2H, J = 4.71 Hz).

(b) Benzo[j]dipyrido[3,2-a:2',3'-c]phenazine (BDPPZ)

1,10-phenanthroline-5,6-dione (0.50 g, 2.38 mmol) was refluxed in ethanol for 15 minutes. 2,3-diaminonaphthalene (0.38 g, 2.38 mmol) was added to the dione and the mixture was allowed to reflux for 4 hours. The solution color changed from yellow to orange. The solution was cooled to room temperature and then filtered to collect the solid product. The product was washed with methanol, and concentrated in vacuum. The average yield of BDPPZ was 80%. ^H NMR (CDCl₃, 400 MHz) δ: 9.73 - 9.70 (d, 2H, J = 7.30 Hz), 9.33 - 9.32 (d, 2H, J = 2.96 Hz), 8.99 (s, 2H), 8.24 - 8.22 (d,d, 2H, J = 2.92 Hz), 7.87 - 7.84 (q, 2H, J = 4.40 Hz), 7.67 - 7.64 (d,d, 2H, J = 3.28).
2.2.2.2 Synthesis of DM-BDPPZ

(a) 2,9-dimethyl-1,10-phenanthroline-5,6-Dione

Concentrated H$_2$SO$_4$ (20 mL) and concentrated HNO$_3$ (10 mL) were added to 2,9-dimethyl-1,10-phenanthroline (1.16g, 5.56 mmol) at 0 °C. The mixture was allowed to reflux at 80 °C for 2 hours then cooled to room temperature. The content of the reaction flask was diluted with deionized water (400 mL), and neutralized with sodium bicarbonate (NaHCO$_3$). The product was extracted with methylene chloride, dried over anhydrous MgSO$_4$, and was concentrated in vacuum, resulting in a yellow-brown solid. Purification was accomplished by recrystallization from methanol to yield yellow-brown crystals. The average yield for this reaction was 95%. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 8.38 - 8.35 (d, 2H, $J = 7.98$ Hz), 7.42 - 7.39 (d, 2H, $J = 8.01$ Hz), 2.84 (s, 6H).

(b) 3,6-dimethyl-benzo[i]dipyrido-[3,2-a:2',3'-c]phenazine (DM-BDPPZ)

2,9-dimethyl-1,10-phenanthroline-5,6-dione (1.13 g, 4.78 mmol) was refluxed in ethanol for 15 minutes. After all the dione had dissolved, one equivalent of 2,3-diaminonaphthalene (0.755g, 4.78 mmol) was added to the contents of the reaction flask. The reaction was refluxed for 4 hours. During the course of the reaction, the solution color changed from yellow to red. The solution was cooled to room temperature and then filtered to collect the solid product. The product was washed with methanol, and concentrated in vacuum. Purification was accomplished by recrystallization from methanol to yield red crystals. DM-BDPPZ was produced in approximately 75-80 % yield. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 9.56 - 9.54 (d, 2H, $J = 8.40$ Hz), 8.94 (s, 2H), 8.21 - 8.20 (m, 2H, $J = 3.32$ Hz), 7.67 - 7.64 (d, 2H, $J =
8.44 Hz), 7.62 - 7.60 (m, 2H, \( J = 3.28 \) Hz), 2.9 - 3.0 (s, 6H).

### 2.2.2.3 Synthesis of Tribenzo[a,c,i]phenazine

**Scheme 2.4** Synthesis of tribenzo [a,c,i] phenazine.

9,10-phenanthrenequinone (0.937 g, 4.50 mmol) was dissolved in 20 mL of ethanol, and refluxed for 10 minutes at 80 °C. One equivalent of 2,3-diaminonaphthalene (0.712 g, 4.50 mmol) was dissolved in 20 mL of ethanol, and refluxed for 10 minutes at 80 °C. Two solutions were mixed, and refluxed for 2 hours. The solution was allowed to cool to room temperature and then filtered to collect the solid product. The product was washed with methanol, and concentrated in vacuum. Purification was accomplished by recrystallization from methanol to yield yellow-orange solid. Tribenzo[a,c,i]phenazine was produced in approximately 90 % yield. \(^1\)H NMR (THF-d\(_8\), 400 MHz) \( \delta \): 9.52 - 9.54 (d, 2H, \( J = 6.6 \) Hz), 9.05 (s, 2H), 8.75-8.76 (d, 2H, \( J = 8.1 \) Hz), 8.33, 7.69 (AA'BB', 4H), 7.82 (m, 4H).

### 2.2.3 Optical Spectra Measurements

A Varian Cary 50 UV-visible absorbance spectrophotometer was used to
acquire steady-state spectra. A Varian Cary Eclipse spectrofluorometer was used to measure fluorescence.

2.2.4 Computational Study

The calculations were carried out by Gaussian 03 program suite.[25] The structures of both BDPPZ and DM-BDPPZ were optimized at the B3LYP level of density functional theory. Initially the structures were optimized using restricted Hartree–Fock method and a small basis set. These structures were then used as the starting point for the density functional calculations. Further optimizations were performed using the 6-31G basis set, expanded to include polarization and diffuse functions (6-31+G(d,p)). Frequency calculations at the same level of theory were also performed to confirm that all stationary points were minima (no imaginary frequencies).

2.2.5 Electrochemical Measurements

Voltammetric measurements were carried out through a three-electrode arrangement using a BAS Epsilon electrochemical analyzer. Differential pulse voltammetry (DPV) data of BDPPZ and DM-BDPPZ were recorded in acetonitrile solution containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF₆) as supporting electrolyte in the absence and presence of three OP pesticides. Measurements were taken under a N₂ atmosphere. A glassy carbon electrode was chosen as the working electrode, while the counter-electrode was a platinum wire and the reference electrode was Ag/AgCl (3 M KCl). Differential pulse voltammograms were obtained at a 0 to -1500 mV (vs. Ag/AgCl) potential window at a scan rate of 20 mV·s⁻¹ and pulse amplitude of 50 mV.
2.3 Results and Discussion

2.3.1 UV-Visible Absorbance and Fluorescence Spectra of BDPPZ and DM-BDPPZ Sensor Molecules

The absorption spectrum of BDPPZ shows two absorption peaks at 390 nm and 410 nm in acetonitrile solution, while for DM-BDPPZ, there is a slight shift to 395 nm and 415 nm (Figure 2.1).

![Absorption Spectrum](image)

**Figure 2.1** Normalized UV-visible absorbance spectra of BDPPZ and DM-BDPPZ in acetonitrile solution.

For both BDPPZ and DM-BDPPZ, an excitation wavelength of 385 nm was used for emission. The emission spectra of both BDPPZ and DM-BDPPZ obtained
in acetonitrile solvent showed a peak centered at 550 nm. The fluorescence quantum yields of BDPPZ and DM-BDPPZ were measured in acetonitrile and found to be 0.15 and 0.12, respectively, using coumarin 153 as a standard. The interactions of the BDPPZ and DM-BDPPZ with three OP pesticides, fenthion, malathion and ethion, were investigated.

![Normalized fluorescence spectra of BDPPZ and DM-BDPPZ. In acetonitrile solution, both BDPPZ and DM-BDPPZ emit in the green with λ<sub>max</sub> = 550 nm.](image)

**Figure 2.2** Normalized fluorescence spectra of BDPPZ and DM-BDPPZ. In acetonitrile solution, both BDPPZ and DM-BDPPZ emit in the green with λ<sub>max</sub> = 550 nm.

### 2.3.2 Titration with OP Pesticides

A 1.4 x 10<sup>-5</sup> M solution of BDPPZ or DM-BDPPZ was prepared in
acetonitrile and titrated with each of the OP pesticides, while monitoring changes in fluorescence spectra. In each case, an acetonitrile solution of the sensor molecules was freshly prepared at room temperature. Fluorescence measurements were taken following each aliquot of OP pesticide added. All measurements were taken at room temperatures.

Figure 2.3a shows the results obtained when BDPPZ and DM-BDPPZ were titrated with fenthion. In each case, the fluorescence was completely quenched. Detection limits of ppm fenthion were obtained. On the other hand, the titration of BDPPZ with malathion resulted in fluorescence quenching, but this time the quenching was accompanied by a 20 nm red-shift from 550 nm to 570 nm (Figure 2.3b (i)). At the end of the titration, the fluorescence color had changed from green to yellow and the solution was dimmer. Surprisingly, the titration of DM-BDPPZ with malathion did not result in fluorescence quenching. Instead, the fluorescence intensity increased and was accompanied by a 25 nm red-shift from 550 nm to 575 nm (Figure 2.3b (ii)). The fluorescence color changed from green to yellow and was brighter.

Similar behavior was observed when BDPPZ and DM-BDPPZ were titrated with ethion. The titration of BDPPZ with ethion resulted in fluorescence quenching accompanied by a 30 nm red-shift from 550 nm to 580 nm (Figure 2.3c (i)), while in the case of DM-BDPPZ titrated with ethion, the fluorescence intensity increased and was accompanied by a 35 nm red-shift from 550 nm to 585 nm (Figure 2.3c (ii)).
Figure 2.3 Changes in the fluorescence spectra of (i) BDPPZ (ii) DM-BDPPZ when titrated with (a) fenthion; (b) malathion, and (c) ethion, respectively. The insets show the corresponding color changes.
We emphasize that there were no further shifts in wavelength or changes in intensity of both sensor molecules at saturation concentrations of the corresponding OP pesticides. We note that there were no changes in the absorbance spectra of BDPPZ or DM-BDPPZ with either of the OP pesticides.

2.3.3 Theoretical Computation

To gain better insight of the binding mechanism, theoretical computations were performed, (Figure 2.4). The calculations were carried out by Gaussian 03 program suite.

Figure 2.4 (I) Electron density of BDPPZ from total SCF with the isovalue 0.01. (II) Electron density of DM-BDPPZ from total SCF with the isovalue 0.01, (a) eclipsed structure, and (b) staggered structure.
The calculation results showed that the phenanthroline nitrogens of the BDPPZ and DM-BDPPZ hold higher electron density relative to the phenazine nitrogens. Therefore, we hypothesized that binding of BDPPZ and DM-BDPPZ to the OP compounds occurred between the phenanthroline nitrogens and the phosphorus atoms, and in doing so, displace the leaving group of the OP compound. It is well-known that the mechanism by which cholinesterase-based enzymes bind to OP compounds is through the P atom followed by displacement of the leaving group.[26-29] We hypothesize that the leaving group becomes the counter anion of the complex formed.

2.3.4 Binding Mechanism

In an effort to investigate the binding mechanism between sensors and OP pesticides, we synthesized the molecule tribenzo[a,c,i]phenazine, and examined changes in its fluorescence with ethion, malathion and fenthion.

A $1.5 \times 10^{-4}$ M solution of tribenzo[a,c,i]phenazine was prepared in acetonitrile and titrated with each of the OP pesticides, while monitoring changes in fluorescence spectra. Figure 2.5 shows the results of the titration of tribenzo[a,c,i]phenazine with fenthion. With the increased concentration of fenthion, fluorescence quenching of the peak centered at 535 nm was obtained, accompanied by the emergence of new peak at 450 nm. At the end of the titration, the fluorescence color changed from yellow green to blue.

Figure 2.6 shows the results obtained when tribenzo[a,c,i]phenazine was titrated with malathion and ethion. The titration with malathion did not result in significant change in fluorescence spectra. Similar behavior was observed when tribenzo[a,c,i]phenazine was titrated with ethion.
Figure 2.5 Changes in fluorescence spectra of tribenzo[a,c,i]phenazine when titrated with fenthion.

Figure 2.6 The fluorescence spectra of tribenzo[a,c,i]phenazine (I) with malathion, (II) with ethion.
The results showed that the addition of saturation concentrations of ethion or malathion to tribenzo[a,c,i]phenazine does not influence the fluorescence, however, the addition of fenthion strongly affects the fluorescence and causes shifts in emission wavelengths. The results thus suggest that ethion and malathion bind to BDPPZ and DM-BDPPZ through the phenanthroline nitrogens, while fenthion binds through the phenazine nitrogens.

We further examined the changes in fluorescence intensity of the BDPPZ and DM-BDPPZ when they were titrated with the electrophile $\text{H}^+$ (in this case, HCl was chosen).

As shown in Figure 2.7, the addition of HCl produces a significant but identical fluorescence response in both BDPPZ and DM-BDPPZ solutions. The fluorescence of both BDPPZ and DM-BDPPZ was quenched and accompanied by a red-shift in the presence of $\text{H}^+$. Furthermore, it was found that HCl formed a 1:2 BDPPZ:$\text{H}^+$ complex and DM-BDPPZ:$\text{H}^+$ complex. The fact that the fluorescence of both BDPPZ and DM-BDPPZ was quenched due to a 1:2 complexation is additional evidence that the reason we see fluorescence enhancement of DM-BDPPZ when bound to ethion and malathion is due to a 1:1 complexation (as shown in Figure 2.3b and 2.3c). Thus, the difference in behaviors of both BDPPZ and DM-BDPPZ with each of the OP pesticides investigated is reflective of the structure of the complex formed upon binding. The results indicate that the difference in emission behaviors results from differences in the structural forms of the complexes.
Figure 2.7 The fluorescence spectra changes of sensors BDPPZ (I) and DM-BDPPZ (II) with the addition of HCl.
2.3.5 Electrochemical Detection

While the fluorescence measurements showed that DM-BDPPZ was selective and sensitive toward three OP pesticides, it was important to determine whether an alternative signal output could be used in addition to determine binding. From a general viewpoint, sensors that provide dual independent signal outputs are advantageous as they minimize the risk of false-positive signals. Electrochemical sensors are advantageous for several applications as they can be developed into miniaturized devices and used for field-based and \textit{in situ} environmental monitoring.[30,31]

Electrochemical measurements provide an alternative signal output for sensing of OP pesticides. Here, differential pulse voltammetry (DPV) was used to investigate the differences in the structures of sensor molecules when bound to OP pesticides. Voltammetric measurements were carried out through a three-electrode arrangement using a BAS Epsilon electrochemical analyzer. DPV data of BDPPZ and DM-BDPPZ were recorded in acetonitrile solution containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF$_6$) as supporting electrolyte in the absence and presence of three OP pesticides. Measurements were taken under a N$_2$ atmosphere. A glassy carbon electrode was chosen as the working electrode, the counter-electrode was a platinum wire and the reference electrode was Ag/AgCl (3 M KCl). Differential pulse voltammograms were obtained at a 0 - 1500 mV (vs. Ag/AgCl) potential window at a scan rate of 20 mV s$^{-1}$ and pulse amplitude of 50 mV.

As shown in Figure 2.8, Figure 2.9 and Table 2.2, significant changes were observed upon addition of excess pesticides. We note that the DPV data shown exclude any influence of the OP pesticides alone.
Figure 2.8 Changes in differential pulse voltammetry (DPV) of BDPPZ with fenthion, malathion and ethion. Measurements were conducted at room temperature using glassy carbon as working electrode, Pt wire as counter electrode, and Ag/AgCl as reference electrode.

Figure 2.9 Changes in differential pulse voltammetry (DPV) of DM-BDPPZ with fenthion, malathion and ethion. Measurements were conducted at room temperature using glassy carbon as working electrode, Pt wire as counter electrode, and Ag/AgCl as reference electrode.
Table 2.2 Summary of the BDPPZ and DM-BDPPZ sensors with various OP pesticides.

<table>
<thead>
<tr>
<th></th>
<th><strong>BDPPZ</strong></th>
<th></th>
<th></th>
<th></th>
<th>Detection Limit (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Before</em> (nm)</td>
<td><em>After</em> (nm)</td>
<td><em>DPV Before V vs. Ag/AgCl</em></td>
<td><em>DPV After V vs. Ag/AgCl</em></td>
<td></td>
</tr>
<tr>
<td><strong>Fenthion</strong></td>
<td>550</td>
<td>550</td>
<td>-0.91, -1.36</td>
<td>-0.91, -1.36</td>
<td>10^-6</td>
</tr>
<tr>
<td><strong>Malathion</strong></td>
<td>550</td>
<td>570</td>
<td>-0.48, -1.07</td>
<td>-0.48, -1.07</td>
<td>10^-5</td>
</tr>
<tr>
<td><strong>Ethion</strong></td>
<td>550</td>
<td>580</td>
<td>-0.09, -0.84</td>
<td>-0.09, -0.84</td>
<td>10^-7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th><strong>DM-BDPPZ</strong></th>
<th></th>
<th></th>
<th></th>
<th>Detection Limit (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Before</em> (nm)</td>
<td><em>After</em> (nm)</td>
<td><em>DPV Before V vs. Ag/AgCl</em></td>
<td><em>DPV After V vs. Ag/AgCl</em></td>
<td></td>
</tr>
<tr>
<td><strong>Fenthion</strong></td>
<td>550</td>
<td>550</td>
<td>-0.96, -1.30</td>
<td>-0.96, -1.30</td>
<td>10^-6</td>
</tr>
<tr>
<td><strong>Malathion</strong></td>
<td>550</td>
<td>575</td>
<td>-0.65, -1.18</td>
<td>-0.65, -1.18</td>
<td>10^-6</td>
</tr>
<tr>
<td><strong>Ethion</strong></td>
<td>550</td>
<td>585</td>
<td>-0.15, -0.82</td>
<td>-0.15, -0.82</td>
<td>10^-8</td>
</tr>
</tbody>
</table>

*a The fluorescence peaks changes before and after the addition of OP pesticides.
*b Differential pulse voltammetry data before and after the addition of OP pesticides. All measurements were made in acetonitrile solvent and at room temperature.

For the 1.73 x 10^-4 M BDPPZ solution, two reduction peaks were observed at -0.91 V and -1.36 V vs. Ag/AgCl. Addition of fenthion did not affect the DPV. However, addition of malathion resulted in two peaks at -0.48 V and -1.07 V vs. Ag/AgCl, while addition of ethion, resulted in two peaks at -0.09 V and -0.84 V vs. Ag/AgCl. The DPV of 1.87 x 10^-4 M DM-BDPPZ in acetonitrile solution displayed peaks at 0.96 V and -1.30 V vs. Ag/AgCl. Addition of fenthion caused an increase in current. In the case of malathion, the DPV data showed shifts to -0.65 V and -1.18 V vs. Ag/AgCl, while addition of ethion, caused shifts to -0.15 V and -0.82 V vs.
Ag/AgCl. The emergence of new peaks in the DPV of BDPPZ and DM-BDPPZ indicates interaction with the OP pesticides. The difference in the peak positions further affirms the selectivity of the sensor, as well as the difference in binding modes.

2.3.6 Binding Constants

Despite the structural similarities between them, the DM-BDPPZ methyl groups significantly influence the interaction of the ‘phenanthroline nitrogens’ with the OP compounds. The method of continuous variation showed that DM-BDPPZ forms a 1:1 complex with the OP pesticides, while the BDPPZ forms a 1:2 complex with the OP pesticides (as shown in Figure 2.10), illustrating the effects of DM-BDPPZ’s methyl groups.

Binding constants were calculated based on a 1:1 or 1:2 sensor:analyte complex.[32] For both BDPPZ and DM-BDPPZ, fenthion acts as the quencher. The fluorescence quenching data are usually analyzed by the Stern-Volmer equation

\[
\frac{F_0}{F} = 1 + K_{SV}[Q]
\]

where Q is the quencher, \(F_0\) and F are the fluorescence intensity in the absence of a quencher and in the presence at \([Q]\) concentration, respectively. \(K_{SV}\) is the Stern-Volmer dynamic quenching constant.

For this study, the quenching occurs within a compound-ligand complex, in which case the Stern-Volmer constant, \(K_{SV}\) is equal to the association constant, \(K_A\). The Stern-Volmer plot for quenching of both BDPPZ and DM-BDPPZ by fenthion showed good linearity in the range of quencher concentration used in our experiments, and provided the binding constant for the association of sensor molecules and fenthion. Based on the calculation, the binding constants of fenthion...
with BDPPZ and DM-BDPPZ are found to be 413.2 ± 2.0 M⁻¹, and 808.5±10.3 M⁻¹, respectively.

Figure 2.10 Continuous variation of the ratios of pesticides/sensor molecules.

The binding constant (K) was calculated from the fluorescence emission titration of both BDPPZ and DM-BDPPZ with malathion and ethion. The basis for the calculation of the binding constant is a 1:1 or 1:2 interaction. For BDPPZ, it forms a 1:2 complex with the OP pesticides; while for DM-BDPPZ, it forms a 1:1 complex with the OP pesticides. The 1:2 interaction of sensor and analyte dictates that K takes the form as listed below in Equation 2.1 where L abbreviates sensor
molecules and G abbreviates OP pesticides. The fluorescence emission data were then used to calculate K. The resulting values were averaged and reported with the respective standard deviation. A correlation coefficient ($r^2$) was also determined between the binding constant and the pesticides concentrations.

$$L + 2G \rightleftharpoons LG + G$$
$$LG + G \rightleftharpoons LG_2$$

$$K_1 = \frac{[LG][G]}{[L][G]^2}$$
$$K_2 = \frac{[LG_2]}{[LG][G]}$$

$$K = K_1 \cdot K_2 = \frac{[LG][G] \cdot [LG_2]}{[L][G]^2 \cdot [LG][G]}$$

$$K = \frac{[LG_2]}{[L][G]^2}$$

$$K = \frac{[LG_2]}{([L]-[LG_2])\left([G]-\frac{[LG_2]}{2}\right)^2}$$

(Eq. 2.1)

Based on the above equations, the binding constants for BDPPZ with malathion and ethion were found to be $3.1 \pm 0.3 \times 10^4$ M$^{-2}$, $8.5 \pm 0.6 \times 10^4$ M$^{-2}$, respectively.

The 1:1 interaction of sensor and analyte dictates that K takes the form as listed below in Equation 2.2 where L abbreviates sensor molecules and G abbreviates OP pesticides. The fluorescence emission data were then used to calculate K. The resulting values were averaged and reported with the respective standard deviation. A correlation coefficient ($r^2$) was also determined between the binding constant and the pesticides concentrations.
Based on the above equations, the binding constants for DM-BDPPZ with malathion and ethion were found to be $4.2 \pm 0.1 \times 10^3 \text{ M}^{-1}$, $1.5 \pm 0.4 \times 10^4 \text{ M}^{-1}$, respectively.

2.4 Conclusions

The optical and electrochemical changes of DM-BDPPZ when exposed to fenthion, ethion or malathion show the potential of phenanthroline derivatives as viable sensors with dual signal transductions. The sensor molecule shows three significant features: (1) high fluorescence quantum yields, (2) remarkable spectral shift or intensity changes in fluorescence, and (3) the complexation with pesticides leads to shifts in redox behavior. These three features are essential toward the development of selective sensors for OP pesticides that yield different signal outputs characteristic of the nature of the organophosphorus compounds.

We have successfully designed a dual fluorescent and electrochemical sensor for OP pesticides. The sensors demonstrate the ability to distinguish three various pesticides by providing different signals with each pesticide.
2.5 References


21. The Pesticide Action Network (PAN) Pesticide Database


CHAPTER III

TUNING THE REDUCTION OF 9, 11, 20, 22-TETRAAZA TETRAPYRIDOPENTACENE (TATPP)

3.1 Introduction

Electron transfer is an essential process that occurs in redox reaction. Numerous processes in biology and chemistry are related to electron transfer reactions.[1] Biological enzymes provide examples of multi-electron transfer (MET) catalysis and of coupled atom/electron transfer, because MET has a more favorable thermodynamic potential compared with a subsequent one. In contrast with single electron transfer, multi-electron transfer involves controlling the multiple numbers of electrons or holes. Metalloenzymes (hemoglobin, nitrogenase, chlorophyll etc.) embedded in protein are known to undergo multiple electron transfer processes, for example oxygen reduction, water oxidation, nitrogen fixation, and sulfate reduction.[2-4] During the MET processes, chemical reactions are able to proceed without forming radical intermediates. However, the mechanism of multi-electron transfer reactions still remains unclear, making such systems theoretically and chemically intriguing.

Multi-electron transfer process avoids high-energy intermediates to yield desired reaction products under mild conditions, and is of great current interest for fundamental studies of energy and electron transfer. MET catalysts possess potential applications in fuel cells, solar energy conversion, small molecule activation, carbon dioxide conversion, water splitting and in molecular electronics.[3,5-11] For instance,
carbon dioxide, a greenhouse gas, causes global warming with increasing atmospheric concentrations. Scientists are making great efforts to economically convert CO$_2$ into fuels or useful chemicals. During the past two decades, intensive research has been carried toward the production of carbon monoxide (CO) and formic acid (HCOOH) from CO$_2$. There is still a challenge to make economical utilization of carbon dioxide as a feedstock for fuels or chemicals, due to the stability of CO$_2$. The potentials, $E^0$ (vs. a normal hydrogen electrode, NHE, at pH = 7), for the reduction of CO$_2$ to HCOOH, CO, C, HCOH, CH$_3$OH and CH$_4$ are shown below (Eq. 3.1— Eq. 3.6).[12] It is to be noted here that the one-electron redox potential of CO$_2$ to CO$_2^{*-}$ ($E^0 = -1.9$ V vs. NHE) is very negative, making the one-electron reduction highly unfavorable.[13,14] In contrast, the proton-assisted, multi-electron routes to these products require much less energy than the one-electron process. This suggests that multi-electron transfer routes have a considerable advantage in practice, and electrolysis in the presence of MET catalysts can be carried out at considerably lower energies.

$$\text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{HCOOH} \quad E^0 = -0.61 \text{ V} \quad \text{(Eq. 3.1)}$$

$$\text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{CO} + \text{H}_2\text{O} \quad E^0 = -0.53 \text{ V} \quad \text{(Eq. 3.2)}$$

$$\text{CO}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow \text{C} + 2\text{H}_2\text{O} \quad E^0 = -0.20 \text{ V} \quad \text{(Eq. 3.3)}$$

$$\text{CO}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow \text{HCOH} + \text{H}_2\text{O} \quad E^0 = -0.48 \text{ V} \quad \text{(Eq. 3.4)}$$

$$\text{CO}_2 + 6\text{H}^+ + 6\text{e}^- \rightarrow \text{CH}_3\text{OH} + \text{H}_2\text{O} \quad E^0 = -0.38 \text{ V} \quad \text{(Eq. 3.5)}$$

$$\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad E^0 = -0.24 \text{ V} \quad \text{(Eq. 3.6)}$$

It is essential to develop molecular systems capable of storing and shuttling multiple electrons. While a few notable examples have been reported,[15-19] there continues to be a need to develop systems that can be controlled either by external environmental factors or by solar energy to provide unique tunability and thus
reactivity toward specific substrates.

MacDonnell et al. have demonstrated that the dinuclear ruthenium complexes \([(\text{phen})_2\text{Ru(TATPP)}\text{Ru(phen)}_2]\) with 9,11,20,22-tetraaza-tetrapyridopentacene (TATPP) as a coordinating ligand undergoes photodriven two-electron and four-electron reductions in the presence of a sacrificial reductant.[20,21] The processes are completely reversible upon exposure to air and consequently, this complex has the potential to be used catalytically in multi-electron transfer reactions.

![Ladder scheme of redox/protonation states of dinuclear ruthenium complexes with TATPP ligand.][20]

Figure 3.1 A ladder scheme of redox/protonation states of dinuclear ruthenium complexes with TATPP ligand.[20]

Surprisingly, not much has been done in terms of understanding the properties of the TATPP ligand alone when exposed to light irradiation. Such information will provide rationale for the design of new systems, while aiding in understanding the use of the ligand in various coordination compounds.

In this chapter, we demonstrate that an organic molecule 9,11,20,22-tetraaza-tetrapyridopentacene (TATPP) in solution, can be tuned to photochemically store
electrons based on the presence of either acid or base. Such molecular systems could yield tunable multi-electron catalysts.

3.2 Experimental Section

3.2.1 Materials and Methods

All chemicals were used as received without further treatment. Ethanol (EtOH) (≥ 99.9%, HPLC grade), dimethyl sulfoxide (DMSO) (≥ 99.7%, HPLC grade), 1,10-phenanthroline (> 99 %), 1,2,4,5-benzenetetramine tetrahydrochloride, potassium bromide (KBr) (> 99.0%) were purchased from Aldrich Chemicals. Nitric acid (concentrated), sulfuric acid (concentrated) and magnesium sulfate (anhydrous) were obtained from Fisher Scientific. Tetrabutylammonium hexafluorophosphate (TBAPF₆) (≥ 98.0%) was purchased from Fluka Co. Deionized Milli-Q water at a pH of 7 was used where aqueous measurements are described.

3.2.2 Instrumentation

TATPP was characterized by UV-visible absorbance (Varian Cary 50 spectrometer) and ¹H NMR spectroscopies using a 400 MHz JEOL NMR spectrometer. Chemical shifts (δ) are quoted in parts per million relative to TMS and the coupling constants (J) are expressed in Hertz (Hz). Spectroelectrochemical measurements were performed with a BAS model CV-50W electrochemical workstation in combination with the Varian Cary 50 spectrometer. A custom-designed quartz cuvette with a 1 cm path length was used for the photochemical studies. To study the photochemical properties of TATPP, a solar simulator was used as the irradiation source. In general, the irradiation process was carried out in a N₂ saturated TATPP-DMSO solution using a solar simulator equipped with 400W Xenon
lamp and a KV-370 (λ > 370 nm) filter purchased from Newport, Inc. (Spectra-Physics, Stratford CT).

3.2.3 Spectroelectrochemistry

For spectroelectrochemical measurements, solutions were placed in a thin layer quartz crystal spectroelectrochemical cell (CH Instruments, Inc). Spectroelectrochemical measurements were conducted on a CV 50W Potentiostat (BAS Bioanalytical System, Inc), to apply the desired potentials in a standard three-electrode arrangement with a Pt counter electrode, Ag/AgCl reference electrode (3M KCl), and Pt gauze electrode (shown in Figure 3.2).

![Figure 3.2](image)

Figure 3.2 The design of SEC-C thin layer quartz glass spectroelectrochemical cell, purchased from CH instruments. (photo from CH instruments)

0.1 M tetrabutylammonium hexafluorophosphate (TBAPF₆) in DMSO solution was used as supporting electrolyte. For each measurement, the sample was placed in an electrochemical cell compartment pre-washed with *aqua regia*. A
solution consisting of supporting electrolyte was used as a blank, and was run prior to each measurement. All measurements were carried out under a N₂ atmosphere. A Cary 50 Spectrophotometer was used to measure absorbance spectra of the solution following an applied potential. Each potential was held until the UV-visible absorbance spectrum remained unchanged.

3.2.4 Synthesis Procedures

Scheme 3.1 Synthesis of 9,11,20,22-tetraaza-tetrapyridopentacene (TATPP).

TATPP was synthesized following a modified literature procedure[22] as shown in Scheme 3.1. Briefly, 1,10-phenanthroline was oxidized to 1,10-phenanthroline-5,6-dione using a sulfuric acid/nitric acid mixture in the presence of potassium bromide (KBr). The dione, produced in 90% yield was reacted with 1,2,4,5-benzenetetramine tetrahydrochloride in ethanol to produce TATPP in 70% yield.
3.2.4.1 Synthesis of 1,10-phenanthroline-5,6-dione

A mixture of 1,10-phenanthroline (5.56 mmol, 1.00 g), and potassium bromide (42.0 mmol, 5.00 g), was placed in a 250-mL round-bottom flask in ice bath. H2SO4 (20 mL), and HNO3 (10 mL) were added dropwise into the flask, respectively. The resulting mixture was heated at 80°C to reflux for 4 hours, then cooled to room temperature. The content of the reaction flask was diluted with deionized water (400 mL), and neutralized with sodium bicarbonate (NaHCO3). The product was extracted with dichloromethane. The extract was filtered, and dried over MgSO4. After evaporation of solvents, the product was dried in vacuum and resulted in a yellow solid. The yield for the reaction was 1.11 g (95%). 1H NMR (400 MHz, CDCl3, δ / ppm): 9.12 (t, 2H, J = 2.85 Hz), 8.50 (d, 2H, J = 1.83 Hz), 7.58 (m, 2H, J = 4.71 Hz).

3.2.4.2 Synthesis of 9,11,20,22-tetraaza tetrapyrrido[3,2-a:2':3'-c:3",2":1':2":3"']pentacene (TATPP)

A mixture of 1,10-phenanthroline-5,6-dione (1.00 g, 4.76 mmol), 1,2,4,5-benzenetetramine tetrahydrochloride (0.329 g, 2.38 mmol), was suspended in ethanol (40 mL) and refluxed for 6 hours. After cooling of the reaction to room temperature, the precipitate was filtered out, washed with 15 mL of boiling ethanol, and dried in vacuum resulting in a brown solid. The reaction yield was 0.81 g (70%). 1H NMR (400 MHz, CDCl3:CF3COOD = 9:1; δ / ppm): 10.21 (d, 4H, J = 8.0 Hz); 9.78 (s, 2H); 9.34 (d, 4H, J = 4.0 Hz); 8.36 (dd, 4H, J1 = 7.3 Hz, J2 = 4.5 Hz).

3.2.5 Photochemical Reduction Process

TATPP solutions were degassed and saturated with N2 atmosphere for measurements of photochemical reduction. Irradiation of TATPP solutions was
carried out using a 400-W Xe lamp with a KV 370 filter. In each case, samples were illuminated for 30 min. Absorption spectra measurements were acquired using a Cary 50 UV-visible spectrophotometer.

3.3 Results and Discussion

3.3.1 UV-Visible Absorbance Spectrum of TATPP

Due to the limited solubility of TATPP in most organic solvents, all studies were conducted in dimethyl-sulfoxide (DMSO). The UV-visible absorbance of TATPP alone shows two peaks at 435 nm and 460 nm (Figure 3.3). The two transitions for the TATPP can be assigned to $\pi-\pi^*$ transitions involving the HOMO, LUMO and LUMO+1. The presence of the two $\pi-\pi^*$ transitions is in good agreement with the spectrum of TATPP studied by MacDonnell’s group.[20] The molar absorptivity for TATPP at 435 nm and 460 nm in DMSO are both 10,300 M$^{-1}$cm$^{-1}$.

![Figure 3.3 UV-visible absorbance spectrum of TATPP in DMSO at room temperature. The inset shows a photograph of TATPP solution.](image-url)
3.3.2 Photoreduction of TATPP

Based on the research on metal-TATPP complexes, TATPP was found to contribute to the multi-electron transfer processes.[20-25] The multi-electron reductive reactions were conducted in both electrochemical and photochemical pathways. In 2005, MacDonnell’s group studied dinuclear ruthenium complex in which TATPP acted as ligands.[20,21] They found that this complex undergoes a photo-driven two-electron reduction in aqueous solution, and stores light energy as chemical potential within its structure. The reduction processes of metal-TATPP complexes are strongly influenced by the pH value. The photochemical pathway favors sequential single-electron reductions under basic conditions, while neutral or slightly acidic conditions give rise to proton-coupled multi-electron transfer.

![Figure 3.4 Changes in the electronic absorption of TATPP in DMSO $\lambda_{max} = 435, 460$ nm (—); TATPP after irradiation in the presence of acid to form TATPPH$_2$ $\lambda_{max} = 560$ nm (— —). The inset shows a photograph of the corresponding color change that occurs after the molecule undergoes photochemical reduction.](image-url)
To study the photochemistry properties of TATPP molecule alone, the irradiation process was carried out in a N₂ saturated DMSO solution using a Xe lamp (λ > 370 nm). Our results showed that TATPP undergoes a photoreduction process, and the resulting products can be tuned based on environmental conditions, in this case, in the presence of either acid or base.

Absorption spectra of TATPP in the presence of acid (in this case 1 equivalent of HCl) or base (in this case 5 equivalents of NaOH) following the irradiation process are shown in Figure 3.4 and Figure 3.5. Irradiation in the presence of acid resulted in a significant change in the spectrum (Figure 3.4). Both the 435 nm and the 460 nm peaks disappeared, while a new broad band centered at λ = 560 nm, ε₅₆₀ = 6,500 M⁻¹·cm⁻¹, was formed and its intensity increased with increasing irradiation time. An isosbestic point at 490 nm provided evidence for the formation of a new species. The color of the solution following the irradiation process changed from yellow to purple, indicating formation of a reduced TATPP species. The product was stable under a nitrogen atmosphere for days.

On the other hand, in the presence of base, the UV-visible absorbance spectrum of TATPP showed prominent differences after irradiation (Figure 3.5). Both the 435 nm and the 460 nm peaks disappeared, and a new peak centered at 665 nm (λ₆₆₅ = 8,450 M⁻¹·cm⁻¹) emerged. The solution color changed from yellow to green, unlike the observation above, suggesting a different TATPP species being formed in the presence of base. This product was stable under a nitrogen atmosphere for days.

The reduction of tetraazapentacene compounds has been investigated by L. Dunsch et al. and S. A. Jenekhe.[26,27] They showed that heterocyclic nitrogen-containing ladder polymers could be protonated either chemically or electrochemically.
Figure 3.5 Changes in the electronic absorption of TATPP in DMSO $\lambda_{max} = 435, 460$ nm (—); TATPP after irradiation in the presence of base to form [TATPPH]$^-$ $\lambda_{max} = 665$ nm (---). Spectra were recorded after 30 mins of irradiation. The inset shows a photograph of the corresponding color change that occurs after the molecule undergoes photochemical reduction.

3.3.3 Computational Study

To understand the structural changes that occur upon TATPP irradiation, and due to significant limitations in the compound solubility, theoretical computations were carried out. All structures were optimized at the B3LYP level of density functional theory. All calculations were done by Gaussian 03 program suite.[28] Initially the structure was optimized using restricted Hartree–Fock and a small basis set. This structure was then used as the starting point for the density functional calculation. Further optimization was performed using the 6-31G basis set, expanded
to include polarization functions (6-31G(d,p)). Frequency calculations at the same level of theory were also performed to confirm that all stationary points were minima (no imaginary frequencies). Application of density functional calculation showed that the HOMO-LUMO band gap of TATPP is 2.80 eV.

Computational calculations based on Time-Dependent Density Functional Theory (TD-DFT) (excluding solvent effects) were applied to provide insights on the reduced TATPP states. Figure 3.6a shows results of calculations for a structure in which TATPP was protonated at the nitrogens in positions 9 and 20 or 11 and 22. The calculations predicted that the low energy transitions occur at 561.15 nm, which is within reasonable agreement of the experimental findings. In addition a comparative structure of TATPP in which two protons were bound to the nitrogens at either positions 9 and 22 or 11 and 20, was calculated. The data suggested that the latter structure was inconsistent with our results, most likely due to increased polarity that the adjacent protons would induce in the TATPP molecule. In addition, a structure in which TATPP was protonated at all four nitrogens in positions 9, 11, 20 and 22 was calculated. Such a structure showed an absorbance peak maximum at 740 nm, which is also inconsistent with our experimental data. Thus, the structure shown in Figure 3.6a is most plausible and further supports the experimental data whereby TATPP irradiated in the presence of acid produces TATPPH2. While phenanthroline protons are known to readily undergo protonation in acidic environments, our results indicate that the corresponding protonated structure would have a much lower energy located in the IR region, and therefore is not consistent with our results.
Figure 3.6 Graphical representation of calculation structure of protonation states of TATPP. (The charge on the nitrogen at positions 22 is drawn for clarity, but was not needed for the calculation)
Further calculations were performed to determine the energy level of the excited state of a structure in which one proton was bound to one of the pentacene nitrogens and one electron was delocalized in the molecule as shown in Figure 3.6b. The results predicted that the low energy transition occurs at 630.70 nm. Our experimental data showed an absorbance peak at 665 nm. While this value is lower in energy relative to the computational value, we note that the theoretical computations, in this case did not account for the solvent effects, but in principle the solvent would further stabilize the structure. Comparative calculations in which two electrons were delocalized in the TATPP molecule showed an absorbance maximum at 1325.05 nm, which is inconsistent with our experimental data. Both the computational results and the experimental results suggest that the structure generated when TATPP is irradiated in the presence of base is [TATPPH]. Furthermore, calculations in which the phenanthroline nitrogens were protonated were conducted, but the results were inconsistent with our experimental data.

3.3.4 Conversion Between Reduced Species of TATPP

The experimental data show that the formation of either of the two photo-generated products can be controlled by environmental conditions; i.e. TATPPH\textsubscript{2} generated in the presence of acid can be easily converted to [TATPPH]\textsuperscript{-} by simply adding excess base followed by irradiation, while [TATPPH]\textsuperscript{-} can be converted to the protonated product TATPPH\textsubscript{2} by simply adding acid as shown in the Eq. 3.7 and Figure 3.7.

\[
\text{TATPP} \underset{\text{H}^+, \text{hv}}{\rightarrow} \text{TATPPH}_2 \underset{\text{OH}^-, \text{hv}}{\rightarrow} \text{TATPPH}^+ \quad (\text{Eq.3.7})
\]
Figure 3.7 Schematic presentation of photochemical reduction of 9,11,20,22-tetraaza-tetrapyridopentacene (TATPP) in various environmental conditions, and the conversion between different reduction forms.

3.3.5 Spectroelectrochemistry of TATPP

In an effort to determine the redox and protonation behavior of the TATPP system, the spectroelectrochemical analysis of TATPP was further investigated. Spectroelectrochemistry combines the techniques of electrochemistry and spectroscopy, and allows monitoring electrochemical reactions in situ by spectroscopic technique.[29,30] For the spectroelectrochemical methods, the major challenge is to design an electrochemical cell that is mutually compatible with the desired spectroscopic technique. The optically transparent thin-layer electrodes which
enable spectral measurements to be made directly through the electrodes, have been routinely used for transmission spectroelectrochemistry.[31-33] The electrochemical cells with this type electrode have been widely used in numerous UV-visible spectroelectrochemical studies. They are advantageous for the specific condition: e.g. only small sample volumes, and the capability for rapid electrolysis.

![Spectroelectrochemical profile](image)

**Figure 3.8** Spectroelectrochemical profile of (a) TATPP in DMSO/0.1 M TBAPF$_6$ with no applied potential (---); (b) upon application of a potential of -0.6 V vs. Ag/AgCl to generate TATPPH$_2$ $\lambda_{max} = 560$ nm (-----), and (c) upon application of a potential of -2.00 V vs. Ag/AgCl to generate [TATPPH]$^-$ $\lambda_{max} = 665$ nm (----).

Spectroelectrochemistry confirmed the findings of reduction states of TATPP. There are two specific reduction potentials observed. When a potential of -0.60 V vs. Ag/AgCl was applied to a solution of TATPP in DMSO/TBAPF$_6$, and equilibrium
was attained, the solution color changed from yellow to purple, and the spectrum shown in Figure 3.8 (solid line) was generated. Application of a more negative potential of -2.00 V vs. Ag/AgCl to TATPP in DMSO/TBAPF$_6$ and allowing the solution to acquire equilibrium resulted in a color change from yellow to green and the spectrum shown in Figure 3.8 (dotted line) was obtained. Both these spectra are consistent with reduced TATPP products as shown by UV-Vis data acquired following irradiation in either acid or base (Figure 3.4 and Figure 3.5).

3.3.6 Potential Applications

The ability to store electrons in a molecule and dissipate the electrons as needed has several significant implications including small molecule activation, solar energy conversion and in electronic devices. To investigate whether the electrons were stored in the TATPP molecule following irradiation, we expose each solution, i.e. TATPPH$_2$ and [TATPPH]$^-$ to dioxygen. We note that the reaction of either TATPPH$_2$ or [TATPPH]$^-$ with dioxygen was carried out in the absence of irradiation.

In each case, the solutions changed color. In the case of TATPPH$_2$ from purple to yellow, and in the case of [TATPPH]$^-$ from green to yellow as shown in the insets of Figure 3.9 and Figure 3.10, respectively. The UV-visible absorbance profiles in both cases showed that the species had been oxidized back to TATPP (Figure 3.9 and Figure 3.10). The data are similar to earlier work by McGovern and coworkers who investigated the photoreduction of dipyrido[3,2-a:2,3-c]phenazine (DPPZ) and its re-oxidation using dioxygen.[34] The oxidized TATPP molecule could be irradiated again and subsequently oxidized by dioxygen and the process could be repeated up to ten times with no observed degradation of the TATPP molecule.
Figure 3.9 TATPP in DMSO in the presence of acid before irradiation (---); TATPP after irradiation to form TATPPH₂ in DMSO solution (-----), and TATPP generated after exposure to O₂ (-----). The inset shows a photograph of the corresponding color change of before and after exposure to O₂.

Figure 3.10 TATPP in DMSO in the presence of base before irradiation (---); TATPP after irradiation to form [TATPPH]⁺ in DMSO solution (-----), and TATPP generated after exposure to O₂ (-----). The inset shows a photograph of the corresponding color change of before and after exposure to O₂.
3.4 Conclusions

In summary, we have demonstrated that TATPP can be selectively reduced in the presence of either acid or base to generate TATPPH₂ or [TATPPH]⁻, respectively. The spectroelectrochemical data suggest that [TATPPH]⁻ is a stronger reducing agent relative to TATPPH₂. The ability to control the structure of reduced TATPP photochemically provides a means to rationally use the molecule either alone or in coordination complexes to design various systems for advanced applications.
3.5 References


4.1 Introduction

4.1.1 Background of Chlorinated Ethylenes

Large amount of volatile organic compounds (VOCs) such as chlorinated ethylenes have been widely used for a kind of metal cleaning and dry cleaning solvents, as well as chemical feed stocks. Not surprisingly, these chemical materials have contaminated air, soil, river and groundwater all over the world, and have become a serious environmental problem.

Based on the survey of water supplies in the United States in 1984, the chloroethenes, such as tetrachloroethene (PCE), trichloroethene (TCE) and dichloroethene (DCE) are among the most common contaminants detected in groundwater systems.[1] Chlorinated ethylenes are heavier than water; therefore, a spill of sufficient magnitude is likely to move downward through the subsurface until lower permeability features impedes its further progress.[2] Research on chlorinated ethylene compounds indicated that they are not significantly biodegradable in groundwater.[3,4]

Recently, based on toxicology, metabolism, animal studies, and human studies, chlorinated ethylenes have been associated with numerous adverse health effects, including liver and kidney toxicity, and carcinogenicity (as shown in Table 1.2 in Chapter 1).[5-7] However, many of these compounds remain in active and
even in large volume use. Therefore, they occur in groundwater systems with the greatest frequency and highest concentration. Due to the reports of the World Health Organization (WHO) and Environmental Protection Agency (EPA) about the cancer risks and other health hazards from exposure to chlorinated ethylenes, their presence in drinking-water aquifers is of concern.

There are various methods developed for the remediation of chlorinated organic pollutants.[8-15] Preliminary assessment of remediation technologies feasible for reclamation of subsurface environmental media contaminated with chlorinated compounds must involve consideration of the compound's physical and chemical properties. These properties are directly responsible for behavior, transport and fate of the chemical in the subsurface environment. Chlorinated ethylenes are highly oxidized chemicals, and susceptible to reduction. The current technology used for treating ground water contaminated with chlorinated ethylenes is zero-valent iron (ZVI) [16-26], where the iron acts as the electron donor to reduce the organohalides. For chloroethene contaminants, the tendency to undergo reductive dechlorination decreases with decreasing number of chlorine substituents.[27,28] One major concern with ZVI is that many chlorinated ethylenes undergo incomplete degradation thus forming products more toxic than the parent compound.[23-25,29,30] For example, both trichloroethylene (TCE) and tetrachloroethylene (PCE) are reduced to dichloroethylene (DCE) and vinyl chloride (VC), and both DCE and VC are much more toxic than TCE or PCE.[31] In addition there are concerns related to the limited lifetime of zero-valent iron due to its susceptibility to corrosion. Consequently, significant efforts have been devoted toward the remediation of chlorinated ethylenes.[32,33]

In 2001 Roberts [34] demonstrated through theoretical calculations that the
degradation of chlorinated ethylenes would undergo a favorable thermodynamic degradation pathway if two-electron transfer reductants were used relative to one-electron transfer pathways. Based on the calculations, two electron reduction potentials were found to be more positive compared to one-electron reduction potentials. Despite this prediction, very few reports have appeared in the literature that show the reduction of chlorinated ethylenes via a two-electron pathway.[35] Identifying two-electron or multi-electron transfer catalysts and understanding their reactivity could potentially yield viable methods for the remediation of organic pollutants at mild conditions.

In this chapter, we report the reactivity of the biological molecule flavin mononucleotide (FMN) in its reduced form, FMNH₂, with the chlorinated ethylenes, cis-dichloroethylene (cis-DCE), trichloroethylene (TCE) and tetrachloroethylene (PCE).

4.1.2 Flavin Mononucleotide

Flavins are redox active chromophores found in many enzymes and photoreceptors.[36,37] Chemists and biochemists began to study the passage of electron transfers of flavin at the beginning of the last century. Riboflavin, also known as vitamin B₂, is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. It is the central component of two flavin coenzymes Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN), which are essential to exert important biological functions. The riboflavin molecule comprises a substituted isoalloxazine moiety with a ribityl side chain (Scheme 4.1).

Riboflavin can be synthesized in bacteria, fungi, plants and some animals.[38] Riboflavin typically participates in redox reactions, and is critical for the metabolism
of carbohydrates, fats, and proteins, synthesis of hemoglobin and maintenance of the visual function of the eyes.[39,40] For instance, FAD is part of the electron transport (respiratory) chain, which is central to energy production. Flavins frequently form parts of multi-redox-center enzymes, such as the succinate and NADH dehydrogenases, xanthine oxidase/dehydrogenase, cytochrome P450 systems and the more recently recognized nitric oxide synthase.[40] In neutral aqueous solution, riboflavin exhibits a strong yellow-green fluorescence. In aqueous solution, riboflavin is able to degrade with the exposure to both UV radiation and visible light. The rate of destruction increases with an increase in temperature and pH.[40,41]

Scheme 4.1 Structures of riboflavin, FMN and FAD.
FMN or riboflavin-5'-phosphate is required for important reactions in all species. FMN can directly accept two-electron two-photon to yield the well-known FMNH₂. When bound at the active site of some enzymes, FMN can accept one electron, converting it to the half-reduced semiquinone radical. The semiquinone can accept a second electron to yield FMNH₂.[37] The structures are shown in Scheme 4.2. The pH values strongly affect the redox properties of FMN, and results in various reduced states of FMN, e.g. anionic or neutral hydroquinone, anionic or neutral semiquinone.[40,42]

Scheme 4.2 Structures of various redox states of FMN, FMNH• and FMNH₂.

Since it can accept/donate either one or two electrons, FMN has an important role in mediating electron transfer between carriers that transfer 2 e⁻ and carriers that
can only accept 1 e\textsuperscript{-}. The redox active behavior of FMN led us to investigate its reactivity with chlorinated ethylenes and to further identify methods to control and enhance its reactivity. The results provide insights on how various biological molecules could potentially be tuned toward environmental remediation of various environmental pollutants. An added and significant advantage of this approach relative to others is the ability to run these degradation reactions in both organic solvents or in aqueous solutions at ambient temperature and pH.

4.2 Experimental Section

4.2.1 Materials and Methods

All chemicals were used as received without further treatment. Methanol (MeOH) (≥ 99.9%, HPLC grade), acetonitrile (CH\textsubscript{3}CN) (≥ 99.9%, HPLC grade), titanium (IV) isopropoxide (Ti(iOPr)\textsubscript{4})(97%), cis-1,2-dichloroethylene (cis-DCE) (≥ 97%), trichloroethylene (TCE) (≥ 99.5%), tetrachloroethylene (PCE) (99.9%), riboflavin (≥ 98%) and riboflavin 5'-phosphate sodium (flavin mononucleotide (FMN)) (~ 85%, HPLC grade) were purchased from Aldrich Chemicals. Nitric acid and microscope glass slides were obtained from Fisher Scientific. Tetrabutylammonium hexafluorophosphate (TBAPF\textsubscript{6}) (≥ 98.0%) was purchased from Fluka Co. Indium doped tin oxide (ITO) coated sheets of glass were purchased from Hartford Glass Company, Inc. Deionized Milli-Q water at a pH of 7 was used where aqueous measurements are described. Colloidal TiO\textsubscript{2} nanoparticles were imaged on a JEOL scanning electron microscope (SEM). A custom-designed quartz cuvette with a 1 cm path length was used as the spectroelectrochemical cell.
4.2.2 Nanocrystalline TiO$_2$ Film Preparation and Functionalization

Transparent TiO$_2$ films consisting of ~12 nm diameter anatase particles were prepared by the hydrolysis of Ti(iOPr)$_4$ using a sol-gel technique.[43] The TiO$_2$ paste was cast as mesoporous thin (~10 μm) films onto microscope glass slides. The attachment of FMN to the TiO$_2$ surface was achieved by soaking freshly prepared TiO$_2$ films for 6 hours in a $1 \times 10^{-4}$ M FMN solution in MeOH. Once FMN was anchored, it remained strongly bound to the surface in both methanol and aqueous solutions. The nanocrystalline thin films were placed diagonally in a standard quartz cuvette. Absorption spectra and steady-state kinetic measurements were acquired using a Cary 50 UV-visible spectrophotometer. Irradiations of FMN/TiO$_2$ films or FMN in solution were carried out using a 400-W Xe lamp with a KV 370 cut-off filter. In each case, samples were degassed, saturated with N$_2$ atmosphere and illuminated for 30 min.

4.2.3 Spectroelectrochemical Measurements

Cyclic voltammetry (CV) and spectroelectrochemistry experiments were performed using a BAS electrochemical analyzer (BAS Bioanalytical System, Inc). For CV measurements, a glassy carbon (1.5 mm diameter disk) working electrode was used. Immediately before use, the electrodes were polished to a mirror finish with wet alumina (Buehler, 0.05 μM) followed by rinsing with Millipore Milli-Q water, dried, and stored in acetonitrile during the preparation of the electrochemical cell. A Pt gauze and a premium “no leak” Ag/AgCl electrode (Cypress, Model EE009) were used as the counter and reference electrode, respectively, and potentials are quoted with respect to this reference. For supporting electrolytes, 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF$_6$) was used in non-aqueous
solution, while 0.1 M NaCl was used in aqueous solution. Each sample was purged for 10 minutes with nitrogen. A solution consisting of supporting electrolytes dissolved in either methanol or aqueous solution was used as a blank, and was run prior to each measurement.

Spectroelectrochemical measurements were conducted on a CV 50W potentiostat to apply the desired potentials in a standard three-electrode arrangement with a Pt counter electrode, Ag/AgCl (3 M KCl) reference electrode, and ITO/TiO₂/FMN as a working electrode on alligator clips. A fresh solution of 0.1 M TBAPF₆ in acetonitrile was used as the supporting electrolyte. A Varian Cary 50 UV-visible absorbance spectrophotometer was used to measure absorbance spectra. Each potential was held until the UV-visible absorbance spectrum became time-independent, and steady state concentrations were assumed.

4.2.4 Monitoring Reactivity of FMNH₂ toward Chlorinated Ethylenes

A Varian Cary 50 UV-visible spectrophotometer was used to acquire absorbance vs. time profiles for the reactions of chlorinated ethylenes with (i) FMNH₂ in methanol solvent, (ii) FMNH₂ anchored to nanocrystalline TiO₂ in an aqueous solution, and (iii) FMNH₂/TiO₂(e⁻) in methanol. In each case the oxidation of FMNH₂ to FMN was monitored via the appearance of a 445 nm absorbance peak corresponding to FMN.

4.2.5 Analysis of Chlorinated Ethylenes Reduction by GC-MS

Loss in concentrations of cis-DCE, TCE or PCE following their reduction by the catalyst FMNH₂/TiO₂ or FMNH₂/TiO₂(e⁻_CB), were analyzed by GC/MS. An aliquot (1 µL) of sample in methanol solution were analyzed by GC using a HP 6890
Series GC System (Hewlett Packard), equipped with a HP 5973 mass selective detector. A capillary column (30.0 m x 250 μm x 0.25 μm nominal) purchased from SGE Forte was used for separation. The sample was kept in a N₂ atmosphere to avoid reactivity with oxygen. The parameters for front inlet were set as follows: split less mode using He carrier gas, heater at 250 °C, 10.5 psi. The GC oven was initially set at 80 °C for 5 min, heated at 8 °C/min to 150 °C and kept for 3 min.

4.3 Results and Discussion

4.3.1 Photoreduction of FMN to FMNH₂ and its Reactivity with Chlorinated Ethylenes

The redox active behavior of FMN presents unique opportunities to explore its potential in environmental remediation processes. Additionally, FMN possesses optical properties characteristic of its redox states, which make UV-visible absorbance spectroscopy and fluorescence spectroscopy ideal techniques for monitoring reactions of FMN and its reduced states, FMNH⁺ and FMNH₂ with chlorinated ethylenes. The UV-visible absorbance spectrum of FMN in aqueous solution shows two peaks at 375 nm and 445 nm, while in MeOH the peaks are at 355 nm and 445 nm. In both solvents, FMN solutions are in bright yellow color. In aqueous solution at pH = 7, the molar absorptivity of FMN was calculated to be 11,000 ± 125 M⁻¹ cm⁻¹ at 375 nm and 11,800 ± 134 M⁻¹ cm⁻¹ at 445 nm. In MeOH, the molar absorptivity of FMN was calculated to be 7,485 ± 85 M⁻¹ cm⁻¹ at 355 nm and 10,400 ± 220 M⁻¹ cm⁻¹ at 445 nm. The emission spectrum of FMN shows a peak at 525 nm in both aqueous solution or in MeOH solvent, with green fluorescence.

In aqueous solution and under a N₂ saturated atmosphere, the photo-reduction of FMN resulted in the formation of a semiquinone radical FMNH⁺. The UV-visible
absorbance spectrum of FMNH* is blue shifted relative to the FMN spectrum and shows two peaks at 345 nm and 400 nm (as shown in Figure 4.1).

![Absorbance Spectrum](image)

**Figure 4.1** Changes in the UV-Visible absorbance spectra of FMN (—) as it is reduced photochemically in aqueous solution to form FMNH* (− • −). Inset is the photograph of the visible color change of FMN to FMNH*.

The reactivities of FMNH* toward the chlorinated ethylenes, *cis*-DCE, TCE and PCE, were investigated. In this case a $1 \times 10^{-5}$ M solution of FMNH* under a N$_2$ saturated atmosphere was used and excess *cis*-DCE, TCE or PCE was added. The results showed that FMNH* was not oxidized to FMN even after several days of stirring. This observation indicated that FMNH* was not strong enough to reduce any of the chlorinated ethylenes investigated.
Figure 4.2 Changes in the fluorescence spectra of FMN (—) as it is reduced photochemically in aqueous solution to form FMNH• (---). Inset is the photograph of the fluorescence color change of FMN to FMNH•.

The fluorescence spectrum of FMNH• further supported the conclusion that FMNH• has no reactivity towards the PCE, TCE and DCE. With the addition of chlorinated ethylenes, the emission peak at 475 nm of semiquinone FMNH• did not change (Figure 4.2).

Since FMNH• was found to be unreactive toward chlorinated ethylenes, we investigated the reactivity of FMNH2 with cis-DCE, TCE and PCE. We found that if FMN is photo-reduced in MeOH as opposed to in water, FMN undergoes two-electron, two-proton reduction to form FMNH2, while the MeOH is oxidized to formaldehyde. FMNH2 thus carries two electrons.[44] Formation of FMNH2 was
confirmed by the appearance of a new peak centered at 320 nm (Figure 4.3), which is consistent with literature reports.[45] In MeOH, FMN fluoresces at 525 nm but after photo-reduction, a new peak centered at 445 nm was observed which was characteristic of FMNH₂.

![Figure 4.3 UV-visible absorbance spectra of FMN in methanol, (I) before irradiation (II) after irradiation (III) after the addition of TCE in the dark. Inset is the photograph of the visible color change of FMN to FMNH₂.](image)

The reactivity of FMNH₂ (1 x 10⁻⁵ M) with the chlorinated ethylenes, cis-DCE, TCE and PCE, was investigated. In each case ~10⁻⁵ M of either cis-DCE, TCE or PCE (which is one equivalent of the FMNH₂) was added to a deoxygenated solution of
FMNH$_2$. Upon addition of either of the chlorinated ethylenes to the FMNH$_2$ solution, the peaks at 355 nm and 445 nm re-emerged indicating that FMNH$_2$ was oxidized back to FMN (Figure 4.3).

**Figure 4.4** Fluorescence spectra of FMNH in aqueous solution, (I) before irradiation (II) after irradiation (III) after the addition of TCE in the dark. Inset is the photograph of the fluorescent color change of FMN (green in color) to FMNH$_2$ (blue in color).

A similar behavior was observed in the fluorescence spectrum of FMN. Photoreduction of FMN in MeOH solvent resulted in the loss of the peak at 525 nm
and formation of a new peak at 445 nm. Upon addition of either of the chlorinated ethylenes to a solution of FMNH₂, the 445 nm peak was quenched and the peak at 520 nm re-emerged confirming oxidation of FMNH₂ to FMN as shown in Figure 4.4. These results indicate that FMNH₂ reacts effectively with the chlorinated ethylenes, cis-DCE, TCE and PCE.

### 4.3.2 Photogeneration of FMNH₂ in Aqueous Solution and its Reactivity with Chlorinated Ethylenes

The observed efficient reactivity of FMNH₂ in MeOH solvent with chlorinated ethylenes is encouraging, but the inability to generate FMNH₂ in aqueous solution provides a limitation for future environmental applications. One way to overcome this limitation is to make use of an electron donor that is capable of reducing FMN to FMNH₂ in an aqueous solution. To accomplish this task we anchored FMN to a surface consisting of nanocrystalline TiO₂ which during band gap irradiation, served as the electron donor to FMN.

TiO₂ nanoparticles were synthesized by a sol-gel method following an established literature procedure.[43] Titanium (IV) isopropoxide was used as a precursor in an acid catalyzed hydrolysis reaction as shown in equation 4.1.

\[
\text{Ti(OR)}_4 + 2 \text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4 \text{ROH} \quad \text{(Eq. 4.1)}
\]

The resulting nanoparticles were 10-12 nm in diameter as shown in the SEM image in Figure 4.5. The TiO₂ nanoparticles were coated on microscope glass slides or ITO slides (each cut to 3 cm x 1 cm l x w to fit into quartz cuvette with a 1 cm path length) to form transparent films that were ~ 10 μm in thickness.
Figure 4.5 Scanning electron micrograph (SEM) image of nanocrystalline TiO$_2$ particles. Particle diameter is an average of 12 nm. The scale bar represents 100 nm.

When the energy was greater than the band gap of TiO$_2$ (for anatase TiO$_2$, band gap $\sim 3.2$ eV), electrons are excited from the valence band to the conduction band, while the holes remain in the valence band (Figure 4.6).[46] The photo-excited electron-hole pairs have the ability of reduction and oxidation, respectively.[47] The photogenerated electrons and holes can escape by direct recombination. The presence of a hole scavenger allows excited electrons to trap in the conduction band. Electron-hole recombination is in direct competition with the trapping process. The rate of trapping and the photocatalytic activity of TiO$_2$ will be enhanced by retarding the electron-hole recombination.
Research found that dye molecules with anchoring groups are capable of binding covalently to semiconductor surfaces. Some organic linkers are typically very good anchoring groups for metal oxides, such as phosphonic acids (P(O)(OH)₂), carboxylic acids (COOH), as well as their derivatives, e.g. esters, acid chlorides, carboxylate salts. The anchoring groups form bonds with the semiconductor by reacting with surface hydroxyl groups. Studies on structure and size of organic linkers indicate that the nature of anchoring groups can influence the binding constant and surface coverage. For instance, strong chemisorption at TiO₂ occurs through phosphonic acid group. As shown in Scheme 4.1, FMN possesses a phosphonic acid group that is capable to bind to the hydroxyl group on the surface of semiconductors.

FMN was attached to the TiO₂ nanoparticles by immersing a methanol solution of 1 x 10⁻⁴ M FMN. The attachment of FMN to the nanocrystalline TiO₂ produced a bright yellow film (as shown in Figure 4.7).
Figure 4.7 Photographs of (I) nanocrystalline TiO₂ film, (II) FMN anchored on nanocrystalline TiO₂ film.

FMN anchored to the TiO₂ surface was found to be stable, in both organic and aqueous solutions for periods of days. The surface coverage of FMN on the TiO₂ surface was 1x10⁻⁹ mol/cm², and the binding constant was calculated to be 2.3 x 10⁷ M⁻¹. Comparative studies with TiO₂ coated glass slides immersed in a solution of riboflavin (which does not possess the phosphonic acid functional groups) showed no evidence of adsorbed riboflavin on the TiO₂ surface. This observation can be explained by the fact that the binding of FMN to the meso-porous nanocrystalline (anatase) TiO₂ thin films occurs through the phosphonic acid functional groups. Phosphonic acid groups are well-known to bind covalently to metal oxides.[54,55] In addition to using TiO₂ to reduce FMN → FMNH₂ in an aqueous solution, and added advantage is that by attaching the molecule to the surface, the system becomes heterogeneous, thus allowing catalyst recovery. Such approaches are especially attractive for environmental applications as they avoid the introduction of catalysts directly into the environment.

Surface coverage of FMN on nanocrystalline TiO₂ was obtained using a modified form of the Beer-Lambert equation.[56] Here, the number of moles of FMN per square centimeter of projected surface area of the nanocrystalline TiO₂ film, Γₚₑₒ
(mol/cm²), was calculated from the relationship,

\[ \Gamma_{\text{pro}} = \frac{[A(\lambda)]}{[\sigma(\lambda)]} \]

where:

- \( \Gamma_{\text{pro}} \) = number of moles of FMN on TiO₂ (mol/cm²)
- \( A(\lambda) \) = absorbance of the film, and
- \( \sigma(\lambda) \) = absorption cross section (cm²/mol)

We note that the absorption cross section was obtained using the dedadic molar absorptivity (ε₄₄₅ nm = 10,400 M⁻¹ cm⁻¹) by multiplication by 1000 cm³/L, as reported earlier by Meyer's group.\[57\] In each experiment the concentration of FMN adsorbed on the TiO₂ surface was typically 3.6 x 10⁻⁵ M.

FMN bound to the TiO₂ surface showed an absorbance maximum peak at 445 nm (the 355 nm peak of FMN lies within the TiO₂ band gap of 3.2 eV and therefore was not observed). Following TiO₂ band gap irradiation, FMN undergoes complete photoreduction to FMNH₂, which is displayed by the disappearance of 445 nm peak, as shown in Figure 4.8. As mentioned earlier, TiO₂ band gap excitation produces an electron-hole pair. Band gap irradiation of FMN/TiO₂ system in aqueous solution was found to yield FMNH₂/TiO₂. In this case, the conduction band electrons (e⁻_{CB}) contribute to the reduction of FMN to FMNH₂ but due to the high rate of TiO₂ electron-hole recombination in aqueous solution, no electrons accumulate in the conduction band.
Figure 4.8 UV-visible absorbance spectra of FMNH$_2$/TiO$_2$ in aqueous solution (I) before irradiation, (II) after irradiation, and (III) after addition of TCE in the dark.

The fluorescence spectrum of FMN anchored to TiO$_2$ on Figure 4.9 shows a peak maximum at 525 nm, corresponding to the emission wavelength of FMN. Following irradiation, FMN is completely reduced to FMNH$_2$ characterized by the loss of emission maximum.

The reactivity of FMNH$_2$/TiO$_2$ in aqueous solution with the chlorinated ethylenes, cis-DCE, TCE and PCE was analyzed. Addition of the chlorinated ethylenes ranging in concentration from 1 equivalent to 5 equivalents, in the dark,
was found to generate the initial FMN/TiO₂ absorption spectrum as seen in Figure 4.8, indicating re-oxidation of FMNH₂ to FMN.

Fluorescence measurements also showed the reappearance of the peak at 525 nm corresponding to FMN upon addition of each of the chlorinated ethylenes. The fluorescence data further confirmed the oxidation of FMNH₂ and consequently the reduction of the chlorinated ethylenes during the process, Figure 4.9.

![Figure 4.9](image)

**Figure 4.9** Fluorescence spectra of FMNH₂/TiO₂ in aqueous solution and its reactivity toward TCE (I) before irradiation, (II) after irradiation, and (III) after addition of TCE in the dark.
Reduction rate constants of the reactions of the chlorinated ethylenes with FMNH₂ in methanol solvent were compared to the values obtained with FMNH₂ anchored to TiO₂ in aqueous solution using the relationship:

\[ \ln A = \ln (A_0) - kt. \]

Second-order rate constants were abstracted for plots of \( k_{\text{obs}} \) vs. [chlorinated ethylenes] for cis-DCE, TCE and PCE. The results were compared to the reduction rate constants obtained from FMNH₂ in methanol solution (in the absence of TiO₂) and are shown in Table 4.1.

**Table 4.1** Rate constants (\( k \)) of TCE reduction by TiO₂ (e⁻_CB), FMNH⁻, FMNH₂, FMNH₂/TiO₂ in water, and FMNH₂/TiO₂(e⁻_CB) in methanol (Data collected at room temperature at multiple error trial).

<table>
<thead>
<tr>
<th>RX</th>
<th>TiO₂ (e⁻) or FMNH⁻ ( k(M^{-1} \cdot S^{-1}) )</th>
<th>FMNH₂ ( k(M^{-1} \cdot S^{-1}) )</th>
<th>FMNH₂/TiO₂ in H₂O ( k(M^{-1} \cdot S^{-1}) )</th>
<th>FMNH₂/TiO₂(e⁻_CB) in CH₃OH ( k(M^{-1} \cdot S^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-DCE</td>
<td>No Reaction</td>
<td>0.03 ± 0.01</td>
<td>0.16 ± 0.09</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>TCE</td>
<td>No Reaction</td>
<td>0.54 ± 0.07</td>
<td>2.61 ± 0.20</td>
<td>67.9 ± 4.6</td>
</tr>
<tr>
<td>PCE</td>
<td>No Reaction</td>
<td>0.68 ± 0.08</td>
<td>3.33 ± 0.08</td>
<td>97.8 ± 9.4</td>
</tr>
</tbody>
</table>

The data showed that the reduction rate constants obtained when FMNH₂ anchored to TiO₂ was used as the catalysts were an order of magnitude higher than the one obtained when FMNH₂ was used in solution. A number of factors could contribute to the observed enhanced reactivity, including activation of the chlorinated ethylenes by the TiO₂ surface or possible shifts in the FMN/FMNH₂ reduction
potential when anchored to the surface. Reports have shown that attachments of molecules onto semiconductor surfaces could have profound effects on the formal reduction potentials.[58,59] If FMN exhibits similar behavior upon attachment to semiconductor surfaces, then FMN could potentially have significant impacts on various environmental remediation processes.

To investigate possible shifts in reduction potentials of FMN/FMNH₂ formal reduction potentials (E⁰) for FMN were measured in fluid solution and compared to E⁰ when anchored onto TiO₂ surfaces. Cyclic voltammetry was used to measure the E⁰ value of FMN in TBAPF₆/CH₃CN/H₂O electrolyte (Figure 4.10). The FMN redox chemistry showed equivalent anodic and cathodic peak currents, iₚa/iₚc ~ 1 (Nernstian behavior). The FMN/FMNH₂ formal potentials were measured to be -570 mV vs. Ag/AgCl (3 M KCl) in 0.1 M TBAPF₆/CH₃CN/H₂O electrolyte.

![Figure 4.10 Cyclic voltammogram of flavin mononucleotide (FMN). Electrolyte: 0.1 M TNAPF₆/CH₃CN/H₂O. Working electron is glassy carbon, counter electron is Pt wire, and reference electrode is Ag/AgCl.](image)
Measuring accurate FMN reduction potentials upon surface binding to TiO₂ using cyclic voltammetry was not feasible due to the strong oxidation of TiO₂ which overlaps with the FMNH₂ oxidation. An alternative route to measuring the formal reduction potential of FMN when anchored to the TiO₂ surface is by spectroelectrochemistry. Special optically transparent electrodes are essential for transmission spectroelectrochemistry. Nanostructured metal oxide films can be deposited on conducting glass supports to yield transparent electrodes, which offers advantage to characterize optical spectroscopy properties of electrons present in these electrodes in local environments.[60] Here we use the ITO/FMN/TiO₂ slide as the working electrode for spectroelectrochemical studies to investigate the formal potential of FMN bound to TiO₂, since indium doped tin oxide (ITO) coated sheet of glass is advantageous for a conductive solid support.

The formal reduction potentials correspond to the equilibrium potentials where the concentration of reduced and oxidized compounds was equal. The results of the spectroelectrochemical measurements (Figure 4.11) show that surface binding has a profound effect on the formal potentials and thus its reactivity with chlorinated ethylenes. The formal potential of FMN bound to TiO₂ was calculated to be -775 mV vs. Ag/AgCl (3 M KCl), whereas the formal potential of FMN alone -570 mV vs. Ag/AgCl (3 M KCl). A significant shift in the formal reduction potential of FMN was observed upon surface binding, such that FMN bound to TiO₂ was always a stronger reductant than FMN in fluid solution. This observation explains the enhanced reactivity on the TiO₂ surface compared to FMNH₂ in fluid solution. The results indicate that FMNH₂ is effective in reacting with the chlorinated ethylenes cis-DCE TCE and PCE, in both MeOH solvent and aqueous solution and could potentially be a viable alternative to current remediation technologies.
Figure 4.11 Representative spectroelectrochemical data of molecular catalyst FMN bound to TiO$_2$. FMN was reduced to FMNH$_2$ in CH$_3$CN/TBAPF$_6$ electrolyte upon applying negative potential. The potential applied were measured against Ag/AgCl (3 M KCl). The plot of FMN/FMNH$_2$ concentrations vs. applied potential is shown in the inset.

4.3.3 Combining Reactivity of FMNH$_2$ with TiO$_2$ Conduction Band Electrons (FMNH$_2$/TiO$_2$(e'))

Enhanced reactivity of FMNH$_2$ was investigated by combining its reactivity with that of the TiO$_2$ conduction band electrons (TiO$_2$(e'$_{CB}$)). The multiple electrons from FMNH$_2$ as well as the TiO$_2$(e'$_{CB}$) were modulated to react with chlorinated ethylenes. In MeOH solution, TiO$_2$ band gap excitation resulted in the reduction of
FMN to FMNH₂ while electrons were trapped in the TiO₂ conduction band. The presence of both FMNH₂ and TiO₂(e⁻CB) was confirmed by UV-visible absorbance measurements taken before and after photolysis as shown in Figure 4.12. The 445 nm peak of FMN disappeared after photolysis indicating reduction of FMN to FMNH₂. In addition, an increase in absorbance intensity in the 500-800 nm region occurred, indicating trapped electrons in the TiO₂ conduction band. With the addition of chlorinated ethylenes, we observe the loss of trapped electrons, as shown by the decrease in absorbance intensity in the 500 – 800 nm regions and the oxidation of FMNH₂ to FMN as shown by the growth of the peak at 445 nm (Figure 4.12).

A typical absorbance vs. time profile of the reaction of the chlorinated ethylenes with FMNH₂/TiO₂(e⁻CB) is shown in the inset of Figure 4.12. The measurement at 700 nm represents the TiO₂(e⁻CB), whereas the measurement at 445 nm represents the FMN absorbance peaks. Addition of excess cis-DCE, TCE or PCE to FMNH₂/ TiO₂(e⁻CB)(MeOH) resulted in the loss of trapped electrons as shown by the decrease in absorbance intensity in the 500 – 800 nm regions. This was further accompanied by the oxidation of FMNH₂ to FMN as shown by the growth of the peak at 445 nm. Steady-state kinetic data (Figure 4.12 inset) were collected and peaks at 445 nm and 800 nm corresponding to the oxidation of FMNH₂ → FMN and TiO₂(e⁻CB) → TiO₂, were monitored. The data show that upon addition of either of the chlorinated ethylenes to FMNH₂/TiO₂(e⁻CB), two significant changes occurred: (1) depletion of the TiO₂ conduction band electrons, followed by (2) oxidation of FMNH₂ to FMN. Since TiO₂(e⁻CB) are unreactive toward the chlorinated ethylenes, the data suggests that as long as TiO₂(e⁻CB) are present, they continue to reduce any FMN formed. This process continues until all the TiO₂ is consumed after which FMNH₂→ FMN oxidation is observed.
Figure 4.12 UV-visible absorbance spectra of FMNH$_2$/TiO$_2$(e$^-_{CB}$) in methanol solvent (i) before irradiation, (ii) after irradiation and (iii) after addition of TCE in the dark. Inset is time resolved absorption changes of FMNH$_2$/TiO$_2$(e$^-_{CB}$) monitored at (a) 445 nm and at (b) 700 nm following the addition of TCE in the dark.

Reduction rate constants of FMNH$_2$/TiO$_2$(e$^-_{CB}$) with cis-DCE, TCE and PCE were abstracted using the relationship $\ln A = \ln (A_0) - kt$ and compared to FMNH$_2$ in solution, and FMNH$_2$ anchored to TiO$_2$ (in the absence of conduction band electrons). As shown in Table 4.1, the presence of TiO$_2$ conduction band electrons increased the rate constants by two orders of magnitude relative to FMNH$_2$ in solution, and by one order of magnitude relative to FMNH$_2$ anchored to TiO$_2$ (in the absence of
conduction band electrons). The enhanced reactivity of FMNH₂ in the presence of TiO₂(e⁻ CB) can be attributed to either (i) direct reduction of the chlorinated ethylenes by TiO₂(e⁻ CB), or (ii) influence of the TiO₂(e⁻ CB) on the FMNH₂ that enhances reactivity toward chlorinated ethylenes.

In an effort to elucidate the nature of the enhanced reactivity, studies of the reactivity TiO₂(e⁻ CB) in the absence of FMN, with the chlorinated ethylenes cis-DCE, TCE and PCE were investigated. In each case, a glass slide coated with nanocrystalline TiO₂ was immersed in a cuvette containing MeOH solvent. The cuvette was sealed, purged with N₂ gas and irradiated for 30 minutes. TiO₂(e⁻ CB) formation (Figure 4.13) was confirmed by changes in the UV-visible absorbance spectra which showed increases in absorbance intensity in the 500-800 nm region accompanied by blue coloration in the film. After irradiation, excess cis-DCE, TCE or PCE were added to the TiO₂(e⁻ CB) using an air-tight syringe. However, there was no change in the blue coloration and the increased absorbance in the 500 – 800 nm region remained unchanged even after 2 days in a N₂ saturated atmosphere. The observation indicated that TiO₂(e⁻ CB) did not react with any of the chlorinated ethylenes investigated. The lack of reactivity of TiO₂(e⁻ CB) with the chlorinated ethylenes investigated suggests that the reactivity arises directly from FMNH₂. However, the fact that the reduction rate constants of FMNH₂/TiO₂(e⁻ CB) are two orders of magnitude higher than TiO₂(e⁻ CB), suggests that the TiO₂ surface profoundly influences the FMNH₂ reactivity. We showed that attachment of FMNH₂ to TiO₂ surfaces shifts the FMN/FMNH₂ formal reduction potential more negative by 205 mV vs. Ag/AgCl. The charged surface is likely to further influence the FMNH₂ reactivity. Moreover, the number of reactive FMNH₂ molecules at the TiO₂(e⁻ CB) surface is higher than FMNH₂ at a TiO₂ surface. This is because, as shown in the inset of Figure
4.12, any FMNH$_2$ that reacts with a molecule of either $cis$-DCE, TCE or PCE, and forms FMN, is immediately reduced back to FMNH$_2$ due to the presence of TiO$_2$($e^-_{CB}$). It is not until all the TiO$_2$($e^-_{CB}$) is consumed that FMNH$_2$ is also consumed. Nonetheless, in each case, the two FMNH$_2$ electrons are involved in the reduction of the chlorinated ethylenes.

**Figure 4.13** Photolysis of TiO$_2$ a) before irradiation, b) after irradiation.

The degradation of chlorinated ethylenes by FMNH$_2$/TiO$_2$(e$_{CB}^-$) was monitored using GC/MS by analyzing the loss in $cis$-DCE, TCE or PCE concentration. Figure 4.14 shows a plot of the decrease in corresponding chlorinated ethylene concentration (% mol/L) over time.
Figure 4.14 Degradation profile of cis-DCE, TCE and PCE by FMNH$_2$/TiO$_2$(e$_{CB}$) at room temperature.

GC/MS analysis of the reaction solution showed consistent decrease in chlorinated ethylene concentration, however, there was no evidence for the formation of any products in the liquid phase. Halogenated intermediates, such as cis-, or trans-dichloroethylene (DCE), (which are typical products formed when zero-valent iron is used to reduce TCE or PCE), in this case were not detected, during the course of TCE or PCE degradation. This prompted head-space analysis, which showed the presence of a mixture of gases including ethylene. The lack of formation of cis-DCE or trans-DCE when TCE and PCE are reacted with FMNH$_2$ is in agreement with that of other catalysts that have been used for TCE and PCE dechlorination via a two-electron transfer reduction pathway. Furthermore, the results show that FMNH$_2$ is an effective catalyst for the degradation of chlorinated ethylenes, and its reactivity can be
controlled by immobilizing it on semiconductor surfaces. Current studies in our group are focused on elucidating the products formed during each reaction, which will enable a clearer elucidation on the mechanism of the two-electron reduction of chlorinated ethylenes by similar biologically relevant molecules.

4.4 Conclusions

Reduced flavin mononucleotide (FMNH₂) has been shown to react effectively with the chlorinated ethylenes cis-DCE, TCE and PCE. The reactivity of FMNH₂ could be controlled by immobilizing it on mesoporous nanocrystalline (anatase) TiO₂ to form FMNH₂/TiO₂, or even a photoreduced TiO₂ surface to form FMNH₂/TiO₂(e⁻ CB). Reduction rate constants for the chlorinated ethylenes with FMNH₂, FMNH₂/TiO₂ and FMNH₂/TiO₂(e⁻ CB) were measured. In each case the reduction rate constant was measured to be one order of magnitude higher than FMNH₂/TiO₂, and two orders of magnitude higher than FMNH₂ in fluid solution. The enhanced reactivity can be attributed to shifts in the FMN reduction potentials upon surface binding. In conclusion, we have shown that simple biological molecules such as FMNH₂ that deliver multiple electrons are effective in the reduction of chlorinated ethylenes and therefore, have significant potential for environmental remediation applications.
4.5 References


CHAPTER V

MODULATING THE OXIDATION OF PHOTO-REDUCED FLAVIN MONONUCLEOTIDE BY ORGANOPHOSPHORUS COMPOUNDS

5.1 Introduction

Organophosphorus compounds have become one of the most widely used insecticides available today and are also amongst the most dangerous chemical warfare agents. Organophosphorus pesticides (OPP) are some of the most useful and diverse in use for almost five decades. They are widely used in agriculture to protect insects for crops and animals. They were used as an alternative to organochlorine pesticides (which are persistent and accumulate in the environment) for their relatively rapid decomposition and low accumulation in biological food chains,[1] because they are relatively easier to degrade via microbial or environmental processes. Organophosphorus compounds can accumulate in soil and aquatic organisms under conditions of low temperature, high acidity, and reduced natural light.[2,3]

Unfortunately, OP compounds are highly toxic and in some cases their degradation products (degraded via microbial or environmental processes) have the potential to be more toxic with chronic exposure.[4] When most OP compounds are used for agricultural purposes, however, only about 1% reaches its target, while the rest enters the environment. Frequent use of OP in agricultural lands has resulted in their presence as residuals in crops, livestock, and poultry products and has further
led to their migration into aquifers. Typical pesticide concentrations that flow into aqueous waste range from 1 – 10,000 ppm. While many OP pesticides can degrade via microbial or environmental processes, some of the pesticides could be consumed by organisms, or they could leach into ground water. In ground water that has little sunlight exposure, the rate of degradation of OP pesticides is low. Thus these pesticides pose potential risks to human health. OP compounds are efficiently absorbed by inhalation, ingestion, and skin penetration and are powerful inhibitors of esterase enzymes, such as acetyl- and butyryl-cholinesterase, or neurotoxic esterase, which affect nerve function.

The structure of OP pesticides is similar to that of chemical nerve agents. These chemicals act by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of both humans and insects. The agents are cholinesterase inhibitor, and thus lead to death. The most common difference between the structure of OP pesticides and that of chemical warfare agents is that the former consists of the less toxic thion (P=S) form, while the latter consists of a more toxic oxon (P=O) form. The oxon form is three orders of magnitude more potent as an inhibitor of acetylcholinesterase. Although the oxon forms of the OP pesticides have greater toxicity, they are less stable and are easily degraded via hydrolysis of one or more of the phosphate ester bonds. This is not the case with thion OP pesticides; they are relatively stable, have a long shelf-life and are easily taken up by organisms including humans. The mechanism of action of the thion OP compounds with organisms is to undergo in vivo transformation reactions using oxidative enzymes to convert the thion form (P=S) to the more potent oxon form (P=O). It has been reported that the thion forms if inhaled or ingested by an organism, are converted via enzymes to the oxon forms.
Due to the toxicity of OP pesticides, it is necessary to remove them from the environment. In this regard a number of methods including the use of surfactants [10,11], iron porphyrins [12], microbial pathways [13-15], and solar irradiation [16], for OP compound degradation have been reported in the literature. Reports using metals [17], or photocatalysts consisting of titanium dioxide [18-20] have been found to be relatively effective.

Research found that similar degradation pathways are followed by all the structures, apart from the nature of the substituents. A major concern is that many of the above described processes involve oxidation pathways for degradation of OP pesticides, which in some cases leads to a number of toxic oxon by-products [21-24]. Surprisingly, not much has been done in terms of developing materials that react with OP pesticides via a reduction pathway. The development of such materials is timely and will open new avenues that could reduce OP exposure.

In this chapter, we report the ability to carefully tune the reactivity of flavin mononucleotide (FMN) to enhance its reactivity with organophosphorus (OP) compounds through the reduction process.

Flavin mononucleotide (FMN), or riboflavin-5'-phosphate, is produced from riboflavin (vitamin B2) by the enzyme riboflavin kinase and functions as prosthetic group of various oxidoreductases including NADH dehydrogenase (Scheme 5.1). Riboflavin and its derivatives have been used as biocatalysts [25-29], but remain largely unexplored for environmental applications. As mentioned in the previous chapter, FMN stores two electrons, and possesses a functional group to anchoring on the surface of nanocrystalline TiO₂.

Here we use a hybrid catalyst consisting of riboflavin 5'-phosphate sodium (flavin mononucleotide (FMN)) anchored to nanocrystalline titanium dioxide (TiO₂)
particles assembled as shown in Figure 5.1. The described system can be modulated to have multiple electrons because FMN can be reduced to FMNH₂ thus carrying two electrons, and TiO₂ photo-reduction under appropriate conditions leads to electrons being trapped in the TiO₂ conduction band. Systems that deliver multiple electrons to a substrate, for example organophosphorus pesticide, are advantageous because they allow reactions to be carried out under mild conditions.

Scheme 5.1 Structure of flavin mononucleotide (FMN).

Figure 5.1 Schematic representation of the attachment of FMN to TiO₂, and the electron transfer during photolysis that leads to electron accumulation (prior to reactivity with the OP pesticides).
We have investigated the effects of the hybrid catalysts toward the reduction of the organophosphorus pesticides, fenthion (O,O-dimethyl O-4-methylthio-m-tolyl phosphorothioate), ethion (O,O,O',O'-tetraethyl S,S'-methylene bis( phosphorodithioate) and the chemical warfare agent mimic diethylchlorophosphate (DCP).

5.1.1 Fenthion

![Scheme 5.2 Structure of fenthion.](image)

Fenthion is a widely used insecticide, especially in orchards, and is frequently found in environmental samples.[30,31] Its primary degradation products include fenthion sulfone, fenthion sulfoxide, fenoxon, fenoxon sulfoxide, and fenoxon sulfone, with the effects of environment and microorganisms.[32,33] Fenthion has been classified in toxicity class II (Table 1.1 in Chapter 1). While it is an effective insecticide, it is also moderately toxic to mammals, but highly toxic to birds.

5.1.2 Ethion

![Scheme 5.3 Structure of ethion.](image)
Ethion is common insecticides, used to on citrus trees, fruit trees, cotton and some vegetables.\[34\] It may be used on a wide variety of food, fiber, and ornamental crops, including greenhouse crops, lawns, and turf. The possible degradation of ethion products include ethion monoxon, ethion dioxon, O,O-diethylthiophosphate and thio-formaldehyde.\[35,36\] Ethion has been classified in toxicity class II.

5.1.3 Diethylchlorophosphate (DCP)

![Scheme 5.4 Structure of diethylchlorophosphate (DCP).](image)

Some of the most dangerous chemical warfare agents (as shown in Scheme 1.3 Chapter 1) are organophosphorus compounds. Due to their extremely high toxicity most studies are performed on less toxic models, which mimic the most important molecular features of the analytical targets, such as diethylchlorophosphate (DCP). These organophosphates have been widely used as simulants as they display a similar reactivity that nerve agents, yet they lack their toxicity compare with typical nerve agents.

5.2 Experimental Section

5.2.1 Materials and Methods

HPLC grade methanol (MeOH), titanium (IV) isopropoxide, fenthion, riboflavin and riboflavin 5’-phosphate sodium (flavin mononucleotide (FMN)) were
purchased from Aldrich Chemicals and used as received. Nitric acid was obtained from Fisher Scientific and used as received. Indium doped tin oxide (ITO) coated sheets of glass were purchased from Hartford Glass Company, Inc. Deionized Milli-Q water at a pH of 7 was used where aqueous measurements are described. Colloidal TiO$_2$ nanoparticles were imaged using a JEOL scanning electron microscope (SEM). A custom-designed quartz cuvette with a 1 cm path length was used as the spectroelectrochemical cell.

5.2.2 Instrumentation

UV-visible absorption spectra were acquired using a Varian Cary 50 spectrophotometer. Fluorescence measurements were acquired using Varian Cary Eclipse spectrofluorometer. Irradiations of FMN solutions or FMNH/TiO$_2$ slides in either methanol or water, were degassed and carried out using a solar simulator equipped with 400W Xenon lamp and a KV-370 filter purchased from Newport, Inc. (Spectra-Physics, Stratford CT). Certain solutions were placed in a standard 10 mm x 10 mm quartz cuvette, sealed with a rubber septum, and purged with ultra high pure compressed N$_2$ (Airgas, Inc). In each case, samples were irradiated for 30 min under a N$_2$ atmosphere. Irradiation was continued until the sample showed no fluorescence under UV light, thus indicating complete reduction.

5.2.3 Nanocrystalline TiO$_2$ Film Preparation and Functionalization

Transparent TiO$_2$ films consisting of ~12 nm diameter anatase particles were prepared by the hydrolysis of Ti(iOPr)$_4$ using a sol-gel technique as shown in Figure 5.2. The TiO$_2$ paste was cast as mesoporous thin (~10 µm) films onto microscope glass slides. The attachment of FMN to the TiO$_2$ surface was achieved by soaking
freshly prepared TiO₂ films for 6 hours in a 1 x 10⁻⁴ M FMN solution in MeOH. Once FMN was anchored, it remained strongly bound to the surface in both methanol and aqueous solutions. The nanocrystalline thin films were placed diagonally in a standard quartz cuvette. Absorption spectra and steady-state kinetic measurements were acquired using a Cary 50 UV-visible spectrophotometer. Irradiations of FMN/TiO₂ films or FMN in solution were carried out using a 400-W Xe lamp with a KV 370 filter. In each case, samples were illuminated for 30 min.

Figure 5.2 SEM image of nanocrystalline titanium dioxide (TiO₂) nanoparticles synthesized via a sol-gel method. Average particle diameter is 12 nm. Scale bar = 100 nm.

5.2.4 Spectroelectrochemistry/Electrochemistry

Spectroelectrochemical measurements were conducted on a CV 50W potentiostat to apply the desired potentials in a standard three-electrode arrangement with a Pt counter electrode, Ag/AgCl (3 M KCl) reference electrode, and ITO/TiO₂/FMN as a working electrode on alligator clips. A fresh solution of 0.1 M
TBAPF$_6$ in acetonitrile was used as the supporting electrolyte. A Varian Cary 50 UV-visible absorbance spectrophotometer was used to measure absorbance spectra. Each potential was held until the UV-visible absorbance spectrum became time-independent, and steady state concentrations were assumed.

5.2.5 Monitoring FMNH$_2$ Oxidation by OP Compounds

A Varian Cary 50 UV-visible spectrophotometer was used to acquire absorbance vs. time profiles for the reactions of photo-generated FMNH$_2$ with OP compounds. In each case the oxidation of FMNH$_2$ to FMN was monitored via the appearance of a 445 nm absorbance peak corresponding to FMN.

5.2.6 GC-MS Analysis of OP Compound Reduction

Loss in OP compound concentration following their reduction by the catalyst FMNH$_2$/TiO$_2$(e$^{-}\text{CB}$), were analyzed by GC/MS. An aliquot (1 µL) of sample in methanol solution was analyzed using a HP 6890 Series GC System (Hewlett Packard), equipped with a HP 5973 mass selective detector. The sample was kept in a N$_2$ atmosphere to avoid reactivity with oxygen. The column used was a capillary column (30.0 m x 250 µm x 0.25 µm nominal) purchased from SGE Forte. The parameters for the front inlet were set as follows: splitless mode using He carrier gas, heater at 250 °C, 10.5 psi. The GC oven was initially set at 50 °C for 3 min, heated at 10 °C/min to 200 °C and kept for 3 min.

5.3 Results and Discussion

Flavin mononucleotide is a well-known redox system with significant
potential for several environmental remediation processes, though it has not been extensively exploited for such applications. The photo-reduction of flavin mononucleotide (FMN) to form FMNH• or the well-known two-electron two-photon FMNH₂ [37,38] is shown in Figure 5.3.

The UV-visible absorbance spectrum of FMN in aqueous solution shows two peaks at 375 nm and 445 nm, while in MeOH the peaks are at 355 nm and 445 nm. In aqueous solution at pH = 7, the molar absorptivities of FMN was calculated to be 11,000 ± 125 M⁻¹ cm⁻¹ at 375 nm and 11,800 ± 134 M⁻¹ cm⁻¹ at 445 nm. In MeOH, the molar absorptivities of FMN was calculated to be 7,485 ± 85 M⁻¹ cm⁻¹ at 355 nm and 10,400 ± 220 M⁻¹ cm⁻¹ at 445 nm. The emission spectrum of FMN shows a peak at 525 nm in both aqueous solution or in MeOH.

Figure 5.3 Chemical structures of FMN, FMNH• and FMNH₂.
5.3.1 Photoreduction of FMN in Aqueous Solution to Form FMNH·

In aqueous solution and under a N₂ saturated atmosphere, the photo-reduction of FMN resulted in the formation of a semiquinone radical FMNH·. The UV-visible absorbance spectrum of FMNH· is blue shifted relative to the FMN spectrum and shows two peaks at 345 nm and 400 nm (Figure 5.4a).

![Absorption spectra](image)

**Figure 5.4** (a) UV-visible absorption spectra of FMN before and after irradiation in aqueous solution showing the conversion of FMN → FMNH·, and (b) UV-visible absorption spectra of FMN before and after irradiation in methanol solution showing the conversion of FMN → FMNH₂.

This data are consistent with literature values.[38-40] The oxidation of FMNH· using the OP compounds fenthion, ethion or DCP was investigated. In each case a 1 x 10⁻⁴ M solution of FMNH· under a N₂ saturated atmosphere was used and excess OP compound was added. In each case, no oxidation of FMNH· to FMN was observed after several days of stirring. This observation indicated that the OP compounds investigated were not strong enough to oxidize FMNH· to FMN in aqueous solution.
5.3.2 FMNH$_2$ in Solution and its Oxidation by OP Compounds

The photo-reduction of FMN in a MeOH solution resulted in the formation of FMNH$_2$. FMNH$_2$ formation in was confirmed by the shifts in UV-visible absorption peaks from 355 nm and 445 nm to a single peak centered at 320 nm (Figure 5.4b), which is consistent with literature reports. [38,39]

A solution of FMNH$_2$ in MeOH was stable in a N$_2$ saturated atmosphere for over a week. While FMNH$^\cdot$ was found to be unreactive with fenthion, ethion and DCP, we investigated their reactivity with FMNH$_2$, a two-electron two-proton species. For each experiment, a 1 x 10$^{-4}$ M solution of FMNH$_2$ was prepared under a N$_2$ saturated atmosphere and addition of excess OP compound was found to result in a rapid shift in the UV-visible absorbance spectrum from 320 nm to form two peaks at 355 nm and 445 nm corresponding to the oxidation of FMNH$_2$ $\rightarrow$ FMN. The results indicate that the two-electron species FMNH$_2$ is oxidized at a greater rate with the OP compounds investigated, relative to FMNH$^\cdot$. These results are surprising since most radical species such as FMNH$^\cdot$ are expected to be quite reactive and could react easily with OP compounds. However, when FMNH$_2$ is reacted with one equivalent or more of OP compounds, it was found to be oxidized back to FMN, indicating reduction of the OP compounds (Figure 5.5).

In addition, the observation suggests that the reaction pathway between FMNH$_2$ and the OP pesticides may not follow a one-electron transfer process as is expected if the reductant was FMNH$^\cdot$, but may rather follow a two-electron transfer mechanism. Indeed Roberts [41] has shown through theoretical calculations, at least in the case of some organohalides, that the two-electron transfer reaction occurs through a much more favorable thermodynamic pathway compared to the one-electron transfer process. The loss of two-electron in the oxidation of FMNH$_2$ $\rightarrow$
FMN rather than FMNH$_2$ → FMNH$^*$ is evidence for a two-electron transfer pathway. Thus, the difference in reactivity of fenthion with the two photo-reduced species FMNH$^*$ and FMNH$_2$ is an important step toward recognizing efficient materials with potential to degrade OP compounds.

![Figure 5.5](image_url)

**Figure 5.5** UV-visible absorbance spectra of FMN in methanol solution (a) before irradiation, (b) after irradiation, and (c) after addition of OP pesticides in the *dark*.

### 5.3.3 FMNH$_2$ Oxidation at TiO$_2$ Interfaces in Aqueous Solution

The inability to generate FMNH$_2$ in aqueous solution provides a limitation for future environmental applications. One way to overcome this limitation is to anchor FMN to a surface that will provide an electron source to drive its reduction to FMNH$_2$. An added advantage to the attachment of FMN and other molecular catalysts
to solid supports is that they provide a heterogeneous system thus allowing catalyst recovery. Such approaches are especially attractive for environmental remediation applications as they avoid the introduction of catalysts directly into the environment. A microscope slide coated with transparent nanocrystalline TiO$_2$ was immersed in a methanol solution of $1 \times 10^{-4}$ M FMN. After about 6 hours of TiO$_2$ film immersion in FMN, the films were yellow corresponding to FMN immobilization and had an absorbance peak at 445 nm in the UV-visible spectrum. (We note that the typical second absorbance peak of FMN at 355 nm in this case was not observed because it overlapped with the TiO$_2$ band gap (3.2 eV), (for example, see corresponding spectra in Figure 5.6). The FMN remained on the TiO$_2$ surface when the slides were immobilized in either aqueous solutions or methanol solvent. The surface coverage of FMN on the TiO$_2$ surface was $1 \times 10^{-9}$ mol/cm$^2$, and the binding constant was calculated to be $2.3 \times 10^7$ M$^{-1}$. The calculation of surface coverage is mentioned in Chapter 4. Comparative studies with TiO$_2$ coated glass slides immersed in a solution of riboflavin (which does not possess the phosphonic acid functional groups) showed no evidence of adsorbed riboflavin on the TiO$_2$ surface. This observation can be explained by the fact that the binding of FMN to the mesoporous nanocrystalline (anatase) TiO$_2$ thin films occurs through the phosphonic acid functional groups. Phosphonic acid groups are well-known to bind covalently to metal oxides. [42,43]

Further characterization of the FMN bound onto nanocrystalline TiO$_2$ was conducted to investigate and characterize how the properties of FMN anchored to the surfaces influenced various properties including electrochemical, optical, etc. Since the reactivity of photo-reduced FMN with OP compounds depends on its electrochemical properties, formal reduction potentials ($E^0$) for FMN were measured in fluid solution and compared to the formal reduction potential when anchored onto
TiO₂ surfaces. Cyclic voltammetry was used to measure formal potentials in TBAPF₆/CH₃CN/H₂O electrolyte. The FMN redox chemistry showed equivalent anodic and cathodic peak currents, $i_{pa}/i_{pc} \sim 1$ (Nernstian behavior). The FMN/FMNH₂ formal potentials were measured to be $-570$ mV vs. Ag/AgCl (3M KCl) in 0.1 M TBAPF₆/CH₃CN/H₂O electrolyte.

Figure 5.6 Representative spectroelectrochemical data of molecular catalyst FMN bound to TiO₂. FMN was reduced to FMNH₂ in CH₃CN/TBAPF₆ electrolyte upon applying negative potential. From top to bottom the applied potentials are -500 mV, -525 mV, -550 mV, -600 mV, -650 mV, -700 mV, -750 mV, -800 mV, -850 mV, -900 mV, -950 mV, -1000 mV. The potential applied were measured against Ag/AgCl (3 M KCl).

Measuring accurate FMN reduction potentials upon surface binding to TiO₂ using cyclic voltammetry was not feasible due to the presence of the TiO₂ reduction peak which overlaps with the FMN reduction peak. An alternative route to measure the formal reduction potential of FMN when anchored to the TiO₂ surface is by
spectroelectrochemistry. The UV-visible absorbance changes due to an applied voltage are shown in Figure 5.6.

The formal reduction potentials correspond to the equilibrium potentials where the concentration of reduced and oxidized compounds was equal (Figure 5.7). The results of the spectroelectrochemical measurements show that surface binding has an intense effect on the formal reduction potentials and their subsequent reactivities with analytes of interest. The formal potential of FMN bound to TiO$_2$ was calculated to be $-775$ mV vs. Ag/AgCl (3 M KCl), whereas the formal potential of FMN alone $\sim570$ mV vs. Ag/AgCl (3 M KCl). A significant shift in the formal reduction potential of FMN was observed upon surface binding, such that FMN bound to TiO$_2$ was always a stronger reductant than FMN in fluid solution. This shift in reduction potential upon surface binding is a phenomenon that is not well-understood but is thought to result due to electrostatic interaction and the presence of hydroxyl groups on the surface that affect the formal reduction potential. This shift in reduction potential should have a profound influence on the reactivity of photo-reduced FMN on the TiO$_2$ surface.

Figure 5.7 Plot of FMN/FMNH$_2$ concentrations vs. applied potential.
To investigate this effect, a flavin anchored TiO₂ slide was irradiated for 30 minutes in a N₂ saturated aqueous or methanol solutions. In an aqueous solution (Figure 5.8), the photolysis of FMN anchored to TiO₂ in nitrogen-saturated aqueous solutions resulted in a color change of the film from yellow to colorless, indicating reduction of FMN to FMNH₂. Prolonged irradiation following FMN → FMNH₂ in aqueous solution did not yield the well-known absorption of reduced TiO₂, and no further absorption changes were observed, suggesting TiO₂ electron-hole recombination (Figure 5.8).

![UV-Visible absorbance spectra of FMN anchored on TiO₂ surface in aqueous solution, (a) before photolysis, (b) after photolysis, and (c) after addition of OP pesticides in the dark.](image)

**Figure 5.8** UV-Visible absorbance spectra of FMN anchored on TiO₂ surface in aqueous solution, (a) before photolysis, (b) after photolysis, and (c) after addition of OP pesticides in the dark.

Each of the OP pesticides fenthion, ethion, and DCP (in concentration ranging from 1 equivalent of FMN to excess OP) was added to the FMNH₂ anchored to TiO₂ in a nitrogen-saturated solution. In each case, within a few minutes, the color of the
film slowly changed from colorless to yellow indicating $\text{FMNH}_2 \rightarrow \text{FMN}$ oxidation by the OP compounds. The results are important as they show the reduction of organophosphorus compounds in aqueous solution. Current studies are focused on identifying the reaction products.

5.3.4 FMNH$_2$/TiO$_2$ Oxidation by OP Compounds in Methanol Solution

We further investigated the effect of combining the TiO$_2$ conduction band electrons with FMNH$_2$ toward the reactivity of the OP compounds ethion, fenthion and DCP. The presence of a net two electrons from FMNH$_2$ as well as the TiO$_2$ conduction band electrons were modulated to react with each of the OP compounds. In MeOH solution, TiO$_2$ band gap excitation resulted in the reduction of FMN to FMNH$_2$ while electrons were trapped in the TiO$_2$ conduction band. The presence of both FMNH$_2$ and TiO$_2(e^-)$ was confirmed by UV-visible absorbance measurements taken before and after photolysis as shown in Figure 5.9, spectra (a) and (b), respectively.

The 355 nm peak of FMN lies within the TiO$_2$ band gap of 3.2 eV and therefore was not observed. However, the 445 nm peak of FMN disappeared after photolysis indicating reduction of FMN to FMNH$_2$. In addition, an increase in absorbance intensity in the 500-800 nm regions occurred, indicating trapped electrons in the TiO$_2$ conduction band. Both reduced equivalents, FMNH$_2$ and the TiO$_2(e^-)$ were stable under a nitrogen atmosphere at room temperature for over 24 hours.
Figure 5.9 UV-visible absorbance spectra of (a) FMN anchored to TiO₂ in MeOH before photolysis, (b) after photolysis, here loss of the peak at 445 nm indicates reduction of FMN to form FMNH₂, while the increase in absorbance in the 500 – 800 nm region is due to electron trapped in the TiO₂ conduction band.

Varying aliquot concentration of fenthion, ethion or DCP (ranging from 1 equivalent of FMNH₂ to excess OP) were added to FMNH₂/TiO₂(e⁻)(MeOH) system. In each case OP addition resulted in first, the loss of trapped electrons as shown by the decrease in absorbance intensity in the 500 – 800 nm regions and second, the oxidation of FMNH₂ to FMN as shown by the growth of the peak at 445 nm as shown in Figure 5.10.

The absorbance vs. time profiles (Figure 5.11) showed depletion of the TiO₂ conduction band electrons, followed by oxidation of FMNH₂ to FMN. These results suggest one of two possible mechanisms: either (1) the reduction of OP compounds
occurs via the FMNH$_2$ reduced equivalents and in this case, as FMNH$_2$ → FMN, the 
TiO$_2$ conduction band electrons continue to reduce the FMN → FMNH$_2$ so FMN is 
not observed until all the TiO$_2$(e$^-$) is depleted, or (2) both TiO$_2$(e$^-$) and FMNH$_2$ reduce 
the OP compounds. We have already confirmed that FMNH$_2$ in fluid MeOH solution 
is capable of reducing OP compounds.

Figure 5.10 UV-visible absorbance spectra of (I) FMN anchored to TiO$_2$ in MeOH 
before photolysis, (II) after photolysis. After addition of OP compound 
in this specific example the data corresponds to fenthion ~ 1 
equivalents, but the trend is similar with the other OP compounds 
investigated) (III) 15 min, (IV) 30 min, (V) 50 min, (VI) 1hr, 10 min, 
and (VII) 3 hrs.
To investigate whether TiO$_2$(e$^-$) are effective OP compound reductants, we irradiated (non-functionalized) TiO$_2$ films in a N$_2$ saturated MeOH solution. After 30 minutes of irradiation we observed an increase in the 400 – 800 nm absorbance intensity accompanied by blue coloration of the film, indicating that conduction band electrons were trapped. Addition of either ethion, fenthion or DCP in the dark resulted in the loss of trapped electrons. However, quantification of the reaction proved difficult due to the possible binding of OP compounds to TiO$_2$ surfaces. It is well known that phosphates have a tendency to bind to metal oxide surfaces such as TiO$_2$.[42,43] To assess whether the OP compounds investigated in this study could bind to the TiO$_2$ surface, we prepared known concentrations of either ethion, fenthion, or DCP. We immersed a TiO$_2$ slide in each of the solutions, and after 24 hours removed the slide and measured the concentration of each of the OP compounds. In each case, there is loss in the concentration of the OP compound...
(assessed by UV-visible absorbance or GC-MS analysis of the solution after the TiO$_2$ slide was removed), which suggests that some of the compound may be adsorbed to the TiO$_2$ surface. On the other hand, comparable experiments conducted with FMN functionalized TiO$_2$ showed no significant changes in the concentration of any of the OP pesticides.

The observed reactivity of TiO$_2(e^-)$ with OP compounds thus suggests that the second mechanism is most plausible especially if we assume that the TiO$_2(e^-)$ can not only be used to reduce FMN $\rightarrow$ FMNH$_2$ but also be directly transferred to the OP compounds. Given these observations we measured the reduction rates of OP compounds with the FMNH$_2$(MeOH) in fluid solution and compared the results to FMNH$_2$/TiO$_2(e^-)(MeOH)$ using the relationship $\ln A = \ln (A_0) - kt$. Second-order rate constants were abstracted for plots of $k_{obs}$ vs. [OP] for ethion, fenthion and DCP. The results were compared to the reduction rate constants obtained from FMNH$_2$ in methanol solution (in the absence of TiO$_2$) (Table 5.1).

**Table 5.1** Rate constants for OP compound reduction by FMNH$_2$ or FMNH$_2$/TiO$_2(e^-)(CB)$. All measurements were made in room temperature. The concentration of FMNH$_2$ was constant in both cases.

<table>
<thead>
<tr>
<th>OPs</th>
<th>FMNH$_2$(MeOH) (M$^{-1}$ s$^{-1}$)</th>
<th>FMNH$_2$/TiO$_2(e^-)(MeOH)$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenthion</td>
<td>$(2.0 \pm 0.1) \times 10^{-3}$</td>
<td>$2.1 \pm 0.1$</td>
</tr>
<tr>
<td>Ethion</td>
<td>$(1.3 \pm 0.3) \times 10^{-3}$</td>
<td>$4.5 \pm 0.1$</td>
</tr>
<tr>
<td>DCP</td>
<td>$(7.5 \pm 0.5) \times 10^{-3}$</td>
<td>$12.6 \pm 0.1$</td>
</tr>
</tbody>
</table>

In each case, it was observed that the presence of TiO$_2(e^-)$ in the reaction plays a significant role by increasing the reduction rate constant by two orders of
magnitude, indicating that multiple electrons are responsible for the enhanced reactivity. At the end of each of the reactions shown, i.e. oxidation of FMNH$_2$ by OP compounds starting with FMNH$_2$/TiO$_2$($e^-$)$_{(MeOH)}$, the OP compound was completely consumed (Figure 5.12) suggesting a potentially strong and effective approach for OP degradation under mild conditions. Furthermore, we note that FMN coated TiO$_2$ slides could be recycled up to eight times with no measurable loss in catalytic activity.

![Figure 5.12](image)

**Figure 5.12** The degradation profiles of the OP compounds plotted as percentage of OP compound vs. time during the reaction with FMNH$_2$/TiO$_2$($e^-$)$_{(MeOH)}$. 
5.4 Conclusions

In conclusion, we have shown that FMN anchored to nanocrystalline TiO$_2$ can be photo-reduced to FMNH$_2$ in aqueous solution or methanol solvent. The photoreduction process bears similarities in mechanism to that of other coordination complexes anchored on TiO$_2$ surfaces [44,45]. The photoreduction process of FMN anchored to TiO$_2$ in methanol solvent further results in TiO$_2$ conduction band electron build up accompanied by FMNH$_2$, thus providing multiple redox equivalents. The oxidation of photogenerated FMNH$_2$ by fenthion, ethion and DCP to FMN represents an important step toward the ability to degrade these environmental pollutants under mild condition, in comparison to other methods.[10,11]

It is expected that the enhanced reactivity obtained by tuning the FMN environment could lead to important systems toward the environmental remediation of various pollutants. Current investigations are focused on identifying the OP compounds degradation products under the various reduction conditions. In general, the ability to design catalysts that are tunable and can degrade organophosphorus compounds in aqueous solution and at ambient temperatures opens exciting opportunities toward environmental remediation applications.
5.5 References


APPENDIX A

Acronym Glossary
AChE  Acetylcholinesterase
B3LYP  Becke 3 term, Lee Yang, Parr (Density Functional theory method)
BChE  Butyrylcholinesterase
BDPPZ  Benzo[i]dipyrido[3,2-a:2'-3'-c]phenazine
CV  Cyclic Voltammetry
DCE  Dichloroethylene
DFT  Density Functional Theory
DM-BDPPZ  3,6-dimethyl-benzo[i]dipyrido-[3,2-a:2'3'-c]phenazine
DMSO  Dimethyl-sulfoxide
DPPZ  Dipyrido[3,2-a:2,3-c]phenazine
DPV  Differential Pulse Voltammetry
EPA  Environmental Protection Agency
EtOH  Ethanol
FAD  Flavin Adenine Dinucleotide
FMN  Flavin Mononucleotide
IARC  International Agency for Research on Cancer
ITO  Indium Doped Tin Oxide
MeOH  Methanol
NTE  Neuropathy Target Esterase
OP  Organophosphorus
OPP  Organophosphorus Pesticides
PCE  Tetrachloroethylene
SEM  Scanning Electron Microscope
TATPP  9,11,20,22-tetraaza-tetrapyridopentacene
TBAPF₆  Tetrabutylammonium hexafluorophosphate
<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>TCE</td>
<td>Trichloroethylene</td>
</tr>
<tr>
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<td>Vinyl Chloride</td>
</tr>
<tr>
<td>VOCs</td>
<td>Volatile Organic Compounds</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
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