Optical and Electrochemical Molecular and Nanoscale Sensors for the Selective Detection of Organophosphorus Based Pesticides

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OPTICAL AND ELECTROCHEMICAL MOLECULAR AND NANOSCALE SENSORS FOR THE SELECTIVE DETECTION OF ORGANOPHOSPHORUS BASED PESTICIDES

by

Chandrima De

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Science
Department of Chemistry

Western Michigan University
Kalamazoo, Michigan
August 2008
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I would like to take this opportunity to express my gratitude to individuals for their help, support and motivation for the successful completion of this research.

Honestly, I do not have words to express my gratitude to my research advisor, mentor, and chair of my committee, Dr. Sherine Obare for all her guidance, support, expertise, and encouragement during my entire stay in Western Michigan University. She has been the constant source of inspiration throughout my project work. Her commitment to education and research, in addition to her emphasis on strong personality development are deeply appreciated.

I would also like to thank my other research committee members, Dr. Herb Fynewever, and Dr. Yirong Mo for their significant contribution in my graduate research, and invaluable advices towards the completion of this project. I am highly grateful to all the members of the Obare research group at Western Michigan University (WMU) and University of North Carolina at Charlotte (UNCC) for their enjoyable company, constant support and encouragement. Specifically, I would like to thank Christopher Ciptadjaya, Ruel Freemantle, and Wen Guo for their help with my experiments and making my stay in graduate school as a memorable experience. In addition, I am grateful to Dr. Sherine Obare for the financial support during my graduate studies.
Acknowledgements-Continued

I am thankful to all the faculty, graduate students, and staff of the Department of chemistry at Western Michigan University for their help, encouragement, and constant support during my entire graduate work. The Department of Chemistry is highly acknowledged for giving me an opportunity to continue my higher education and financial assistance to fulfill my childhood dreams.

Last but not the least; I would like to express my gratefulness to my entire family for always supporting me and believing in me. My mother, Mrs. Debjani Sen, father, Mr. Sabyasachi Sen, sister, Miss Debleena Sen, and grandmother, Mrs. Provaboti Sen has been a source of inspiration, encouragement, and motivation in my life. I would like to convey heartfelt thanks to my husband, Dr. Sinjan De, mother-in-law, Mrs. Lakhshmi De, and father-in-law, Mr. Ajit Kumar De for their understanding, and encouragement to pursue my graduate degree. I am highly grateful to all my family members for their patience, eternal support, and above all believing in me. Without their support in every aspect of my life, this work would not have been possible.

Chandrima De
OPTICAL AND ELECTROCHEMICAL MOLECULAR AND NANOSCALE SENSORS FOR THE SELECTIVE DETECTION OF ORGANOPHOSPHORUS BASED PESTICIDES

Chandrima De, M.S.
Western Michigan University, 2008

Organophosphorus based pesticides are highly toxic and represent serious environmental concerns. These compounds tend to easily enter ground water and cause drastic pollution. The toxicity levels of the organophosphorus pesticides however are based on their structures, and hence methods for their selective detection are in high demand. The creation of new materials for sensing and actuation requires careful manipulation of the responsive units required to control analyte selectivity. We have successfully designed and synthesized a molecular sensor based on the family of stilbenes that not only can detect organophosphorus pesticides, but also distinguish between them. Four different pesticides have been investigated, namely, ethion, malathion, fenthion, and parathion. The sensors show significant differences in the optical output and electrochemical signal by interacting with the organophosphorus pesticides. The dual signal transduction is advantageous because it minimizes false positives. Apart from molecular sensors, we have also developed a series of gold nanoparticle-based sensors for detection of organophosphorus pesticides. Both the molecular and nanomaterials as sensors operate in real time and have parts-per-million detection limits.
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CHAPTER 1

INTRODUCTION TO ORGANOPHOSPHORUS COMPOUNDS AND THE NEED FOR NOVEL SENSORS FOR THEIR DETECTION

1.1 Sensors for Detection of Organic-Based Environmental Contaminants

Organic chemical contamination has become a worldwide problem. Modern industrialized societies have developed thousands of synthetic organic compounds and volatile organic compounds (e.g., benzene) for several uses. The most important ones include plastics, lubricants, refrigerants, fuels, solvents, preservatives, and pesticides. However, as a result of improper practices, these contaminants enter the environment and cause air, water, and soil pollution. For instance, pesticides and herbicides are applied directly to plants and soils, and incidental releases of other contaminants can originate from spills, leaking pipes, underground storage tanks, waste dumps, and waste repositories. Some of these contaminants have long half-lives and thus remain in the environment. They migrate through large regions of soil until they reach water resources, where they may present an ecological or human-health threat. In fact organisms including humans are affected by various chemicals through either inhalation or ingestion that have entered water bodies, volatilized in the air, been taken up by vegetation, or remain in the soil. These contaminants pose serious health hazards. The civilian, commercial, and defense sectors of most advanced industrialized nations as well as developing nations are facing tremendous
environmental concerns due to these various contaminants. Environmental monitoring is required to protect the public and the environment from toxic contaminants and pathogens that can be released into a variety of media including air, soil, and water. The United States Environmental Protection Agency (U.S. EPA) has imposed strict regulations on the concentrations of many environmental contaminants in air and water. However, current monitoring methods are costly and time-intensive, and limitations in sampling and analytical techniques exist. Thus, there is a great demand for development of quick and simple methods for the detection of these compounds. Table 1 lists the names of the 48 most commonly used organophosphorus pesticides. This thesis addresses a class of organic pollutants namely organophosphorus based pesticides. Our goal is to design portable sensors for organophosphorus based pesticides that can provide rapid responses (relative to current methods and technologies), ease of operation (for field use), and sufficient detection limits.
Compound  | Compound
---|---
Azinphos-Me | Diazoxon
Carbophenothion | Dichlorvos
Dimethoate | Dicrotophos
Disulfoton | Mevinphos
Malathion | Monocrotophos
Phorate | Nalid
Phosalone | Phosphamidon
Phosmet | Tetrachlorvinphos
Terbufos | Demeton-S
Thiometon | Oxydemeton-Me
Chlorpyrifos | Acephate
Chlorpyrifos-Me | EPN
Demeton-O | Ethephon
Diazinon | Ethoprop
Fenitrothion | Fenamiphos
Fensulfothion | Fonofos
Fenthion | Fosamine Ammonium
Isazofos | Glyphosate
Methyl Parathion | Metamidphos
Parathion | Profenofos
Pirimiphos-Me | Sulprofos
Pirimiphos-Ethyl | Tribufos
Temephos | Trichlorfon
Tolelofos-Me | Trichloronal

Table 1 List of the 48 widely applied organophosphorus pesticides.

1.2 Organophosphorus (OP) Pesticides

1.2.1 Organophosphorus Pesticides and the Environment

Pesticides are used to describe any chemical that kills or slows down the growth of an undesirable organism. Pesticides include herbicides, insecticides,
fungicides, and nematocides. In order to meet the nutritional needs of an increasing world population, there is no doubt about the necessity of application of various pesticides to crops and animals. It is believed nowadays that application of synthetic pesticides is the most effective method of controlling insects that affect crop growth.\textsuperscript{4} It has been estimated that approximately 40,000 new potentially poisonous products enter the market each year.\textsuperscript{5}

Organophosphorus (OP) pesticides constitute the most widely used insecticides available today. This class of compounds has achieved enormous commercial success as a key component in the arsenal of agricultural pesticides and herbicides, and is currently an integral element of modern agriculture. According to the EPA, about 70\% of the insecticides in current use in the US are organophosphorus pesticides.\textsuperscript{1} They were developed to replace organohalide pesticides in the late 1950's because of the fact that these OPs are relatively easier to degrade via microbial or environmental processes. Unlike organohalide pesticides, the OPs do not bioaccumulate due to their rapid breakdown in the environment. For this reason, they are preferred over organohalides for insecticide/pesticide use. However, frequent use of OP compounds 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.\textsuperscript{6} Typical pesticide concentrations that flow into aqueous waste range from 10,000 to 1 ppm.\textsuperscript{2,7} Unfortunately, although these OP compounds are considered safer than organohalides, they are highly toxic to humans and in some cases their degradation products (degraded via microbial or environmental processes) have the potential to be more toxic with chronic exposure. OP compounds are extremely
dangerous to human health; they are powerful inhibitors of esterase enzymes. They are efficiently absorbed by inhalation, ingestion, and skin penetration. Each year OPs poison thousands of humans across the world, causing hundreds of deaths. In fact, in 1994, an estimated 74,000 children were involved in common household pesticide related poisoning or exposures in the United States.¹

OP pesticides are used to control moths, ants, cockroaches, fleas, termites, fruitflies, caterpillars and ticks, only to name a few. Although these OP pesticides are sprayed in agricultural lands with the aim of killing or slowing down the growth of these undesirable harmful pests and insects, however, only 1% reaches its target,¹ while the rest enters the environment. Pesticides can be influenced by a number of processes once it enters into the environment. While many OP pesticides can degrade via microbial or environmental processes, some of the pesticides could get consumed by organisms, or they could leach into ground water. Once a pesticide reaches the ground water it can persist for considerable periods of time. In ground water, there is little sunlight exposure which slows down the degradation of OP pesticides. Thus these pesticides pose potential risks to human health. Figure 1 shows the possible routes of environmental exposure of organophosphorus pesticides to humans and wildlife.⁸
1.2.2 Structures of Organophosphorus Compounds

Organophosphorus pesticides (OP) are synthetic and are usually esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids. There are over 100 organophosphorus compounds currently in the market, representing a variety of chemical, physical, and biological properties. As the name indicates, all OP pesticides have a central phosphorus atom, with either double bonded oxygen (P=O), or a double bonded sulfur atom (P=S). A P=O pesticide is called as an oxon pesticide, and the P=S is termed as a thion pesticide.
OPs have the following general structure:

\[ R_3\text{-}O\text{-}P\text{-}R_1 \]

\[ R_3\text{-}O\text{-}R_2R \]

The main classes of OPs are:

- **Phosphates** \([R_1, R_2 = O]\);
  
e.g. Chlorfenvinphos, Dichlorovos

- **Thiophoaphates (Phosphorothioates)**
  
  \([R_2 = N, S, \text{ or } O]\);
  
e.g. Fenthion, Parathion, Diazinon

- **Dithiophosphates (Phosphorodithioates)**
  
  \([R_1, R_2 = S]\); e.g. Ethion, Malathion, Disulfoton

- **Phosphonates** \([P\text{-}C\text{ bond}]\);
  
e.g. Trichlorfon

- **Phosphoramides**
  
  [containing NH\(_2\) as R group and O as R\(_1, R_2\)];
  
e.g. Fenamiphos, Isophenphos

**Figure 2** Examples of structures of main classes of OP compounds.

Organophosphorus compounds include a number of nerve gas agents as well as agricultural pesticides and insecticides. As mentioned before, agricultural pesticides
or organophosphorus (OP) compounds constitute the most widely used insecticides available today. Common examples of OP pesticides are shown in Figure 3.

Figure 3 Structures of common organophosphorus pesticides.

1.2.3 Health Hazards from Organophosphorus Pesticides

The organophosphorus pesticides are highly neurotoxic to human body. They are strong inhibitors of cholinesterase enzymes, such as, acetylcholinesterase,
butylcholineesterase, and pseudocholinesterase which are neurotransmitters responsible for nerve transmission. The enzymes are inhibited by binding the OP compound which, upon hydrolysis leaves a stable phosphorylated and largely unreacted enzyme. This inhibition results in the accumulation of acetylcholine at the neuron/neuron and neuron/muscle junctions or synapses. The first indication of insecticidal activity among OP compounds was found in 1930, but the first compound of this type, HETP (an impure mixture containing tetraethylpyrophosphate as the active ingredient) was not used as an agricultural insecticide until 1942.  

![Structure of HETP.](image)

**Figure 4** Structure of HETP.

Since the first introduction of HETP, the number of organophosphorus pesticides has risen to hundreds, and the most toxic ones are shown in table 1.2 with their mammalian toxicity levels.
<table>
<thead>
<tr>
<th>Organophosphorus pesticides</th>
<th>Mammalian toxicity (rat) LD$_{50}$ mgkg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipterex</td>
<td>600</td>
</tr>
<tr>
<td>Tetraethyl pyrophosphate</td>
<td>1.12</td>
</tr>
<tr>
<td>Schradan</td>
<td>20</td>
</tr>
<tr>
<td>Dimefox</td>
<td>7</td>
</tr>
<tr>
<td>Malathion</td>
<td>1000</td>
</tr>
<tr>
<td>Diazinon</td>
<td>100</td>
</tr>
<tr>
<td>Parathion</td>
<td>6</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>3</td>
</tr>
<tr>
<td>Systox</td>
<td>1-30</td>
</tr>
</tbody>
</table>

**Table 2** List of the most toxic OP pesticides with their mammalian toxicities.$^9$

1.2.4 Mechanism of Inhibition of Acetylcholinesterase$^9$

Nerve fibers have an electric potential of about 100 mV across their outer wall, and when a nerve gets stimulated, the sign of this potential is momentarily reversed over a small area of the nerve fiber. This small area of reversed potential now travels rapidly along the nerve fiber until it reaches the nerve endings, where it stimulates the release of acetylcholine. If this nerve fiber is now considered to supply impulses to a muscle, the acetylcholine being produced by stimulation of the nerve diffuses into the muscle surface, and causes a change in electrical potential leading to a contraction. On arriving at the muscle surface, acetylcholinesterase hydrolyses the ester linkages
of acetylcholine which stops the stimulation allowing the muscle to relax. Before another muscle contraction takes place, further nerve stimulation and acetylcholine release is required. However, OP pesticides are anti-cholinesterases which interfere with the process of acetylcholine hydrolysis by deactivating the enzyme. Since the hydrolysis is inhibited, acetylcholine can only leave the area of the muscle by diffusion. On a second stimulation, consequent release of acetylcholine occurs which arrives at the muscle surface where the original acetylcholine is still present. This leads to complete confusion of cause and effect, and the muscle being stimulated undergoes continuous stimulation, a condition named tetanus.\textsuperscript{9} Inhibition of acetylcholinesterase is a progressive process depending not only on the concentration of organophosphate concentration, but also on the time of exposure.\textsuperscript{10} The sequence of symptoms varies with the route of exposure. Inhalation of the vapor shows respiratory symptoms first, while ingestion shows gastrointestinal problems at first.

![Inhibition scheme of acetylcholinesterase (AChE) by organophosphates.](image)

\textbf{Figure 5} (A) Inhibition scheme of acetylcholinesterase (AChE) by organophosphates. (B) Mechanism of inhibition of acetylcholinesterase by OP pesticides.
Symptoms of OP poisoning include rapid twitching of voluntary muscles, followed by paralysis, intense pupil contraction, headache, and abdominal cramps. Affected individuals are reported to have poor mental health and difficulties with memory and concentration. The severity of the symptoms however depends on the degree of acetylcholinesterase inhibition. More severe effects include muscle paralysis which leads to difficulty in breathing, and eventually causes death due to respiratory failure. Because of such adverse effects of these OPs, their rapid detection in the environment, public places, or workplaces and the monitoring of individual exposures to chemical warfare agents have become increasingly important for homeland security and health protection.
The structure of OP pesticides is similar to that of nerve gases, as shown in Figure 6. These nerve agents are also cholinesterase inhibitors and thus lead to death. Recently, there has been a major interest in the detection of nerve agents since both soldiers and civilians are at risk of terrorist attacks especially those that may use nerve gas agents. In 1995, a terrorist group launched an attack using sarin nerve gas against commuters in the Tokyo subway station. This attack caused 12 deaths and over 5000 injuries.\textsuperscript{11} The mechanism of inhibition of cholinesterase enzymes by nerve gas agents is similar with OP pesticides.

![Figure 6 Structures of some Nerve Gases.\textsuperscript{12}](image)

Thus, from both a scientific and societal perspective it is of paramount importance to develop a real-time sensor for OP compounds. However, there is a wide range of toxicity in OP compounds, for example the oxon (P=O) form is three orders of magnitude more toxic than the thion (P=S) form and hence is more potent as
an inhibitor of acetylcholinesterase. But, thion OP pesticides are relatively more stable, have a long shelf-life and are easily taken up by organisms including humans. Ideally, sensors that not only detect OP pesticides, but also selectively differentiate between them are in demand.

1.3 Advances in Detection of Organophosphorus Pesticides

Significant advances toward the development of detection methods for OP pesticides have been reported in the literature. Analysis of OPs in environmental and biological samples is routinely conducted using various analytical techniques, including NMR, gas, liquid, or thin layer chromatography, and mass spectrometry. A variety of approaches have been investigated for sensors, including enzymatic assays, molecular imprinting coupled with luminescence of lanthanides, colorimetric methods, surface acoustic waves, fluorescent organic molecules, gas-chromatography-mass spectrometry, and interferometry. The most common methods of detection of OP pesticides are the chromatographic methods coupled with different detectors and different types of spectroscopy, immunoassay, and enzyme biosensors based on inhibition of cholinesterase activity. These two common methods of detection of OP compounds, namely, enzyme based sensors and chromatography will be discussed below in further detail.
1.3.1 Enzyme Based Detection Methods

To date, a number of sensitive biosensors based on acetylcholinesterase (AChE) or butyryl cholinesterase (BChE) inhibition have been developed and used for OP agent detection.\textsuperscript{17, 29, 32-38} Other enzymes including urease and glucose oxidase have also been used in inhibition-based biosensors for organophosphate neurotoxins.\textsuperscript{32, 33} In general, enzyme-based sensors for the detection of organophosphorus compounds can be broadly categorized into two major classes based on the enzyme employed-(1) acetylcholinesterase (AChE) or (2) organophosphorus hydrolase (OPH).

Hydrolysis of acetylcholine by AChE produces one proton per substrate molecule resulting in an increase in the acidity of the solution. This forms the basis for AChE-based sensors. Rogers et al.\textsuperscript{37} used a pH-sensitive fluorescent dye, consisting of AChE linked to the pH-sensitive fluorescein isothiocyanate (FITC). The enzyme-dye adduct was immobilized on a quartz fiber which was attached to a fluorescence spectrometer. In the absence of an OP compound, the labeled AChE was able to hydrolyze acetylcholine leading to the decrease of pH which resulted in the reduction of the FITC fluorescence intensity due to interruption of the fluorophore’s conjugation upon protonation (Figure 7). However, in the presence of diisopropylfluorophosphate (DFP) and subsequently acetylcholine, it was observed that 90% of the enzyme activity has lost, which was quantified by a less pronounced reduction of the fluorescence intensity. This biosensor was found to be very sensitive (capable of detecting paraoxon in the nM range when exposed to the solution
containing the analyte for ten minutes), and it demonstrated some selectivity toward different OP compounds.

![Diagram of fluorescein isothiocyanate (FITC) at different pH](image)

**Figure 7** (A) Structure of fluorescein isothiocyanate (FITC) at different pH, (B) its relative fluorescence intensity at selected pH values.\textsuperscript{38}

The second family of biosensors utilizes OPH as the enzymatic sensor for the detection of OP compounds. The mode of action of OPH is different from AChE; it catalytically hydrolyses the organophosphate instead of covalently binding to it. Thus, instead of measuring the enzyme inhibition, detection methods involving OPH allow for a more direct measurement of OP compounds. Nowadays, OPH is widely used as a CWA biosensor because of the ability of its catalytic site to hydrolyze a wide range of compounds containing P-O, P-F, P-S, or P-CN bonds.\textsuperscript{11, 38} Hydrolysis of the OP compounds lead to the stoichiometric production of two protons which can be
monitored and directly correlated to the amount of OP substrate present. For instance, Cao et al.\textsuperscript{36} labeled OPH with FITC and deposited this material onto silanized quartz slides in the form of Langmuir-Blodgett films to create organized monolayers of the enzyme-based sensors. It was demonstrated that this OPH based enzyme sensor showed enhanced sensitivity, detecting the analyte at nM concentrations.

\begin{center}
\includegraphics[width=\textwidth]{figure8.png}
\end{center}

**Figure 8** Mechanism for the hydrolysis of paraoxon by OPH.

In summary, enzyme based sensors are very sensitive and both selective in their approach to detect organophosphorus compounds. Furthermore, OPH based enzyme sensors offer distinct advantages over AChE-based systems. While these approaches have been significant toward OP detection, the inhibition-based biosensors suffer from three drawbacks; (1) the enzymes easily lose activity in the event of environmental or handling factors, therefore these enzymes may provide false positive signals,\textsuperscript{39, 40} (2) the sensors require baseline testing prior to sample application and lengthy incubation times to allow enzyme-analyte interaction, and (3) due to the irreversible nature of cholinesterase enzyme inhibition, inhibition-based sensors cannot be reused without regeneration of enzyme activity. In addition, the lifetime of these sensors is limited by the degradation of enzyme.
1.3.2 Chromatographic Detection Methods

Analysis of OP pesticides in the environmental and biological samples is routinely carried out using chromatographic methods, such as gas or liquid chromatography coupled with mass spectrometry. Gas chromatography (GC) is usually the method of detection for vapor phase OP compounds. GC was the first method to be used for analysis of OP compounds e.g. nerve agents in the laboratory, hence early references date back to the 1960s. Kovats retention devices are still important tools in GC for screening the presence of nerve gases. Hancock and Peters investigated the use of retention index monitoring of nerve gas agents in environmental samples by thermal desorption GC in conjunction with simultaneous flame ionization detection (FID) and flame photometric detection (FPD). Besides the use of retention indices in combination with different types of GC columns, Kaipainen et al. investigated the use of different detectors to support identification of various OP compounds. Recently, Marx et al. have developed thin films of a molecularly imprinted sol-gel polymer with specific binding sites for parathion. The binding of parathion to the imprinted films was investigated by steady-state experiments with analysis by GC-FPD and cyclic voltammetry. Several new developments in GC are being reported, such as, high-speed GC, comprehensive GC-GC, large volume sample introduction by on-column injection or programmed temperature vaporizing (PTV) solvent split injection and new detection methods. However, inspite of being very sensitive and selective, these high-speed and high-resolution GCs can require up to 30 min for complete separation and detection of a
complex mixture of OP compounds which limits their application under rapid field
detection.\textsuperscript{46}

Liquid chromatography (LC) is a separation technique with many modes of
operation – reversed-phase, normal-phase, ion-exchange, and ion pair and size
exclusion, all of which are able to separate essentially all compounds that are soluble
in a conventional solvent or solvent mixture, with thermolability, polarity and/or
volatility not playing a major role. Thus, LC is the choice of detection method for OP
compounds which are not volatile and thermally labile. However, high pressures and
small particle sizes limit column length and, therefore, the ultimate resolving power
possible by LC.\textsuperscript{46} Also, another drawback of LC is the lack of detectors matching
selectivity and sensitivity of GC detection systems.\textsuperscript{41} These drawbacks demonstrate
the application of GC on OP compound even if aqueous sample matrices are
involved.

Although chromatographic methods provide reliable information regarding OP
pesticides, such analysis is generally performed at centralized laboratories which
require extensive labor and analytical sources, and often results in a lengthy
turnaround time. These disadvantages limit the applications of chromatographic
methods primarily to laboratory settings and prohibit their use for rapid analysis
under field conditions.
1.4 The Need

From a practical agricultural viewpoint, the approaches described above have limitations such as low sensitivity, lack of portability, limited selectivity, difficulties in real-time monitoring, and operational complexity. In addition, in most cases, these tests are qualitative or semiqualitative and show false positive and negative results.\textsuperscript{6}

Both soil and water are likely to contain mixtures of OP pesticides due to heavy urban and rural use of these compounds.\textsuperscript{6} Unfortunately, there have been no reports to demonstrate molecular sensors that differentiate selectively between OP pesticides. The broad range in toxicity levels of OP pesticide necessitates new methods to discriminate between various OP pesticides.

A key toward practical analytical chemistry is to achieve portability and continuous on-site monitoring of a wide range of analytes which is especially necessary in environmental detection. To meet the requirements of rapid warning and field deployment, more compact low cost instruments, coupled to smaller sensing probes are highly desirable for facilitating the task of on-site monitoring of OP compounds.\textsuperscript{6} This goal can be achieved either by miniaturizing established analytical apparatus, for example, separation techniques and spectrometry, as is, for example done in “lab on a chip approaches”, well known examples being the miniature DNA analyzers based on capillary electrophoresis which speeded up the identification of the human genome.\textsuperscript{47} The second approach can be made by development of chemical sensors, i.e. miniaturized measuring devices optimized to interact with a specified analyte.\textsuperscript{48}
1.5 Our Project

Here, we show a class of heterocyclic molecular and nanomaterials as sensors to detect OP pesticides and distinguish between them, based on the pesticide structure. As mentioned previously, organophosphorus pesticides have been extensively used for agricultural purposes for more than 40 years. There are about 200 different compounds on the market, accounting for 45% of the registered pesticides in the USA alone. The OP pesticides that will be investigated for the proposed project are shown in Figure 1. All the pesticides that have been chosen for the present case are OP thion insecticides. The reason behind selecting these four is that they are some of the most commonly used OP pesticides. The properties of each of the chosen OP pesticides will be discussed in further detail in the next section.

![Structures of the organophosphorus pesticides investigated for the project.](image)

**Figure 9** Structures of the organophosphorus pesticides investigated for the project.
**Table 3** Physicochemical Properties of the Selected Organophosphorus Pesticides.


**Ethion**: This OP pesticide is used to kill aphids, mites, scales, thrips, and maggots. It is also used on a wide variety of food, fiber, greenhouse crops, lawns, and turf. Ethion is highly toxic by inhalation, dermal exposure, and ingestion. It is almost non-volatile at room temperatures, but when sprayed it can be easily inhaled. The common symptoms of ethion intake are runny nose, irritation to the eye, and a sensation of tightness in the chest. High environmental temperatures or exposure of ethion to visible or UV light may enhance its toxicity.

**Malathion**: It is a non-systemic, wide-spectrum OP insecticide. It is one of the earliest OP insecticides developed (introduced in 1950). Malathion is used for the...
control of sucking and chewing insects on fruits and vegetables, and also applied to control mosquitoes, flies, and household insects.\textsuperscript{49} It is an OP pesticide with moderate toxicity (LD$_{50}$ value of 1000 mg/kg in rats). Symptoms of acute exposure to malathion include numbness, incoordination, headache, dizziness, tremor, nausea, and difficulty in breathing.\textsuperscript{49}

**Fenthion:** This compound is a widely used insecticide, especially in orchards, and is also frequently used in environmental samples.\textsuperscript{49} It is an example on an environmental contaminant for which degradation processes are important. The primary degradation products of fenthion are fenthion sulfone, fenthion sulfoxide, fenoxon, fenoxon sulfoxide, and fenoxon sulfone, which are formed through a variety of biological and abiotic pathways. It is an effective pesticide being moderately toxic to mammals, but highly toxic to birds. Symptoms include irritation in eyes and mucus membranes, and difficulty in breathing.\textsuperscript{49}

**Parathion:** This OP is widely recognized as the cheapest and most effective broad spectrum insecticide.\textsuperscript{49} Large quantities of parathion are used to protect crop plant roots from soil insects, such as rootworms and wireworms. Its popularity exists primarily due to its high insect mortality rate. It is an aromatic type OP, whose chemical name is O,O-diethyl O-p-nitrophenyl phosphorothioate. Parathion is slightly soluble in water, but completely miscible with many organic solvents. It is a dark, brown liquid with characteristic odor. It can be readily oxidized to its oxon derivative, paraoxon, which is more toxic than the parent compound.
Relative toxicity of Parathion: Parathion was the first OP to appear on the market in 1944, and is found to be 70 times as toxic as DDT, and hence is capable of producing severe toxic effects and deaths in animals and man. The LD$_{50}$ value for Parathion as tested against rats is 6 mg/kg. Parathion can enter human body by swallowing and breathing of mists, dusts or vapors or by absorption through skin. Pharmacological studies have shown that parathion can perform dual effect after entering into human body: a stimulation of the parasympathetic nervous system and anticholinesterase activity. Inactivation of acetylcholinesterase enzyme due to the presence of Parathion results in accumulation of acetylcholinesterase at the motor end plates of the parasympathetic and the voluntary nervous system, and in the ganglia of the sympathetics. Symptoms include giddiness, headache, nausea, vomiting, diarrhea, muscular fasciculation, and respiratory failure.\(^5\)

1.5.1 Selective Colorimetric and Electrochemical Detection of Organophosphorus Based Pesticides

An alternative to classical methods of OP pesticides detection is the design of optical detectors, i.e. colorimetric or fluorimetric chemosensors or reagents. One of the most convenient and simple means of chemical detection is the generation of an optical event, i.e. changes in absorption or emission bands in the presence of the target analyte. Optical outputs are being used extensively in recent years for the development of chemosensors for ion or neutral molecule recognition and sensing based on supramolecular concepts.\(^{51}\) Unfortunately, although the utility of optical
detection are becoming increasingly appreciated in terms of both qualitative and quantitative analysis, the number of optical sensors available at present for OP compounds detection is quite limited. Of particular interest in this regard are colorimetric OP sensors, species that would allow the so-called naked-eye detection of OP pesticides without having to resort to any sophisticated spectroscopic instrumentation. Currently, there is a surge of interest in the development of tailor-made sensor molecules, able to act as chromogenic sensors for a specific analyte. A selective sensor molecule should essentially have a receptor component specific for a selected analyte and a signaling unit capable of translating the analyte-binding induced changes into an output signal. The binding event of the analyte and the sensor moiety occurs through intramolecular charge transfer processes which lead to a change of color visible by eye. Materials capable of reversible OP pesticides induced selective changes in color are in great demand as they require little or no instrumentation for practical use. Unfortunately, to our best knowledge, there have been no reports of a selective colorimetric sensor for OP pesticides detection. As mentioned previously, since the toxicity of organophosphates varies by structure, it is important to develop materials that can detect and discriminate between them easily and in real-time. Additionally, materials or techniques that provide independent dual signals are ideal in terms of minimization of false-positive signals. If one of these signals employs an optical technique (i.e. colorimetric or fluorescence) as a signal output, it allows rapid screening and easy user access.

Lately, some innovative methods of detection of OP compounds based on optical methods have been reported in the literature by R.S. Pilato, T. M. Swager,
The first fluorescent chemosensor for detection of OP compounds was reported by Pilato et al where a series of non-emissive platinum 1,2-enedithiolate complexes with an appended primary alcohol were synthesized. Upon addition of electrophilic OP analyte to this compound and an activation agent (triazole) in dichloromethane, the alcohol was converted into a phosphate ester, which arranges by intramolecular ring closure reaction to form a fluorescent cyclic product. Although the method was found to be very effective in detection of a variety of nerve agents, the limitation of this work was that the fluorescence of the cyclic platinum complexes was quenched by oxygen, and hence the reactions needed to be performed in ambient conditions which restrict its application in real field purposes.

**Figure 10** Mechanism of the chemically reactive sensor developed by Pilato et al.²⁶

Swager and his co-workers developed a series of thienylpyridyl and phenylpyridyl systems which undergo intramolecular cyclization reactions upon exposure to nerve agents resulting in a bathochromic shifts in absorption and fluorescence spectra. The fluorescence color changes were observed using a UV lamp under ambient atmosphere. These sensors were found to be both sensitive and
selective to chemical warfare agents detection showing a complete response to 10 ppm DFP vapor within five minutes.

![Fluorescent Chemical Warfare Indicators](image)

**Figure 11** The mechanistic scheme of the chemosensor developed by Swager et. al.²⁸

Rebek et al⁵³ also did similar work on fluorescent sensors for nerve agents where he examined a series of pyrene based compounds as the fluorescent receptor. These sensors utilized a similar reaction mechanism designed by Pilato and Swager, however the mechanism in this case was based on the suppression of a photoinduced electron transfer (PET) process to trigger a fluorescent signal. Saturated aliphatic chains ranging from one to four methylene units were employed to determine how the spacer linking the fluorophore and the amine affected the efficiency of the sensors. Pyrene was used as the fluorophore since it can accept electrons from tertiary amines in PET processes. Upon binding to DCP, the sensors resulted in a significant increase in fluorescent intensity which could be observed visually using a handheld UV lamp. The sensor displayed practically instantaneous (5 seconds) fluorescence upon exposure to vapor comprising 10 ppm of DCP.
Recently in 2007, Simonian et. al\textsuperscript{54} reported a fluorescence based sensor for OP pesticides. They have used a new sensor Coumarin1 which is a competitive inhibitor of organophosphorus hydrolase (OPH). Coumarin1 in the presence of p-nitrophenol substituent organophonates leads to fluorescence quenching due to fluorescence resonance energy transfer (FRET). However, the drawback of this method is that it is applicable only to nitrophenyl substituted pesticides like methyl parathion and fenitrothion.

Here we show a series of four heterocyclic sensors designed not only for detection of OPS, but also to distinguish them, based on the pesticides structure. The
sensors display three significant features that make them ideal for this purpose and the hypothesis of this work is driven by these three features:

1) the highly conjugated structure leads to high absorbance of the sensor.

2) the nitrogen atoms within the structure are nucleophilic, and are capable of binding to the electrophilic phosphorus atoms present in the OP pesticides.

3) the nitrogens being directly fused with the chromophore, allow redox-active behavior that could be altered upon OP binding.

1.5.2 Application of Nanoscale Materials as Sensors toward Detection of Organophosphorus Based Pesticides

Metal nanoparticles are very attractive due to their size- and shape- dependent properties. The presence of extraordinary properties in metal nanoparticles arising from their size-dependent properties has been the area of intense research and has promoted a great deal of excitement since the last few decades. Interestingly, these properties are not present in the bulk. As the size of the particle decreases to the 1-100 nm range, it is well known that the electronic, optical, catalytic, and thermodynamic properties of metal nanoparticles deviate from bulk properties. This has led to the application of metal nanoparticles into various functions as chemical catalysts, sensors, adsorbents, biological stains, and elements of novel nanometer scale optical, electrical, and magnetic devices. Specifically, nanoparticles of silver, gold, and copper show distinct and well-defined plasmon absorption in the visible spectrum. As these noble metals are reduced in size to tens of nanometers, a
new very strong absorption is observed resulting from the collective oscillation of the electrons in the conduction band from one surface of the particle to another. This oscillation has a frequency that is able to absorb light in the visible range, which is widely known as the surface plasmon absorption. This absorption is strong enough to give rise to vivid characteristic color to the metal nanoparticles. For example, the gold nanoparticles give rise to a brilliant rose color as the size is reduced to the nanometer regime.

Lately, metal nanoparticles, especially gold nanoparticles have emerged as excellent colorimetric reporters due to their high extinction coefficients (the molar absorptivity for the 8 and 13 nm-diameter gold particles was reported to be $7.5 \times 10^7$ M$^{-1}$cm$^{-1}$ at 520 nm and $4.7 \times 10^9$ M$^{-1}$cm$^{-1}$ at 524 nm, respectively) which is several orders of magnitude higher than organic dyes and because the transition of the nanoparticles from dispersion to aggregation exhibits a distinct change in color. These properties have led to considerable potential of gold nanoparticles to be applicable as colorimetric reporters of ions and molecules as well as macromolecules. The wide variation of optical properties of gold nanoparticles with particle size, particle-particle distance, and the dielectric properties of the surrounding medium due to surface plasmon resonance enables the construction of simple but sensitive sensors for various analytes. Attention has recently focused on functionalizing gold nanoparticles with molecular recognition components for potential sensing applications. Gold nanoparticles possess distinct surface plasmon absorption bands which depend on their size and shape, and the color change upon aggregation is due to the coupling of the plasmon absorbances as a result of their proximity to each
other.\textsuperscript{62} Aggregation of particles results in color changes of the nanoparticle solutions accompanied by a shift in the SPR peak, due to either change in the dielectric constant around the nanoparticles as a result of adsorption of analyte molecules, or due to analyte-induced agglomeration of the nanoparticles. This aggregation of gold nanoparticles induced by analytes has been utilized for detection of DNA,\textsuperscript{63} several metal ions,\textsuperscript{62,64,65} and anions,\textsuperscript{61} For example, Beer et. al have reported the synthesis of a amide-functionalized zinc metalloporphyrin which was self-assembled on to gold nanoparticles to produce a novel anion-selective optical sensing system.\textsuperscript{61} Figure 14(A) shows the sketch of the anion-sensing porphyrin gold nanoparticle. For detection of heavy metal ions (Pb\textsuperscript{2+}, Hg\textsuperscript{2+}, Cd\textsuperscript{2+}), Kim et al. have successfully employed gold nanoparticles capped with 11-mercaptoundecanoic acid (MUA) which functions as an appropriate metal ion receptor, figure 14(B) showing the schematic representation of the reaction scheme.\textsuperscript{64}

![Figure 14](image)

\textbf{Figure 14} (A) Schematic of the anion-sensing by Au NP functionalized with zinc porphyrin. (B) Scheme of the interaction of MUA capped Au NP with heavy metal ions.
Recently, various research groups have utilized Au NPs for the detection of OP compounds. However, these methods suffer from several drawbacks. Firstly, although some of the methods were found to be very sensitive, however, none of them are able to detect OP pesticides selectively, but are applicable to a broad range of compounds. Secondly, gold surfaces had to be functionalized in order to bind with the OP compounds which make the methods very tedious and time consuming. In addition, the solvent medium used in most of the cases was organic solvents which prevent advancement of these sensors in practical applications.

Here, we have made use of the aggregation-induced color changes of Au NPs in aqueous solutions as an optical sensor for the detection of organophosphorus based pesticides. The interaction of three different sizes of Au nanoparticles was investigated with ethion, malathion, and fenthion. Citrate is a weak capping ligand. On the other hand, gold is known to have a high affinity to bond with sulfur atoms because of their soft characters. Thus, when the OP pesticides containing several sulfur atoms come in contact with the gold nanoparticles, the sulfur atoms bind to the Au surface releasing the capped citrate into the solution. This leads into aggregation of Au nanoparticles resulting in visible color changes which can be readily detected by the naked eye. The novel method of sensing developed by us offer three essential features (i) enables to use Au NPs capped with citrate as sensors for OP detection (ii) the detection was found to be specific with different OPs based on their structures and (iii) ensures proper functioning of the sensors in aqueous environment.
1.6 Research Objectives and Hypothesis

The research objectives of this work are:

1) Design and synthesize a molecular sensor that undergoes changes in optical properties upon interaction with organophosphorus pesticides.

2) Identify sensor with electrochemical properties that could potentially yield an additional signal output upon OP pesticides interaction, thus minimizing false-positives.

3) Development of nanoscale sensors based on gold nanoparticles for detection of organophosphorus pesticides via optical signal transductions, while ensuring proper function in aqueous environments.

4) Optimize sensor characteristics so that the sensors distinguish between various organophosphorus based pesticides.

5) Determination of the detection limits, to ensure that sensors function within the desired range.

The construction of receptors which can selectively recognize and sense organophosphate guest species via macroscopic physical response is a current area of chemical sensor technology receiving considerable attention. The described research is driven by designing and investigating a series of nitrogen containing heterocycles based on stilbene family. The interactions of these compounds were then examined with four different kinds of OP pesticides as mentioned, namely, ethion, malathion, fenthion, and parathion. These molecular sensors have a highly conjugated structure so as to yield high fluorescence quantum yields and absorb in the UV-visible region. The presence of the nucleophilic nitrogen atoms within the structure
allows binding to an electrophilic phosphorus atom in the OP pesticides. The directly fused heteroatom on the chromophore also allows redox-active behavior that could be altered upon OP binding, and therefore producing independent dual signal outputs to minimize false-positive signals.

The second part of our research dealt with the development of nanomaterials as sensors based on gold nanoparticles for the detection of OP pesticides. Three different sizes of Au nanoparticles capped with sodium citrate were synthesized, and their interaction with ethion, malathion, and fenthion was investigated. The mode of detection was once again based on optical methods. We have made use of the aggregation-induced color changes of Au NPs in aqueous solutions as an optical sensor for the detection of organophosphorus based pesticides.
1.7 References

5. Gibson, W. L. Microbial degradation of Parathion.


SELECTIVE COLORIMETRIC AND ELECTROCHEMICAL DETECTION OF ORGANOPHOSPHORUS BASED PESTICIDES

2.1 Introduction

2.1.1 Chemical Sensors and Molecular Recognition

Over the past two decades, chemical sensors, which are defined as reagents that interact with an analyte with high affinity and yield a measurable signal in response, have developed as viable alternatives to traditional methods of analysis.\(^1\) They play a crucial role in the detection of water, oxygen, carbon-dioxide, pH, metal ions, contaminants in waste water, and in the elucidation of cellular mechanisms. An ideal chemical sensor offers the advantage of in situ measurements, and provides information in real-time. It must recognize the analyte with high specificity, and have an affinity commensurate with the average concentration of the analyte solution.\(^2\)

Molecular recognition, "the process involving both binding and selection of substrates by a given receptor molecule as well as possibly a specific function"\(^3\), is opening the way to several desirable goals, such as responsive or intelligent materials, molecular devices, and new sensors.\(^4\) The ideas of molecular recognition have been used in applications in biological and material sciences and the emerging field of supramolecular chemistry. Molecular recognition is a question of information storage
and read out at the supramolecular level. The recognition process involves binding with a purpose, implying a structurally well-defined pattern of intermolecular interactions.

Just as there is a field of molecular chemistry based on the covalent bond, there is a field of supramolecular chemistry, the chemistry of molecular assemblies and of the intermolecular bond. Molecular receptors are organic structures, held by covalent bonds, which are able to bind selectively with various ions or molecules. This binding makes use of various intermolecular interactions, namely, electrostatic interactions, hydrogen bonding, Vander Waals forces, short range repulsions, etc. and results to the formation of an assembly of two or more molecules, a supermolecule. The design of the receptor determines which substrate is bound. The substrate-specific synthesis of a supermolecule thus involves organic (regioselectivity; stereospecific) synthesis of a receptor by formation of covalent bonds, followed by one or several binding steps using intermolecular bonds in an arrangement predetermined in the design of the receptor.

The work described herein, uses the concepts of molecular recognition to develop selective receptor molecules for organophosphorus pesticides. The receptors are comprised of aromatic heterocycles with nitrogen atoms that serve as nucleophiles. The binding process involves signal transduction, and we measure this signal as a change in the optical property of the ligand. The processes of molecular recognition are summarized in Figure 15. We are interested in designing chemical sensors that upon binding to the substrate produce an optical signal.
Figure 15 Schematic representation of the process of molecular recognition: a receptor is synthesized with recognition units or binding sites for the substrate. The receptor recognizes the substrate with high specificity and binds to it through intermolecular interactions. The binding process takes place with high recognition resulting in signal transduction.

### 2.1.2 Advances in Optical Methods of Detection of Organophosphorus Compounds

An alternative to classical methods of OP pesticides detection is the design of optical detectors, i.e. colorimetric or fluorimetric chemosensors or reagents. Unfortunately, although the utility of optical detection are becoming increasingly appreciated in terms of both qualitative and quantitative analysis, the number of optical sensors available at present for OP compounds detection is quite limited. Of particular interest in this regard are colorimetric OP sensors, species that would allow the so-called naked-eye detection of OP pesticides without resort to any sophisticated spectroscopic instrumentation. A selective sensor molecule should essentially have a
receptor component specific for a selected analyte and a signaling unit capable of translating the analyte-binding induced changes into an output signal. Materials capable of reversible OP pesticides induced selective changes in color are in great demand as they require little or no instrumentation for practical use. Unfortunately, to our best knowledge, there have been no reports of a selective colorimetric sensor for OP pesticides detection. Since the toxicity of OP pesticides varies by structure, it is important to develop materials that can detect and discriminate between them easily and in real-time. Additionally, materials or techniques that provide independent dual signals are ideal since they could minimize false-positive signals. If one of these signals employs an optical technique (i.e. colorimetric or fluorescence) as a signal output, it allows rapid screening and easy user access.

Lately, some innovative methods of detection of OP compounds based on optical methods have been reported in the literature. R.S. Pilato, T. M. Swager, and J.R. Rebek are worth-mentioning names here. However, all these methods were directed to design optical sensors for chemical warfare agents, and not OP pesticides. As mentioned before, there have been no reports of sensors which selectively differentiate between various OP pesticides. Here, we show a heterocyclic sensor based on the family of stilbenes. The sensor has been designed not only for detection of OP pesticides, but also for selectively distinguishing them, based on the pesticides structure. The sensor display three significant features that make it ideal for this purpose and the hypothesis of this work is driven by these three features:

1) The push-pull stilbene system leads to high molar extinction coefficients due to an intramolecular charge transfer transitions leading to absorption in the visible region.
2) the highly conjugated structure leads to high fluorescence quantum yields.

3) the nitrogen atoms within the structure are nucleophilic, and are capable of binding to the electrophilic phosphorus atoms present in OP pesticides.

4) the nitrogens being directly fused with the chromophore, allow redox-active behavior that could be altered upon OP binding.

Figure 16 shows the structure of the molecular sensor designed by us.

![Structure of the molecular sensor.](image)

**Dimethyl-[4-(2-quinolin-2-yl-vinyl)-phenyl]-amine**

*(DQA)*

**Figure 16** Structure of the molecular sensor.

2.2 Experimental Section

2.2.1 Materials and Instrumentation

Quinaldine (≥90%), benzaldehyde (≥99.5%), dimethylsulfoxide (anhydrous DMF, ≥99.8%), potassium tertiarybutoxide (≥97%), lithium hydride (≥95%), ammonium chloride (≥99.5%), hydrochloric acid (≥37%), ethion, malathion, fenthion, and parathion were obtained from Sigma-Aldrich. Tetrabutylammonium hexafluorophosphate (TBAPF$_6$, ≥99%) was obtained from Fluka. The solvents
fenthion, and parathion were obtained from Sigma-Aldrich. Tetrabutylammonium hexafluorophosphate (TBAPF$_6$, $\geq 99\%$) was obtained from Fluka. The solvents dichloromethane (anhydrous, $\geq 99.5\%$) and acetonitrile ($\geq 99.5\%$) 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 and $^{31}$P NMR was recorded on a 400 MHz JEOL spectrometer at room temperature, and deuterated acetonitrile ($\geq 99.5\%$) was used as the solvent.

2.2.2 Synthesis of DQA

\[
\begin{align*}
\text{Quinaldine} & \quad \text{Benzaldehyde} \\
\text{DMF, Potassium tertiarybutoxide} & \quad \text{Stirred for 6hrs at room temperature} \\
\rightarrow & \quad \text{(DQA)} \\
\end{align*}
\]

\[
\begin{align*}
\text{(Quinaldine)} & \quad \text{(Benzaldehyde)} \\
\text{+ H}_2\text{O} & \quad \text{(DQA)} \\
\end{align*}
\]

\textbf{Scheme 1} Synthesis of Dimethyl-[4-(2-quinolin-2-yl-vinyl)-phenyl]-amine (DQA).

Equivalent moles of quinaldine (0.677 ml, 0.005 mole) and p-dimethylaminobenzaldehyde (0.74595 g, 0.005 mole) were measured out in a glass vial. To this mixture, 5 ml of fresh and dry DMF was added, and shaken to dissolve completely. To an oven-dried 25 ml round bottom flak, a magnetic stir bar was added,
Lithium hydride (0.099 g, 12.5 mmol) and potassium t-butoxide (0.70131 g, 6.25 mmol) were added to the flask one after another, making sure that the drying tube remains attached after each addition. The reaction mixture was stirred for 5 minutes. After stirring was done, the mixture previously prepared in the glass vial was added to the round bottom flask, once again making sure that the drying tube was replaced on the flask after the addition. This reaction mixture was then stirred for 24 hours.

About 15 g of ice was taken in a large beaker to which 10 ml of saturated ammonium chloride (NH\textsubscript{3}Cl) was added. The stirred reaction mixture was then poured into the beaker of ice, and 5 ml of saturated ammonium chloride was added to it to dissolve any remaining product in the flask. The mixture in the beaker was stirred for about 15 minutes, and the resulting solid was collected using a vacuum filter. The solid product was washed with cold water while the vacuum was on, and left in the vacuum to air dry the product overnight. The average yield of this product was 75%. The product was characterized by \textsuperscript{1}H NMR and UV-visible spectra. \textsuperscript{1}H NMR (CD\textsubscript{3}CN, 400 MHz) \textdelta: 8.1890-8.1679 (d, 1H, J = 8.44 Hz), 7.9527-7.9316 (d, 1H, J = 8.44 Hz), 7.8519-7.8309 (d, 1H, J = 8.4 Hz), 7.7210-7.6715 (q, 1H, J = 19.8 Hz), 7.5680-7.5460 (d, 1H, J = 8.8 Hz), 7.5067-7.4865 (q, 1H, J = 8.08 Hz), 7.2017-7.1614 (d, 1H, J = 16.12 Hz), 6.8253-6.7639 (d, 1H, J = 24.65 Hz), 1.9429 (s, 6H).

2.2.3 UV-visible Absorbance Measurements

A Varian Cary 50 spectrophotometer was used to measure the UV-Visible absorbances of the sensors, as well as OP-induced UV-visible absorbance changes. In
each case, an acetonitrile solution of the sensors was freshly prepared at room temperature. The sensor solution was then titrated with a solution of the pesticide. In most cases, neat pesticide was used for the titration experiment. The titrations were done to observe the UV-visible absorbance changes that occurred upon interaction of the sensor and the OP pesticides. UV-visible absorbance measurements were taken immediately following each addition of OP pesticides added. All measurements were taken at room temperature.

2.2.4 Fluorescence Measurements

Varian Cary Eclipse spectrofluorometer was used to measure fluorescence of the sensors as well as OP-induced fluorescence changes using an excitation wavelength of 330 nm. In the case of titration with fenthion, an excitation wavelength of 375 nm was used. In each case, an acetonitrile solution of the sensors was freshly prepared at room temperature. The sensor solution was then titrated with a diluted solution of the pesticide. Fluorescence measurements were taken immediately following each addition of OP pesticides added.

2.2.5 Electrochemical Measurements

Cyclic voltammetry was used to measure redox potentials of the sensor (\(\sim 10^{-4}\) M) in \(10^{-4}\) M TBAPF\(_6\)/acetonitrile solutions. A BAS model CV-50W
electrochemical workstation was used in a standard three-electrode arrangement consisting of a glassy carbon working electrode, a Pt wire counter electrode, and a Ag/AgCl reference electrode.

2.2.6 Computational Modeling

All studies were optimized at the B3LYP level of density functional theory. The standard 6-31+G(d,p) was used. Frequency calculations at the same level of theory have also been performed to confirm that all stationary points were minima (no imaginary frequencies). To acquire the mapped electrostatic potential, the isosurface values are set to 0.010. All calculations were performed with the Gaussian software package.23

2.2.7 $^1$H AND $^{31}$P NMR Measurements

All NMR experiments were recorded on a 400 MHz JEOL spectrometer with chemical shifts (ppm) relative to trimethylsilane (TMS).
2.3 Results and Discussion

The interactions of DQA with four OP pesticides, ethion, malathion, fenthion, and parathion were investigated.

The absorption spectrum of DQA in acetonitrile shows a peak at 385 nm which can be assigned due to intramolecular charge transitions from the dimethylamine nitrogen to the quinaldine nitrogen.\textsuperscript{15} Moreover, in acetonitrile medium, DQA emits in the green with $\lambda_{\text{max}} = 524$ nm, as shown in figure 18. The emission intensity is readily observed with the naked eye under UV-light.
**Figure 17** UV-visible absorbance spectrum of DQA in acetonitrile.

**Figure 18** Fluorescence spectrum of DQA in acetonitrile.
2.3.1 UV-visible Absorbance Spectroscopy Studies

2.3.1.1 Investigation of the Interaction of DQA with Ethion

A 2.4×10⁻⁵ M solution of DQA in acetonitrile solvent was prepared. Changes in the UV-Visible spectrum were monitored, as neat ethion (Aldrich) was added to the DQA solution in 2 µL (2.1×10⁻³ M) increments. As shown in Figure 19, addition of ethion resulted in the quenching of the 385 nm peak of the sensor, along with the formation of two new peaks at 500 nm and 325 nm indicating the formation of a new species upon complexation of the sensor and ethion. Also, the appearance of two isosbestic points at 340 nm and 425 nm in the titration profile indicates that two species are coexisting at the equilibrium with a 1:1 stoichiometric ratio. The formation of the new species is also evident from a change in color of the solutions, the yellow color of the sensor solution changes results in a bright red colored solution upon addition of ethion.
Figure 19 Changes in the UV-visible absorbance titration profile of DQA in the presence of increasing concentrations of ethion. From top to bottom, [ethion] = 0, 2.1, 4.2, 6.3, 8.5, 10.6, 12.7, 14.8, 16.9, 19.0, 23.3, and 25.4 mM. Inset showing the color change of DQA upon addition of ethion.

2.3.1.2 Investigation of the Interaction of DQA with Malathion

A 2.4×10⁻⁵ M solution of DQA in acetonitrile solvent was prepared. Changes in the UV-visible spectrum were monitored, as neat malathion (Aldrich) was added to the DQA solution in 2 µL (2.5×10⁻³ M) increments. As shown in Figure 20, addition resulted in the quenching of the 385 nm peak of the sensor, along with the formation of two new peaks at 500 nm and 320 nm indicating the formation of a new species upon complexation of the sensor with malathion. Also, the appearance of two isosbestic points at 335 nm and 420 nm in the titration profile indicates that two
species coexist at the equilibrium with a 1:1 stoichiometric ratio. The formation of the new species is also evident from a change in color of the solutions, the yellow color of the sensor solution changes resulting in an orange colored solution upon addition of malathion.

Figure 20 Changes in the UV-visible absorbance titration profile of DQA in the presence of increasing concentrations of malathion. From top to bottom, [malathion] = 0, 2.5, 4.9, 7.4, 9.9, 12.3, 14.8, 17.3, 19.7, 22.2, 24.8, 27.3, 29.7, 32.3, 34.7, 37.2, and 39.7 mM. Inset showing the color change of DQA upon addition of malathion.
2.3.1.3 Investigation of the Interaction of DQA with Fenthion

A $2.4 \times 10^{-5}$ M solution of DQA in acetonitrile solvent was prepared. Changes in the UV-visible spectrum were monitored, as neat fenthion (Aldrich) was added to the DQA solution in $2\mu$L ($3.0 \times 10^{-3}$ M) increments. As shown in Figure 21, at the end of the titration, the absorbance intensity was relatively similar to the original absorbance of DQA. The yellow colored DQA still remained yellow after fenthion addition. This indicates the lack of any complex formation between the sensor and fenthion.

![Figure 21](image)

**Figure 21** Changes in the UV-visible absorbance titration profile of DQA in the presence of increasing concentrations of fenthion. From top to bottom, [fenthion] = 0, 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, and 21.0 mM. Inset showing the color change of DQA upon addition of fenthion.
2.3.1.4 Investigation of the Interaction of DQA with Parathion

A $2.4 \times 10^{-5}$ M solution of DQA in acetonitrile solvent was prepared. Changes in the UV-visible spectrum were monitored, as neat parathion (Aldrich) was added to the DQA solution in 2 µL ($2.9 \times 10^{-3}$ M) increments. As shown in Figure 22, addition resulted in the formation of a new peak at 505 nm indicating the formation of a new species. The new peak continually grows as more parathion is introduced into the solution. The formation of the new species is also evident from a change in color of the solutions, the yellow color of the sensor solution changes results in a yellow-orange colored solution upon addition of parathion.

**Figure 22** Changes in the UV-visible absorbance titration profile of DQA in the presence of increasing concentrations of parathion. From bottom to top, [parathion] = 0, 2.9, 5.8, 8.7, 8.5, 11.6, 14.5, 17.4, 20.3, 23.2, 26.1, 29.0, 31.9, 34.8, 37.7, and 43.5 mM. Inset showing the color change of DQA upon addition of parathion.
2.3.1.5 Summarized Results of the UV-visible Titration Data

Table 2.1 summarizes the results obtained from the UV-visible absorbance spectroscopy measurements. It was found that DQA is very successful in distinguishing all the four OP pesticides resulting in different colored solutions with different \( \lambda_{\text{max}} \) values. This is very desirable since no reports for a selective colorimetric sensor for detection of OP pesticides has been reported before. In order to understand the strength of the binding taking place between DQA and the corresponding OP pesticides, binding constants were calculated. It was found that DQA undergoes strongest binding with ethion, followed by malathion, and fenthion.

<table>
<thead>
<tr>
<th>OP Pesticide</th>
<th>Color of the final solution</th>
<th>( \lambda_{\text{max}} ) for the new peaks (nm)</th>
<th>Detection limits (M)</th>
<th>Binding constant (M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethion</td>
<td>Red</td>
<td>500, 325</td>
<td>( 10^{-6} ) M</td>
<td>( 6.5 \times 10^4 )</td>
</tr>
<tr>
<td>Malathion</td>
<td>Light pink</td>
<td>500, 320</td>
<td>( 10^{-6} ) M</td>
<td>( 1.1 \times 10^4 )</td>
</tr>
<tr>
<td>Fenthion</td>
<td>Yellow</td>
<td>None</td>
<td>N/A</td>
<td>No interaction</td>
</tr>
<tr>
<td>Parathion</td>
<td>Yellow-orange</td>
<td>505</td>
<td>( 10^{-6} ) M</td>
<td>( 0.2 \times 10^4 )</td>
</tr>
</tbody>
</table>

Table 4 Summary of results for the interaction of DQA with the OP pesticides.

Furthermore, in order to support the data obtained from UV-visible absorbance spectroscopy results, further studies were carried on with DQA which will be discussed in the next sections.
2.3.2 Fluorescence Spectroscopy

2.3.1.1 Investigation of the Interaction of DQA with Ethion

Changes in the fluorescence spectra of DQA with the OP pesticides were investigated.

A 2.4×10^{-5} M solution of DQA in acetonitrile solvent was prepared. Changes in the fluorescence spectrum were monitored, as neat Ethion (Aldrich) was added to the DQA solution in 2 µL (2.1×10^{-3} M) increments. As shown in Figure 23, addition resulted in the quenching in fluorescence intensity of the 524 nm peak of the sensor. At the saturation point, the blue emission of DQA was 100% quenched and the solution was colorless. It is possible that the donor (DQA) and the acceptor (ethion) in this case results in the formation of a new DQA-ethion complex which is non-fluorescent.
2.3.2.2 Investigation of the Interaction of DQA with Malathion

A $2.4 \times 10^{-5}$ M solution of DQA in acetonitrile solvent was prepared, and changes in the fluorescence spectrum was monitored, as neat malathion (Aldrich) was added to the DQA solution in 2 µL ($2.5 \times 10^{-3}$ M) increments. As shown in Figure 24, addition resulted in the fluorescence quenching of the 524 nm peak of the sensor. At the saturation point, the blue emission of DQA was 100% quenched and the solution was colorless. It is possible that the donor (DQA) and the acceptor (malathion) in this case results in the formation of a new DQA-malathion complex which is non-
fluorescent. It is worth mentioning here that it requires much higher concentration of malathion compared to ethion in order to result in 100% quenching, indicating that ethion undergoes a stronger binding with DQA than malathion, which follows our expectation as judged by the binding constant values.

**Figure 24** Changes in the fluorescence titration profile of DQA in the presence of increasing concentrations of malathion. From top to bottom, [malathion] = 0, 2.4, 4.9, 7.4, 9.9, 12.3, 14.8, 17.3, 19.7, 22.2, 27.1, 29.5, 32.1, 34.5, and 37.0 mM.
2.3.2.3 Investigation of the Interaction of DQA with Fenthion

A $2.4 \times 10^{-5}$ M solution of DQA in acetonitrile solvent was prepared, and changes in the fluorescence spectrum was monitored using an excitation wavelength of 375 nm, as fenthion (diluted in acetonitrile) was added to the DQA solution in 5 µL ($7.47 \times 10^{-5}$ M) increments. As shown in Figure 25, addition resulted in no significant changes in the fluorescence intensity of DQA indicating that fenthion is non-interactive with DQA in accordance with the UV-visible absorbance data.

![Figure 25](image)

**Figure 25** Changes in the fluorescence titration profile of DQA in the presence of increasing concentrations of fenthion. From top to bottom, [fenthion] = 0, 3.0E-4, 6.0E-4, 9.0E-4, 12.0E-4, and 15.0E-4 M.
2.3.2.4 Investigation of the Interaction of DQA with Parathion

A 2.4×10⁻⁵ M solution of DQA in acetonitrile solvent was prepared, and changes in the Fluorescence spectrum was monitored, as diluted parathion (diluted in acetonitrile) was added to the DQA solution in 5 µL (3.61×10⁻⁵ M) increments. As shown in Figure 26, addition resulted in the quenching in fluorescence intensity of the 524 nm peak of the sensor. At the saturation point, the blue emission of DQA was 100% quenched and the solution was colorless. The binding of a nitro (⁻NO₂) group to a fluorophore is well-known to quench the emission of most fluorophores.¹ The observed quench in fluorescence may be attributed to DQA-parathion complex formation, whereby the −NO₂ group results in fluorescence quenching of DQA.

Figure 26 Changes in the fluorescence titration profile of DQA in the presence of increasing concentrations of parathion. From top to bottom, [parathion] = 0, 3.6E-5, 7.2E-5, 1.1E-4, 1.4E-4, 1.8E-4, 2.2E-4, 2.9E-4, 3.6E-4, 4.3E-4, 5.1E-4, 5.8E-34, 7.2E-4, 8.7E-4, 1.0E-3, 1.2E-3, 1.3E-3, 1.4E-3, 1.7E-3, 2.0E-3, and 2.3E-3 M.
2.3.3 Electrochemical Measurements

While the UV-visible absorbance spectroscopy measurements provided significant results and showed the selectivity toward OP pesticides, it was important to determine whether an alternative signal output could additionally be used to demonstrate whether binding had occurred. 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 in-situ environmental monitoring. These type of sensors are concerned with the interplay between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential or charge and their relationship to chemical parameters where the applied potential between a reference and a working electrode causes the oxidation or reduction of an electroactive species.

To obtain a second 'validating' signal output, the binding of the OP pesticides by the sensors were investigated by cyclic voltammetry (CV). DQA was found to be electroactive, being able to get oxidized and reduced. Figure 27 shows the example of the cyclic voltammogram obtained for DQA (8.75×10⁻⁸ M) alone in acetonitrile medium (E₁/₂ = -845 mV vs. Ag/AgCl). Therefore, we investigated whether the new complexes formed upon binding of the different OP pesticides with DQA would yield different electrochemical properties in comparison to the parent compound alone.
As discussed before, DQA was found to yield distinct changes in the colors of the solutions with ethion, malathion, and parathion, but it did not show any interaction with fenthion. CV data also did not show any changes in the redox activity of DQA upon addition of fenthion, indicating once again that fenthion is not capable of undergoing binding with DQA in the ground state. However, addition of ethion did not show any cathodic or anodic peaks in the spectrum which might be due to the fact that the DQA/ethion complex is non-electroactive. But addition of malathion resulted in the formation of DQA/malathion complex which was accompanied by a specific change in redox potential, as well as formation of a new redox peak (DQA/malathion complex: $E_{1/2} = -1498$ mV vs. Ag/AgCl, $E_{1/2} = -870$ mV vs. Ag/AgCl).
Figure 28 Changes in redox properties of DQA upon addition of $7 \times 10^{-4}$ M malathion.

On the other hand, addition of parathion resulted in the formation of DQA/parathion complex demonstrating significant changes in the redox behavior ($E_{1/2} = -1072$ mV vs. Ag/AgCl, $E_{1/2} = -773$ mV vs. Ag/AgCl) in comparison to the parent compound. The reduction waves show cathodic shifts, indicating that the DQA complex is difficult to reduce. Moreover, addition of parathion to the DQA solution resulted in a gradual increase in current which can be attributed to the formation of the DQA/parathion complex with a higher diffusion coefficient. Figure 29 shows an example of the CV of DQA titrated by parathion.
2.3.4 NMR Studies

$^1$H and $^{31}$P NMR studies were performed before and after titration of DQA with the OP pesticides in order to determine the structures of the new complexes. DQA and the corresponding OP pesticides were mixed in varying molar ratios in deuterated acetonitrile solvent and changes in the NMR spectra were recorded.

In the case of $^1$H NMR, the chemical shifts of the C-H proton in the quinoline ring ($H_1$, $\delta = 8.1890$ ppm) and the ethylene proton ($H_2$, $\delta = 7.2017$ ppm) right next to the nitrogen atom of DQA was found to be sensitive to the addition of ethion. The chemical shifts of these two protons are progressively downfield shifted and remained
unchanged upon addition of 1 equivalent of ethion. The final displacement of the protons was 0.0017 and 0.0018 ppm respectively. The downfield shifts can be assigned due to binding of the nitrogen atom in DQA with the phosphorus atom in ethion which creates a shielding effect to these protons changing their surrounding chemical environment. Similar downfield shifts were also observed with the addition of malathion and parathion to DQA, however the chemical shifts in these cases were less prominent compared to the ethion complex. No changes were observed for fenthion-DQA complex, once again demonstrating that fenthion is non-interactive in the ground state.

Figure 30 Structure of DQA showing the protons susceptible to undergo chemical shifts upon binding with the OP pesticides.
Figure 31 $^1$H NMR shifts in the quinaldine nitrogen of $3.3 \times 10^{-5}$ M of DQA in CD$_3$CN upon addition of ethion in (a) 1:0.4 (b) 1:0.8, and (c) 1:1 molar ratios.

The $^{31}$P NMR of the OP pesticides in acetonitrile was compared with the $^{31}$P NMR of DQA with the corresponding OP pesticides in acetonitrile. Surprisingly, no significant differences were observed. This can be attributed to the fact that chemical shifts of $^{31}$P NMR are greatly affected by the unbalance of the σ-bonds as determined by the electronegativities of the substituents attached to the phosphorus atom. Based on the Pauling electronegativity scale, the difference in the electronegativity of nitrogen (3.066), oxygen (3.610), and sulfur (2.589) atoms is not much. It is possible that due to close proximity of these values, the changes in the chemical environment of the phosphorus atom are insignificant when it binds to the nucleophilic nitrogen on DQA, and hence no significant changes in the $^{31}$P NMR were observed.
2.4 Investigation on the Binding Sites in DQA

DQA has two nucleophilic sites in its structure— the quinoline and the dimethylamine group, and hence a 1:2 stoichiometry complex was expected to form upon interaction of DQA with the OP pesticides. Technically both the sites are capable of donating their lone pair of electrons to the electrophilic phosphorus atoms in the OP pesticides structure. Usually, due to presence of the electron donating methyl groups on the dimethylamine nitrogen, this site is expected to be more basic than the quinoline site and hence should have higher tendency to attack the phosphorus atom. However, with the involvement of intramolecular charge transfer (ICL) in the ground state of DQA, the quinoline site becomes more basic compared to the dimethylamine site.\textsuperscript{15} Thus the quinoline nitrogen atom is more prone to attack the electron deficient phosphorus atoms of the OP pesticides.

In order to identify the molecular static potential and to determine the nucleophilicity of the two nitrogen atoms, computational calculations on DQA was performed with the Gaussian 03 software package.\textsuperscript{23} As discussed above, the quinoline nitrogen was expected to be more nucleophilic, and computational studies confirmed this hypothesis. The data in Figure 2.17 shows that the electrostatic potential at the quinoline nitrogen is much higher compared to the dimethylamine nitrogen atom showing that formation of a 1:2 stoichiometric complex is not very feasible.
In addition, $^1$H NMR studies were investigated in order to understand whether the computational data translates well with the experimental data. $^1$H NMR studies show that chemical shifts of $H_1$ and $H_2$ in DQA progressively downfield shifts with gradual addition of the OP pesticides. However, the downfield shift remains unchanged upon addition of 1 equivalent of the OP pesticides, as shown in figure 33. This confirms a 1:1 stoichiometry upon interaction of DQA and the OP pesticides.
Furthermore, we synthesized an analogue of DQA, 1-quinoli-2-phenethylene (QPE) without containing the dimethylamine group in order to investigate the role of the two nitrogen binding sites. QPE in acetonitrile gives a colorless solution with an absorbance peak at 320 nm. A $2.1 \times 10^{-5}$ M solution of QPE was prepared in acetonitrile and changes in the UV-visible spectrum were monitored as neat ethion (Aldrich) was added to the QPE solution in 2 µL ($2.1 \times 10^{-3}$ M) increments. With the introduction of ethion into this solution, the 320 nm absorbance peak of QPE gradually decreases, along with the formation and growth of a new peak at 370 nm. The appearance of an isosbestic point at 345 nm indicates the formation of a new species. However, because of the formation of the new peak at 370 nm (not in the visible range), there was no change in the color of the solution with ethion. This demonstrates that the quinaldine nitrogen is the only binding site with the OP
pesticides, but the dimethylamine nitrogen is only involved in the intramolecular charge transfer in the molecule resulting in formation of colors in the visible region. This concludes that a 1:1 stoichiometry takes place upon binding of DQA with ethion. Figure 34 shows the structure of QPE and its UV-visible titration data with ethion.

Figure 34 (A) Structure of QPE, and (B) Changes in the UV-visible absorbance titration profile of QPE with the addition of ethion, inset showing the colors of the solutions before and after titration.
2.5 Mechanism of Interaction

The mechanism of interaction of DQA occurs through the nucleophilic attack of the pyridine nitrogen at the electrophilic phosphorus center present in the OP pesticides. This reaction leads to the elimination of the leaving group, X. Figure 2.19 shows the reaction mechanism for OP pesticides in general.

Scheme 2 Mechanism of interaction of DQA with the OP pesticides in general.
The feasibility of the interaction of DQA is determined by the stability of the leaving group present in the OP pesticide. A better leaving group makes the reaction faster in comparison to a pesticide containing a poor leaving group. The leaving group which comes off more easily, the more stable it is as a free entity. This is usually inverse to its basicity, and the best leaving groups are the weakest bases.\(^{14}\)

If the structures of the individual four OP pesticides are taken into consideration, it is seen that ethion contains two electron deficient phosphorus atoms in its structure where nucleophilic attack of the sensor can take place. This explains why ethion binds with the sensor more strongly (and hence possesses the highest binding constant) compared to the other OP pesticides which contain a single electrophilic phosphorus atom. The non-interactive behavior of fenthion can also be explained by the stability of the leaving group. Both fenthion and parathion contain an aromatic system in their structures. However, as seen before, parathion interacts with DQA producing visible color changes, whereas fenthion is incapable of doing so. In the case of parathion interaction, the leaving group (2) is highly stable because of the presence of the electron withdrawing nitro group stabilizing the negative charge on the oxygen atom. However, for fenthion, the leaving group is (1) where both -SCH\(_3\) and -CH\(_3\) are electron donating groups, which increases the negative charge on the anion, making it very unstable resulting in the formation of a very unstable leaving group. This in part, explains the lack of interaction between fenthion and the sensor.
**Figure 35** Structure of the leaving group in the case of parathion interaction.

**Scheme 3** Mechanism of interaction of DQA with fenthion.
2.6 Conclusions

Sensors that can selectively detect and distinguish between various OP pesticides are in great demand for both environmental and agricultural applications. We have successfully developed a new design of sensor that selectively binds to OP pesticides and upon binding, yields dual optical and electrochemical signal outputs based on the sensor-OP complex formed. Here, the sensor show three significant features: (1) the highly conjugated structure lead to strong visible colors, (2) the nitrogen atoms within the structure are nucleophilic due to presence of the lone pair of electrons, and hence permit binding to the electrophilic phosphorus atoms within the OP pesticides structure, and (3) the nitrogens being directly fused with the chromophore allows redox-active behavior that could be altered upon OP binding. These three features were essential toward the development of selective sensor for OP pesticides.

Eventually, these sensors may be able to be used in a sensor array along with other sensors. This would improve the determination of the OP pesticides present because there would be more signals to observe which would reduce the risk of false positives.
2.7 References


CHAPTER 3

WAVELENGTH-SPECIFIC SURFACE PLASMON RESONANCE SHIFTS IN GOLD NANOPARTICLES CAUSED BY ORGANOPHOSPHORUS PESTICIDES

3.1 Introduction

3.1.1 Advantages of using Gold Nanoparticles as Sensors toward Detection of Organophosphorus Pesticides

Metal nanoparticles are currently of great interest due to their applications in various fields of science, namely, chemical catalysts, adsorbents, biological stains, and elements of novel nanometer optical, electronic, and magnetic devices.\(^1\)\(^-\)\(^5\) Lately, these metal nanoparticles are emerging as important colorimetric reporters due to their high extinction coefficients, which are several orders of magnitude larger compared to organic dyes\(^6\)\(^,\)\(^7\) and because the transition of the nanoparticles from dispersion to aggregation exhibits a distinct change in color.\(^7\) Application of Gold nanoparticles is worth-mentioning in this respect. This is because of the characteristic plasmon absorption bands of gold nanoparticles which depend on their size and shape.\(^9\)\(^,\)\(^10\) The wide variation of the optical properties of gold nanoparticles with particle size, particle-particle distance, and the dielectric properties of the surrounding media due to surface plasmon resonance enables the construction of simple, yet sensitive colorimetric sensors for various analytes.\(^9\)\(^-\)\(^12\) The color change upon aggregation is due to the coupling of the plasmon absorbances as a result of their
proximity to each other. By taking advantage of the mechanism of color transformation induced by the presence of analytes, gold nanoparticles have been used for the detection of DNA, \textsuperscript{14-18} several metal ions, \textsuperscript{7,19,20} and antibodies. \textsuperscript{21}

Recently, Organophosphorus based pesticide sensors based on nanoparticles are being designed and reported by various groups.\textsuperscript{22-26} The first work in this context was done by Pavlov et al. where Au NP plasmon resonance was utilized for optical determination of OP pesticides.\textsuperscript{22} On the other hand, Liu and Lin\textsuperscript{23} have reported an electrochemical OP pesticide sensor whose working principle is based on the affinity of the analyte for ZrO\textsubscript{2} nanoparticles. Blanchard et al.\textsuperscript{24,25} have reported the synthesis of simple Au NP sensors functionalized to form a zirconium-phosphate (ZP) terminated surface for detection of OP pesticides. Zr\textsuperscript{4+} have a high affinity to bind with OP pesticides,\textsuperscript{23,27} whereas Au NPs served as optical reporting reagents, where the plasmon resonance band of the Au NP is sensitive to the condition of the Zr-P linkage to its surface. However, the above mentioned nanoparticle based sensors suffered from several limitations. Firstly, the electrochemical method of detection reported by Liu and Lin\textsuperscript{23} is applicable only for nitroaromatic electroactive OP pesticides. On the other hand, the sensing platform developed by Blanchard et al.\textsuperscript{24,25} although simple and inexpensive, however the work presented is sensitive to the broad family of phosphate and phosphonate compounds, and hence suffers from lack of selectivity. Also, no there has been no reports on investigation of the interaction of gold surface alone without functionalization when it comes in contact with the OP pesticides.
The stability of a metal nanoparticle in solution results from the potential barrier that develops as a result of the competition between Van der Waals attraction and Coulomb repulsion between two nanoparticles. However, the stability against aggregation is largely a kinetic effect due to the large Coulombic repulsive barrier resulting from the localization of charged stabilizer capping the nanoparticle, making the effective charge on the nanoparticle zero. Various reports show that the addition of a neutral species to the stable nanoparticle solution can result in the destabilization of the nanoparticle. Addition of a neutral analyte which has high affinity to bind to the gold surface will replace the capping ligand which reduces the Coulombic barrier of repulsion between the nanoparticles, bringing them close together resulting in aggregation of the particles. In our work, we have utilized the aggregation-induced color changes of Au nanoparticles in aqueous solutions as an optical sensor toward the detection of OP pesticides. Gold nanoparticles of three different sizes capped with citrate were synthesized. Citrate is a negatively charged weak capping ligand. On the other hand, gold is known to have a high affinity with sulfur atoms because of their soft character. Thus, when the thion pesticides containing sulfur atoms come in contact with the gold nanoparticles, the sulfur atoms will replace the negatively charged citrate ligand and bind to the gold surface releasing the capped citrate into the solution. This leads to reduction in the Coulombic repulsion barrier between two gold nanoparticles, which brings them in close proximity to each other, resulting in aggregation of Au nanoparticles accompanied by SPR shifts and visible color changes which can be readily detected by the naked eye.
Synthesis of aqueous suspensions of Au NPs involves the chemical reduction of HAuCl$_4$ solution by tri-sodium citrate to form gold crystals, which grow to form gold nanoparticles by the process of Ostwald ripening.$^{13}$ Varying the temperature and the ratio of HAuCl$_4$ and citrate used, three different sizes of Au NPs were obtained. The interaction of these synthesized Au NPs was then investigated with three OP compounds, namely, ethion, malathion, and fenthion. Figure 36 shows the detection scheme of OP pesticides using the citrate capped gold nanoparticles.

Figure 36 Detection scheme for OP pesticides with gold nanoparticles capped with citrate. Au NPs are stabilized with citrate which is a negatively charged weak capping ligand. Upon introduction of OP (small green circles) into the solution, nanoparticle aggregation is induced, which is manifested as a visible color change in the solution.
The novel method of sensing developed here has overcome several drawbacks over the conventional methods of OP pesticides detection and offer three essential features. (i) Firstly, the method is very simple and inexpensive since the synthesized Au NPs do not need to through the tedious processes of functionalizing the gold surface. Gold nanoparticles capped with citrate are very simple to synthesize, and also possess high reproducibility and monodispersity. (ii) Secondly, the method was found to be very selective toward specific pesticides, different optical signals were obtained with each of the three OP compounds. (iii) Finally, the experiments involve water as the solvent medium, thus ensuring proper functioning of the sensors in aqueous environment. Figure 37 shows the structures of the OP compounds investigated in this work.

**Figure 37** Structures of the investigated Organophosphorus Pesticides.
3.2 Experimental Section

3.2.1 Materials

Sodium citrate, sodium borohydride, HAuCl₄.3H₂O, ethion, malathion, and fenthion were purchased from Aldrich chemicals and were used with no further purification. Deionized Milli-Q water at a pH of 7 was used to prepare all the aqueous solutions. Glassware were cleaned with acetone and dried in an oven before use.

3.2.2 Transmission Electron Microscopy (TEM)

Gold nanoparticles were imaged using a JEOL transmission electron microscope (TEM), Model JEM-1230, Voltage 80 kV. Samples were prepared for electron microscopy by evaporating 1µl of the nanoparticle solution on formvar-coated copper grids.

3.2.3 UV-visible Absorption Spectroscopy

A Varian Cary 50 UV-visible absorbance spectrophotometer was used to measure absorbance spectra. Quartz cuvettes with a 1 cm path length were used for all measurements. For a typical titration experiment, 1µL aliquots of the OP
pesticides (ethion: $1 \times 10^{-6}$ M, malathion: $1.2 \times 10^{-6}$ M, and fenthion: $1.4 \times 10^{-6}$ M) were titrated to a 3 mL solution of the Au nanoparticle solution and SPR shifts were monitored until no change occurred. All titrations were performed at room temperature.

3.2.4 Synthesis of 4 nm Au Nanoparticles

Gold nanoparticles less than 4 nm were synthesized following an established procedure.\textsuperscript{28} Briefly, in a clean Erlenmeyer flask, 18.5 mL of deionized milli-Q water, 0.5 mL of a $1.0 \times 10^{-2}$ M aqueous HAuCl$_4$.3H$_2$O solution, and 0.5 ml of a $1.0 \times 10^{-2}$ M aqueous sodium citrate solution were placed. The resulting yellow colored solution was stirred for 2 min, and then 0.5 mL of a $1.0 \times 10^{-2}$ M freshly prepared aqueous NaBH$_4$ solution was added to it. Stirring was stopped as soon as NaBH$_4$ was added to the solution resulting in a brownish red solution. This reaction resulted in the production of approximately 4 nm Au particles which was confirmed by transmission electron microscopy (TEM) as shown in Figure 38. The plasmon resonance band of these nanoparticles showed a maximum at 520 nm.
3.2.5 Synthesis of 13 and 30 nm Au Nanoparticles

Gold nanoparticles with average diameter 13 and 30 nm were synthesized following a modified of published procedure.\textsuperscript{29} Briefly, 95 mL of 1.21\times10^{-6} M HAuCl\textsubscript{4}.3H\textsubscript{2}O solution was heated at 75°C. While stirring this solution vigorously, 5 mL of 3.4\times10^{-4} M sodium citrate was added. After about a minute a faint pink colored solution was obtained, which gradually darkened over a period of 5 min. The resulting color of the solution was wine red. 30 nm gold nanoparticles were synthesized using the same procedure except the solution was heated at 85°C. TEM images confirmed that the resulting gold nanoparticles had average diameters of 13 and 30 nm respectively. The plasmon resonance band showed a maximum at 520 nm and 525 nm for 13 and 30 nm Au NPs, respectively.

**Figure 38** (A) TEM images of 4 nm gold nanoparticles (B) UV-visible absorbance spectrum for 4 nm gold nanoparticles (inset showing the color of the 4 nm Au NP solution).
**Figure 39** (A) TEM images of 13 nm gold nanoparticles (B) UV-visible absorbance spectrum for 13 nm gold nanoparticles (inset showing the color of the 13 nm Au NP solution).

**Figure 40** (A) TEM images of 30 nm gold nanoparticles (B) UV-visible absorbance spectrum for 30 nm gold nanoparticles (inset showing the color of the 30 nm Au NP solution).
3.3 Results and Discussion

The interaction of three different sizes of Au nanoparticles with the OP pesticides ethion, malathion, and fenthion was investigated. Citrate is a negatively charged capping ligand. On the other hand, gold is known to have a high affinity to bond with sulfur atoms because of their soft characters. Thus, when the thion pesticides containing several sulfur atoms come in contact with the gold nanoparticles, the sulfur atoms bind to the gold surface releasing the capped citrate into the solution. This leads into aggregation of Au nanoparticles because of the reduction in Coulombic repulsion between nanoparticles, resulting in visible color changes accompanied by SPR shifts.\textsuperscript{3-9}

3.3.1 Interaction of the 4 nm Gold Nanoparticles with Ethion, Malathion, and Fenthion

The interaction of each of the OP pesticides with 4 nm sized Au nanoparticles was investigated. 3 mL of the 4 nm Au NP solution was individually titrated with ethion, malathion, and fenthion, and the changes in the Au NP SPR were monitored by UV-visible absorbance spectroscopy. As shown in Figure 41, the addition of ethion in 1 µL (1×10\textsuperscript{-6} M) increments to the 4 nm Au NP solution resulted in the decrease in the intensity of the 520 nm SPR peak, accompanied by the formation of a new peak at 790 nm. Increase in ethion concentration resulted in broadening of the 790 nm peak accompanied by red shifts. The color of the final solution was purple
indicating particle aggregation. Transmission electron micrograph of the Au nanoparticles after their interaction with ethion showed aggregation.

Figure 41 (A) UV-visible absorbance spectra of 4 nm Au nanoparticles in the presence of increasing concentrations of ethion. From top to bottom, [ethion] = 1, 2.1, 3.2, 4.2, 5.3, 6.3, 7.4, 8.5, 9.5, and 10.6 µM, respectively. (B) Plot of [Ethion] vs. change in SPR at 520 nm.
Titration of malathion in 1 µL (1.2×10^{-6} M) increments with the 4 nm Au NP solution produced similar results as with ethion. However, in this case, as shown in figure 42, addition of malathion resulted in a decrease in the intensity of the 520 nm Au NP SPR peak, accompanied by formation of a new peak at 770 nm. Increase in malathion concentration resulted in broadening of the 770 nm peak accompanied by red shifts. The color of the final solution was bluish black indicating particle aggregation. Transmission electron micrograph of the Au nanoparticles after their interaction with malathion showed aggregation.

Figure 42 (A) UV-visible absorbance spectra of 4 nm Au nanoparticles in the presence of increasing concentrations of malathion. From top to bottom, [malathion] = 1.2, 2.4, 3.6, 4.8, 5.9, 7.1, 8.3, 9.5, 10.7, and 11.9 µM, respectively. (B) A plot of [Malathion] vs. change in SPR at 520 nm.
Titration of fenthion in 1 µL (1.4×10⁻⁶ M) increments with the 4 nm Au NP solution produced exactly similar results as obtained with malathion. In this case, as shown in figure 43, addition of fenthion resulted in a decrease in the intensity of the 520 nm Au NP SPR peak, accompanied by formation of a new peak at 770 nm. Increase in fenthion concentration resulted in broadening of the 770 nm peak accompanied by red shifts. The color of the final solution was bluish black indicating particle aggregation. Transmission electron micrograph of the Au nanoparticles after their interaction with fenthion showed aggregation.
Figure 43 (A) UV-visible absorbance spectra of 4 nm Au nanoparticles in the presence of increasing concentrations of fenthion. From top to bottom, [fenthion] = 1.4, 2.8, 4.3, 5.7, 7.1, 8.5, 9.9, 11.36, 12.8, and 14.2 µM, respectively. (B) Plot of [Fenthion]*10^-6 M versus change in SPR at 520 nm.
Figure 44 shows the aggregation of the 4 nm Au NPs after titration with ethion, malathion, and fenthion, and figure 45 shows the color changes in solution of 4 nm Au NPs after addition of the OP pesticides.

Figure 44 TEM image of (A) 4 nm Au NPs in the absence of any OP pesticides, and (B), (C), and (D) after titration with ethion, malathion, and fenthion respectively, indicating particle aggregation.
3.3.2 Interaction of the 13 nm Gold Nanoparticles with Ethion, Malathion, and Fenthion

The interaction of three OP pesticides with 13 nm Au nanoparticles was investigated in a similar fashion by monitoring the changes in the Au NP SPR using UV-visible absorbance spectroscopy. 3 mL of the 13 nm Au NP solution was individually titrated with ethion, malathion, and fenthion. However, 13 nm Au nanoparticles was found to be very selective in terms of binding with ethion, malathion, and fenthion, producing different colors of solutions with different $\lambda_{\text{max}}$ which could also be differentiated visually by naked eye.

As shown in figure 46 A, increase of ethion concentration in 1µL (1×10^{-6} M) increments to a solution of 13 nm Au NPs resulted in a decrease of the 520 nm resonance absorption band. Furthermore, a new broad peak appears at 700 nm and is accompanied by red shifts as more ethion is introduced into the solution. The color of
the final solution was bluish black. No precipitation or cloudiness was observed. A plot of the red-shift in the visible extinction band maximum of the gold nanoparticle concentration versus [Ethion] reveals a linear relationship (Figure 46 B), indicating that this system can be used to quantitatively detect ethion in an aqueous medium. Transmission electron microscopy clearly confirmed that particle aggregation had occurred upon ethion addition.
Figure 46 (A) Changes in the SPR peak of 13 nm Au nanoparticles titrated with ethion. From top to bottom, [ethion] = 0, 1, 2.1, 3.2, 4.2, 5.3, 6.3, 7.4, 8.5, 9.5, and 10.6 µM, respectively. (B) Plot of [Ethion] vs. change in SPR at 520 nm.
Addition of malathion to the 13 nm Au nanoparticles in 1 µL (1.2×10^{-6} M) increments did not show an immediate change in the Au SPR peak. Also, there was no change in the color of the solution at the end of titration, and TEM images did not show any significant changes with malathion addition. However, after 1 day, the 520 nm absorption peak decreased in intensity accompanied by formation of a new peak forms at 670 nm indicating particle aggregation. The color of the solution also changed to blue further confirming that the 13 nm Au nanoparticles were aggregated due to their interaction with malathion. We note that the 13 nm Au NPs in the absence of malathion were stable for days, at room temperature.

![Absorbance vs Wavelength graph](image)

**Figure 47** Changes in the SPR peak of 13 nm Au nanoparticles with increase in malathion concentrations. From top to bottom, [malathion] = 1.2, 2.4, 3.6, 4.8, 5.9, 7.1, 8.3, 9.5, 10.7, 11.9 µM, and after 1 day respectively.
Addition of fenthion to a solution of 13 nm Au NPs in 1 µL (1.4x10^{-6} M) increments resulted in a red-shift of the SPR from 520 nm to 530 nm, accompanied by formation of a new peak at 640 nm. At the end of the titration, the color of the solution was purple. TEM images of the Au NPs after titration with fenthion showed particle aggregation.

![Graph showing changes in SPR peak](image)

**Figure 48** Changes in the SPR peak of 13 nm Au nanoparticles with increase in fenthion concentrations. From top to bottom, [fenthion] = 1.4, 2.8, 4.3, 5.7, 7.1, 8.5, 9.9, 11.36, 12.8, and 14.2 µM, respectively. (B) Plot of [Fenthion] versus change in SPR at 520 nm.
Figure 49 shows the TEM images of the 13 nm Au NPs before and after titration with the different OP pesticides, and figure 3.15 shows the color changes in solution of 13 nm Au NPs after addition of the OP pesticides.

\[
\begin{array}{cc}
\text{A} & \text{B} \\
\text{C} & \text{D}
\end{array}
\]

**Figure 49** TEM image of (A) 13 nm Au NPs before, and (B), (C), and (D) after titration with ethion, malathion, and fenthion respectively. Particles have aggregated after titration.
3.3.3 Interaction of the 30 nm Gold Nanoparticles with Ethion, Malathion, and Fenthion

The interaction of the three OP pesticides was investigated with 30 nm Au nanoparticles in a similar fashion. 3 mL of the 30 nm Au NP solution was individually titrated with ethion, malathion, and fenthion. In each case no immediate changes in the SPR peak or color of the 30 nm Au NP solution was observed. TEM images of the 30 nm Au NPs taken after ~ 6 hours showed no indication of aggregation. However, after 24 hours, the Au NP solutions left at room temperature containing ethion and fenthion changed from wine red to purple with a decrease in intensity of the 520 nm SPR peak. No changes were observed with malathion.
Figure 51 UV-visible absorbance spectra of 30 nm Au nanoparticles with increasing concentrations of ethion. From top to bottom, [ethion] = 1, 2.1, 3.2, 4.2, 5.3, 6.3, 7.4, 8.5, 9.5, and 10.6 µM, respectively.
Figure 52 UV-visible absorbance spectra of 30 nm Au nanoparticles with increasing concentrations of malathion. From top to bottom, [malathion] = 1.2, 2.4, 3.6, 4.8, 5.9, 7.1, 8.3, 9.5, 10.7, and 11.9 µM, respectively.
Figure 53 UV-visible absorbance spectra of 30 nm Au nanoparticles with increasing concentrations of fenthion. From top to bottom, [fenthion] = 1.4, 2.8, 4.3, 5.7, 7.1, 8.5, 9.9, 11.36, 12.8, and 14.2 µM, respectively.

Figure 54 TEM images of the ~30nm Au NPs before addition of OP. No changes were observed in the images after addition of OPs.
3.4 Summary

In summary, we have demonstrated a new design of nanosensors which can distinguish between the organophosphorus pesticides ethion, malathion, and fenthion based on different sizes of gold nanoparticles. The results are summarized in Table 5.

<table>
<thead>
<tr>
<th>Au NP size</th>
<th>SPR shifts with ethion</th>
<th>SPR shifts with malathion</th>
<th>SPR shifts with fenthion</th>
<th>Detection limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 nm</td>
<td>775 nm</td>
<td>790 nm</td>
<td>790 nm</td>
<td>$10^{-6}$ M</td>
</tr>
<tr>
<td>13 nm</td>
<td>700 nm</td>
<td>520 nm (no changes immediately) 670 nm (after 1 day)</td>
<td>525 nm (shoulder like peak forms ~640 nm)</td>
<td>$10^{-6}$ M</td>
</tr>
<tr>
<td>30 nm</td>
<td>No changes immediately</td>
<td>No changes even after several days</td>
<td>No changes immediately</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 5 Summary of results for the interaction of the Au NPs of three different diameters with the OP pesticides.

Thus, as it can be seen, the 13 nm gold nanoparticles are very selective in binding with OP pesticides resulting in different $\lambda_{\text{max}}$ with each of them, whereas the 4 nm particles although is sensitive toward OP addition, however it lacks from selectivity; Figure 55 illustrates this data.
Figure 55 (A) SPR shifts in 4 nm Au NPs caused by ethion, malathion, and fenthion. (B) SPR shifts in 13 nm Au NPs caused by ethion, malathion, and fenthion.
The observation that the 4 nm and 13 nm Au particles are more sensitive to OP pesticides in comparison to 30 nm particles can be understood from simple surface area arguments. 4 nm nanoparticles have a surface area of ~50 nm$^2$, whereas 13 nm and 30 nm particles have surface areas of ~531 nm$^2$ and ~2820 nm$^2$ respectively. Hence fewer molecules of OP pesticides are required to aggregate the smaller 4 nm and 13 nm nanoparticles. However, 4 nm Au NPs are so small that it starts to show aggregation instantaneously with addition of all the three OP pesticides that have been investigated here. On the contrary, the surface area of 30 nm Au NPs is so large that it requires more than a day to aggregate. But 13 nm Au NPs were found to be very selective in interaction with the OP pesticides producing different color changes with different $\lambda_{\text{max}}$.

The selectivity in interaction can also be accounted from the point of consideration of the individual OP pesticide structures. Ethion possesses four sulfur atoms in its structure, and hence can bind with more than one Au nanoparticle simultaneously at a time bringing the nanoparticles closer which leads to instantaneous aggregation with both 4 nm and 13 nm Au NP. Fenthion possesses two sulfur atoms binding with two Au nanoparticle and bringing them closer enough to cause aggregation. Due to smaller surface area in 4 nm Au NPs, the aggregation with fenthion occurs instantaneously, but in the case of 13 nm Au NPs, interaction is much slower, taking more time (one day to develop a peak at longer wavelengths at 640 nm), although the change in color of the solution was evident instantaneously with addition of fenthion to 13 nm Au NP. On the other hand, the position of sulfur atoms in malathion is such that it does not enable to bind with more than one Au
nanoparticle simultaneously at a time because of steric hindrance, and hence the interaction of malathion with 13 nm Au NP was found to be very slow, visible changes not being observed before a day. In order to confirm that Au-S interaction is taking place, titration experiments were carried out with paraoxon, containing no sulfur atom in its structure. It was observed that none of the three sizes of Au NPs were able to produce changes in color and UV-visible absorbance spectrum with paraoxon indicating that the presence of sulfur atoms causes the Au NPs to aggregate and produce visible color changes.

![Figure 56 Structure of Paraoxon.](image)

3.5 Conclusion

We have shown that Au NPs can detect and discriminate OP pesticides in an aqueous environment. The detection is based on different color changes of Au nanoparticles induced by OP pesticides producing wavelength-specific surface plasmon resonance in the UV-visible spectra. 13 nm Au NPs were found to be very selective toward binding OP pesticides producing specific color changes and $\lambda_{\text{max}}$ with each of the pesticides. 4 nm Au NP was also found to be very sensitive toward OP addition; however the very small surface area limits its selectivity toward OP
pesticides. On the other hand, nanoparticles of larger diameter ~ 30 nm are limited to provide 'real-time' results, which restrict its application on the field. Thus, the effectiveness of the Au NPs toward OP detection is dependant on the particle size. The system developed here can be effectively used to detect concentrations of OP pesticides in the \(\sim 10^{-6}\) M range and can be further exploited in future for selective detection of organophosphorus pesticides.
3.6 References

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