Nitrogen Heterocyclic Compounds as Receptors in Nanosensors for Nerve Gas Agent Analogs

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NITROGEN HETEROCYCLIC COMPOUNDS AS RECEPTORS IN NANOSENSORS FOR NERVE GAS AGENT ANALOGS

by

Swapna Katram

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

Western Michigan University
Kalamazoo, Michigan
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Swapna Katram
NITROGEN HETEROCYCLIC COMPOUNDS AS RECEPTORS IN NANOSENSORS FOR NERVE GAS AGENT ANALOGS

Swapna Katram, M.S.
Western Michigan University, 2007

The research is focused on developing nanosensors consisting of different components that are chemically linked, namely, nanoparticle, fluorescent monomer, and receptor (NMR sensors) and nanoparticle, monomer, nanomolecule, and receptor (NMNR sensors) for the sensitive and selective detection of nerve gas agents. These sensors detect the target toxins by fluorescence change which is amplified by signal transduction. Model nerve gas toxins (DCP) and (DMMP) and HCl were employed as analytes, and the nitrogen heterocyclic compounds, 5-aminoindazole and dipyrido[3,2-a:2',3'-c]phenazine (dppz) as receptors in the NMR and NNMR nanosensors. The monomers were (E)-4-(4-formylstyryl) benzoic acid (NMR sensor) and 2-mercaptosuccinic acid (NNMR sensor). The nanoparticles used were quantum dots which were Zn, Cd alloys with Zn:Cd ratios of 1:1 ZnS:CdS (NMR) and core shell quantum dots, ZnS:Mn/ZnS (NNMR). The NMR sensors (Sensor I) were more effective for the detection of DCP and HCl than the NNMR sensors (Sensor II).
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CHAPTER I

INTRODUCTION

1.1 Chemical warfare agents

Chemical warfare agents are defined as chemical substances which can be used either in the liquid, solid or gaseous form to kill people. They have been classified as nerve vesicant, or blood borne agents. The nerve agents- tabun, sarin and soman (figure 1.1) act by disrupting the nervous system through the inhibition of a key enzyme, acetyl cholinesterase. The vesicant agents such as sulfur mustard gas, lewisite, nitrogen mustard gas and phosgene oxime are responsible for blistering action. Blood borne agents-such as prussic acid or cyanogens chloride prevent utilization of oxygen by tissues through inhibition of cytochrome oxidase [1, 2]. Among these chemical warfare agents, nerve agents are considered to be the most dangerous chemical warfare agents and nearly the ideal terrorist weapon. Nerve agents are organophosphates, esters of phosphoric acid chemically related to many commercially available pesticides.

1.2 History of nerve gas agents

The use of chemical weapons started as early as the First World War. For example on Apr 22, 1915 near the town of Ypresi, Belgium, German forces opened nearly 6000 cylinders of nerve chlorine gas on opposing French troops [3]. The
development of chemical weapons during the World War II has lead to the discovery of so called nerve agents. In 1936 Gerhard Schrader, a German scientist while working towards his aim of improved insecticides, accidentally discovered the first agents of nerve gases, tabun (figure 1.1). He quickly recognized the potential military application of this chemical and the development of nerve agents as weapons soon began. Eventually sarin and soman (figure 1.1) were secretly developed in Germany during World War II. In spite of possessing tons of these nerve agents, German forces chose not to use them during the World War II due to reasons that were not very clear.

After the war in the early 1950s Great Britain had synthesized the second generation of nerve agents, V-series and was later weaponized by the US. Several countries had stockpiled these compounds in the decades that followed and the first documented use of nerve agents took place during 1984-1988 when the Iraqi forces used them against Iranian forces and then in 1988 against Iraqi Kurds [4]. Later nerve agents were reportedly used in terrorist attacks on June 27, 1994 in a residential area in Mastumoto, Japan [5]. Again in March 1995, the Aum Shinrikyo cult used sarin gas in terrorist attacks in a Tokyo subway and caused several deaths [6].

1.3 Classification of nerve gas agents

There are two main classes of nerve agents, G-series and V-series. G-series are considered as the first generation of nerve agents and were first synthesized by Gerhard Schrader. G agents are derivatives of phosphoramidocyanidic or methylphosphono
fluridic acid and include tabun (ethyl-N-dimethyl phosphoramidocyianidate), sarin (isopropyl methyl phosphonofluoridate) and soman (pinacolyl methylphosphonofluoridate). They are also called by a two letter name designated by NATO as GA, GB and GD, respectively [7].

![Tabun, Sarin, Soman](image)

Figure 1.1 Various nerve gas agents

V agents ("V" for venomous) are derivatives of methylphosphonothioic acid and include VX (ethyl S-2-idisopropyl amino ethyl methyl phosphorothiolate). VX is ten times as toxic as G agents and is also more persistent. Most likely route of exposure for most nerve agents is inhalation. VX is the exception, it is the least volatile hence can be absorbed through skin and can persist on surfaces for hours, days or weeks. LD-50 values of some of the nerve agents are listed in Table 1.1.
<table>
<thead>
<tr>
<th>Name</th>
<th>First made (year)</th>
<th>Lethal dose Breathing (mg × min/m³)</th>
<th>Lethal dose Skin (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tabun (GA)</td>
<td>1936</td>
<td>150-400</td>
<td>1,000-1700</td>
</tr>
<tr>
<td>Sarin (GB)</td>
<td>1938</td>
<td>75-100</td>
<td>1,000-1700</td>
</tr>
<tr>
<td>Soman (GD)</td>
<td>1944</td>
<td>35-50</td>
<td>50-100</td>
</tr>
<tr>
<td>VX</td>
<td>1952</td>
<td>10</td>
<td>6-10</td>
</tr>
</tbody>
</table>

Table 1.1 LD-50 values of various nerve gas agents

1.4 Mechanism of action of nerve gas agents

Figure 1.2 Mechanism of action of nerve gas in humans [8]
As their name implies nerve agents attack the nervous system of the human body. As shown in figure 1.2, under normal conditions when a nerve cell is triggered it releases a neurotransmitter called acetylcholine (figure 1.3), which helps in the transmission of nerve impulse to a muscle or organ. Once the impulse is sent, acetylcholine is hydrolyzed by an enzyme, acetylcholine esterase in order to allow the muscle or organ to relax [3, 9, 10]. However it can be seen from figure 1.2 that in the presence of a nerve gas agent this normal transmission of nerve cell is disrupted as nerve agents inactivate the enzyme, acetylcholine esterase by covalently binding to the hydroxyl group of the amino acid, serine present in the active site of the enzyme. This results in build up of acetylcholine and it continues to act so any nerve impulses are continuously transmitted. As a result, muscle contractions do not stop. This can lead to paralysis of the central nervous system and eventually to death depending upon dose levels. Most common treatments available when exposed to low levels of nerve agents include use of nerve gas antidotes such as atropine, anticholinergic drugs [9, 10].
Various methods used in the literature for the detection of nerve gas agents

There is a potential threat that these nerve agents could be used in terrorist attacks and it is essential to have a sensitive and selective sensor system to protect civilians and military personnel from attack by nerve agents. As a consequence intense research has been focused on developing methods to detect the presence of these compounds at very low concentrations levels, with minimum fault signals.

There have been many analytical methods for the detection of nerve agents. Some of these methods are described here. Enzyme based detection involves, acetyl and butyryl cholinesterase and organophosphorous hydrolase (OPH-an enzyme capable of hydrolyzing various nerve agents). These enzymes were adsorbed onto some solid support and when exposed to nerve gas sample influenced the formation of hydrolysis products, which are detected or measured through various devices [11-14]. However enzyme based sensors suffer from some disadvantages such as loss of stability and sensitivity resulting from contaminations in the samples that destroy the enzyme activity. Also enzyme based sensors exhibit longer response times due to relatively slow hydrolysis kinetics. Another detection method that makes use of transition metal ions and amino acids is based on a microcantilever. In this work, the gold surface of the microcantilever was coated with Cu$^{2+}$/L-cysteine bi-layer. As a result of strong affinity between P=O and Cu$^{2+}$, nerve gas analogs were absorbed on to the gold surface where they could be detected by the deflection of microcantilever due to a change in its surface tension [15]. A surface acoustic wave (SAW) based sensor involves the change in
frequency of the SAW sensor upon exposure to a nerve gas analog. In particular acid hydroxyl groups (-OH) of the polymers, that are coated on the SAW sensor, interact with nerve agent analog causing a mass increase followed by a change in the frequency of the sensor [16]. Techniques such as gas chromatography (GC) [17], ion mobility time-of-flight mass spectrometry [18] and infrared spectroscopy [19], interferometry [20] are used to analyze the presence of nerve agents in water. All these methods are found to be sensitive and reliable but have significant disadvantages. They are time consuming, expensive and require skilled personnel for operation. Consequently methods have been sought to develop simpler means of detection with functional group specific chemosensors based on fluorescence spectroscopy such as the one developed by Swager, et al. [21]. These sensors have receptor molecules such as thienylpyridyl and phenylpyridyl that contain a primary alcohol group in very close proximity to a tertiary amine. Reaction of hydroxyl group of these receptors with the nerve agents results in formation of a phosphate ester, which is a good leaving group when compared to the free hydroxyl group. This leads to intramolecular cyclization of the receptors and results in a change in the fluorescence emission intensity as well as emission maxima which can be monitored with the use of a spectrofluorimeter. They have been able to detect micromolar (µM) concentrations of nerve agent analogs using these receptors [21].

Similarly Rebek, et al. [22] demonstrated a fluorescence sensor which is based on an extension of the concept developed by Swager [22]. These receptors also have a primary alcohol group located near a tertiary amine group. In addition, these receptors have a well known fluorophore, pyrene appended near the amine group. In the absence of
a nerve agent analog there is a photoinduced electron transfer (PET) from the amine group of the receptor to pyrene which results in quenching of fluorescence emission of the latter. Interaction of nerve agent with these receptors makes the lone pair electrons on nitrogen unavailable for photoinduced electron transfer and thus the emission of pyrene is restored, which forms the basis for their detection of nerve agents. In other words this sensor acts as a “switch on” sensor for nerve agent [22].

Toxicity of nerve gas agents arises from their ability to undergo a nucleophilic attack by hydroxyl group of the amino acid, serine, present in the active site of the enzyme, acetyl cholinesterase. Sensors with receptors possessing hydroxyl group as interacting group with the phosphorous atom of nerve agent analogs shown to be sensitive, however, they suffer from oxygen-phosphorous interaction being irreversible [21, 22].

The present study deals with synthesis and characterization of nanosensors with nitrogen heterocyclic compounds as receptors for the detection of nerve gas agent analogs. The nanosensors have 3 to 4 components namely, nanoparticle, nanomolecule, monomer and receptor (figure 1.4). The nanoparticles chosen for the present study are quantum dots. Quantum dots are semiconductor nanoparticles with unique photophysical properties [23, 24]. More detailed description of quantum dots and their application in sensors are discussed under section 1.8, chapter I. The receptors are nitrogen heterocyclic compounds and are fluorescent by nature and are attached to photo luminescent quantum dots through either a fluorescent monomer (sensor I) or through a thiol linker (sensor II).
A fluorescence change accompanying the interaction of the receptor part of a nanosensor with a target molecule could be amplified by other components that do not have significant interaction with the target molecule. Thus the concept is based on the construction of nanosensors capable of signal transduction through \( \pi-\pi \) interaction or ionic interactions between quantum dots and other components of nanosensor. [25, 26, 27].

1.6 Central hypothesis

Nitrogen heterocyclic compounds and their complexes are appropriate receptors for the detection of nerve gas agents and their analogs. Integration of such receptors in nanosensor composites such as NMR and NNMR sensors could provide sensitive and selective sensors for the detection and quantitation of nerve gases and their analogs through signal transduction.

![Figure 1.4 General Nanoparticle, Nanomolecule, Monomer and Receptor (NNMR) concept](image)

Two types of sensors have been designed for the present study, sensor I and sensor II.
1.7 Sensor I, NMR (Nanoparticle, Monomer and Receptor)

Sensor I consists of 3 components termed as receptor, monomer and nanoparticle (NMR).

![Diagram of Sensor I components: Nanoparticle - Zn_{0.5}Cd_{0.5}S quantum dot, Fluorescent monomer - (E)-4-(4-formylstyryl) benzoic acid (FSB), Receptor - 1H-indazol-5-amine.]

Figure 1.5 Sensor I- Nanoparticle, Monomer and Receptor (NMR)

Figure 1.5 shows the schematic representation of sensor I and structures of components of sensor I are shown in figure 1.6. Previous work from our lab indicates that the nitrogen containing aromatic compounds such as isoquinoline amine have good interaction with the nerve agent analogs and moreover they have been shown to have reversible interaction with nerve agent analogs [7]. Also receptors containing, amide bonds [28-30], imidazole rings [31], have been shown to have good association with anionic and
Figure 1.6 Structures of components of sensor I

Neutral phosphates. Thus, the receptor chosen for sensor I is a nitrogen heterocyclic compound named as 1H-indazol-5-amine (5-IA). This is covalently linked to a fluorescent monomer, (E)-4-(4-formylstyryl) benzoic acid (FSB) through an imine bond to give rise to a Schiff base adduct, 4-(4-((Z)-(1H-indazol-5-ylimino) methyl) styryl) benzoic acid (ISBA). ISBA is then attached to a nanoparticle, Zn_{0.5}Cd_{0.5}S alloy quantum dots through ionic interactions between carboxylic acid and metal cations of Zn_{0.5}Cd_{0.5}S quantum dots [32].
Quantum dots and their use in various sensor applications

Quantum dots are semiconductor nanoparticles with unique photophysical properties. In particular they have broad absorption spectra and size tunable photoluminescent spectra. Broad absorption of the quantum dots continues from the visible to the ultraviolet region, as a result they can be excited over a wider selection of wavelengths [23-27]. In addition luminescence lifetime of quantum dots is one order of magnitude larger than those of the organic fluorophores. As a result many researchers have been using them in sensing schemes to signal specific analytes on the basis of electron (photoinduced electron transfer-PET) and energy transfer (fluorescence resonance energy transfer-FRET). Quantum dots can act as electron acceptors or donors through PET from a wide variety of ligands or metal ions [33-36]. These processes lead to quenching of quantum dot luminescence and, thus, can be exploited to regulate their emissive behavior. In particular sensing schemes can be designed to activate or suppress a photoinduced electron transfer process switching the luminescence off or on, respectively. For example the recognition properties of maltose-binding protein (MBP) can be engineered to switch the luminescence of a conjugated quantum dot on the basis of PET. In this study, a chimeric MBP-metallothionin with surface cysteine residues was conjugated to (tetraamine)-(5maleido-phenanthroline) ruthenium(II). This modified protein was then attached to CdSe quantum dots capped with 6-mercaptohexadecanoate. The association of MBP of this assembly with maltose moves the ruthenium away from the CdSe, thereby preventing the electron transfer process and switching on the luminescence of CdSe quantum dot [35].
Semiconductor quantum dots can also be involved in energy transfer processes through FRET (fluorescence resonance energy transfer). In most cases they have been used as energy donors in FRET experiments. For example, a CdSe-ZnS core-shell quantum dot, coated by dihydrolipoic acid was conjugated to an antibody for sensing of an explosive chemical, TNT (2,4,6-trinitrotoluene). This particular antibody has an oligohistidine residue, which binds the zinc cations on the surface of the quantum dots. The addition of a dye labeled TNT analog results in the association of its 2,4,6-trinitrophenyl appendage with the antibody-nanoparticle assembly. The dye absorbs in the same range of wavelengths where the quantum dot emits, as a result, the excited quantum dot transfers energy to this particular dye, which is not emissive and dissipates the transferred energy nonradiatively. This results in a decrease in the luminescence intensity of the quantum dot. However the association of TNT with the antibody moves the dye away from the quantum dot, preventing the energy transfer process and switching the luminescence of the quantum dot, which increases significantly with the concentration of TNT [36]. On the other hand very few studies have dealt with the use of quantum dots as energy acceptors. For example lanthanide metals such as europium and terbium have been demonstrated to be energy donors, to semiconductor CdSe/ZnS core/shell quantum dots [34].

Similarly for the present study alloy quantum dots, Zn$_{0.5}$Cd$_{0.5}$S have been chosen as nanoparticle for sensor I, and core shell quantum dots, ZnS:Mn/ZnS for sensor II as
the spectral properties of these quantum dots are complimentary to the spectral properties of the components of these sensors.

1.9 Sensor II, NNMR (Nanoparticle, Nanomolecule and Receptor)

![Figure 1.7 Sensor II - Nanoparticle, Nanomolecule, Monomer, Receptor (NNMR)](image)

Figure 1.7 shows the schematic representation of Sensor II and structures of components of sensor II are shown in figure 1.8. Similar to receptor of Sensor I, the receptor chosen for Sensor II is also a heterocyclic nitrogen containing compound, dipyrido[3,2-a:2',3'-c]phenazine (dppz) shown in figure 1.8. Dppz is an interesting ligand that has attracted
Figure 1.8 Structures of components of sensor II

growing attention in recent years. Due to its extended π-conjugation and planarity, it has been used as a DNA intercalator in many studies [37, 38]. Recent works have focused on the synthesis of metal complexes containing dppz ligand to function as metallo intercalators and DNA photoswitch [39]. For the present study it has been chosen as the receptor as it possesses aromatic nitrogen atoms which are required to be able to sense nerve agent analogs. In addition dppz has been complexd to the metal, Zn. Metal complexes based on Zn, Eu and La have been demonstrated to be used as sensors for nerve agents [40]. Zinc complex of dppz, Zn(dppz)$_2$[PF$_6$]$_2$ was then attached to the nanoparticle (ZnS:Mn/ZnS) thorugh thiocarboxylic acid such as mercapto succinic acid as a linker. Quantum dots such as CdSeZnS and ZnS:Mn have been capped with thiols in many studies for various applications [41, 42].

The quantum dots used for sensor II are a type of doped semiconductor quantum dots ZnS:Mn/ZnS. In these materials a small amount of metal ions such Mn$^{2+}$ is incorporated into the lattice of the semiconductor, e.g, ZnS or CdS quantum dots.
Commonly denoted as ZnS:Mn or CdS:Mn. The doped particles have interesting magnetic and electro optical properties. The host semiconductor usually absorbs light and transfers energy to the metal ion, which can then emit photons with characteristic energies [43, 44, 45]. The ZnS:Mn/ZnS quantum exhibit a characteristic emission peak corresponding to the doped metal ion, Mn$^{2+}$ at 590 nm as shown in figure 3.7c.

1.10 Analytes employed for the present study

1) Diethyl chloro phosphate (DCP) shown in figure 1.9, is a nerve agent analog and is used as an analyte for studies involving nerve agent sensors [21].

2) Dimethyl methyl phosphonate (DMMP) shown in figure 1.9, which is known as simulant of nerve gas Sarin [46].

3) Hydrogen chloride (HCl), as shown in figure 1.9 is a by product of hydrolysis of DCP and hence been employed as one of the target analytes [47, 48].

\[ \text{DCP} \quad \text{DMMP} \quad \text{HCl} \]

Figure 1.9 Structures of analyte molecules
Figure 1.10 Reaction of hydrolysis of DCP

1.11 Research objectives

The research has been carried out along the following objectives

1) Construct the nanosensors sensor I and sensor II and characterize their absorption and emission properties and emission lifetimes.

2) Determine the efficacies of sensor I and sensor II for the detection of DCP, DMMP and HCl and their selectivities for the three model compounds.

3) Model the association of the model compounds with the sensors and their components to calculate the association constants to gain fundamental understanding of their interaction and signal transduction.
CHAPTER II

SENSOR I

2.1 Experimental Procedures

2.1.1 Materials

The compounds 5-aminoindazole, cadmium acetate, zinc acetate dihydrate, sodium sulfide, methanol, diethylchlorophosphosphate, dimethylmethylphosphonate were obtained from Sigma-Aldrich and used as received. Concentrated HCl was obtained from Fisher Scientific, Inc. The compound (E)-4-(4-formylstyryl) benzoic acid was prepared according to a procedure developed in our laboratory. Absolute alcohol was obtained from Aaper Alcohol. Acetonitrile HPLC grade was purchased from EM Science. All solvents used for NMR studies were obtained from Sigma-Aldrich.

2.1.2 Instruments for characterization

All compounds were characterized by $^1$H-NMR, mass spectroscopy, uv-vis spectroscopy and fluorescence spectroscopy. The $^1$H-NMR spectra were obtained with Eclipse 400 FT-NMR spectrometer with Delta NMR software. LC/MS data were collected by Schimadzu LC/MS-2010 EV High Performance Liquid Chromatograph/Mass Spectrometer with LC/MS solution software and negative
ionization method. Mass spectrum of ISBA (ionization method-Fast Atom Bombardment) was obtained with help of mass spectrometry facility at MSU, Lansing, Michigan. UV-Visible spectra were recorded using Perkin-Elmer UV-Vis Lambda 20 spectrometer and Shimadzu UV-Vis Scanning spectrometer and are reported as absorbance (A) versus wavelength \( \lambda \) (nm). Luminescence and lifetime data were collected by Edinburgh time resolved and steady state fluorescence spectrometer with F 900 data acquisition and analysis software.

2.1.3 Synthetic methods

2.1.3.1 Synthesis of 4-(Diethoxy-phosphorylmethyl)-benzoic acid (DPBA, figure 2.1)

To a solution of 1 g (0.0046 moles) of 4-(bromo methyl) benzoic acid in toluene, 1.03 mL (0.006 moles) of triethyl phosphite was added and refluxed overnight under an argon atmosphere. After reflux the white crystals that formed were filtered and dried in a vacuum desiccator and the mass of dried sample was found to be 1.00 g (80% yield) [49, 50]. Figure 2.2 shows the NMR spectrum of DPBA and the chemical shifts are, (\(^1\)H NMR 400 MHz d\(_3\)-CHCl\(_3\); TMS reference; \( \delta \) ppm); a protons in benzene ring; 8.01 (d, 2H, J=7.3Hz); b protons in benzene ring; 7.38 (d, 2H, J=2.6 Hz); c protons in ethyl group 4.05 (q, 4H, J=6.96 Hz); d protons in methylene group; 3.23 (d, 2H, J=22.3 Hz); e protons in ethyl group; 1.25(t, 6H, J=7.0 Hz). Figure 2.3 shows the mass spectrum of DPBA (LC/MS) which has a peak at m/z= 271 corresponding to the molecular ion peak, MW=272)
2.1.3.2 Synthesis of (E)-4-(4-formyl styryl) benzoic acid (FSB, figure 2.1)

The product obtained from step I, DPBA 1.37 g (0.005 moles) was dissolved in 40 mL of freshly distilled THF and to this solution 1.35 g (0.01 moles) of potassium tertiarybutoxide was added and the mixture was stirred at room temperature for 15 minutes under argon atmosphere. To this solution 0.9 mL (0.004 moles) of 4-(diethoxy methyl) benzaldehyde was added slowly and the mixture stirred at room temperature for 12 hours. After 12 hours the reaction mixture was neutralized with 6N HCl, the resulting solids were washed with H_2O/ether mixture. The pale yellow colored solids thus obtained were filtered and kept in vacuum oven for drying (yield 70%) [49, 50]. Figure 2.4 shows the \(^1\)H NMR spectrum of FSB and the chemical shifts are, \(^1\)H NMR ( 400 MHz d_3-CHCl_3; TMS reference; \(\delta\) ppm ) ; a proton of aldehyde group 10.00 (s, 1H); b protons of benzene ring 1; 7.96( d, 2H, J=8.4 Hz); b protons of benzene ring 2 ;7.94(d, 2H, J=8.4 Hz0; c protons of benzene ring 1; 7.82 (d, 2H, J= 8.4 Hz); d protons of benzene ring 2; 7.78 (d, 2H, J=8.4 Hz); e protons of double bond; 7.55( d, 2H, J=6.24). Figure 2.5 shows the mass spectrum of FSB by LC/MS which, has a peak at m/z=251 that corresponds to the molecular ion peak.

2.1.3.3 Synthesis of 4-(4-((Z)-(1H-indazole-5-ylimini) methyl) styryl) benzoic acid (ISBA, figure 2.1)

To a solution containing 0.026g (0.2 mmol) of 5-aminooindazole in 50 mL ethanol and 0.05 g (0.2 mmol) of (E)-4-(4-formylstyryl) benzoic acid 2-3 drops of glacial acetic acid were added and refluxed for 12 hrs. The yellow colored solid that formed was
collected by filtration and dried in vacuum oven overnight to give 0.065 g of the schiffbase (yield = 85%). A general procedure for the preparation of schiffbase is available in the available in the literature [51]. Figure 2.12 shows the NMR spectrum of DPBA and the chemical shifts are, $^1$H NMR (400 MHz $d_3$-CHCl$_3$; TMS reference; $\delta$ ppm ); a protons in imine group; 8.74(s, 1H); b protons in benzene ring of 5-IA ; 8.10(s, 1H); c protons in benzene ring 1; 7.98(d, 2H, J = 8.4 Hz); c protons in benzene ring 2; 7.95(d, 2H, J=8.4); d protons in benzene ring 1; 7.80(d, 2H, J=8.4 Hz); e protons in benzene ring 2; 7.75(d, 2H, J=8.4Hz); f protons in benzene ring of 5-IA ; 7.67(s, 1H, J=1.08); g protons in benzene ring of 5-IA; 7.58(d, 1H, J=8.7 Hz); h protons in benzene ring of 5-IA; 7.48(s, 1H); i protons in double bond bridge; 7.45 (d, 1H, J=1.8 Hz); 7.43 (d, 1H, J=1.8 Hz). Figure 2.13 shows the FAB mass spectrum of ISBA with a molecular ion peak at m/z=368.

2.1.3.4 Synthesis of Zn$_{0.5}$Cd$_{0.5}$S quantum dots

The Zn$_{0.5}$Cd$_{0.5}$S alloy quantum dots were synthesized according to a modified procedure available in the literature [52]. Zinc acetate (Zn (CH$_3$COO)$_2$) 0.0458 g (0.00025 moles) and 0.0576 g (0.00025 moles) of cadmium acetate, Cd(CH$_3$COO)$_2$ were taken in 50 mL of methanol and stirred until the salts were dissolved. In a separate vial 0.058g (0.00075 moles) of dried Na$_2$S was dissolved in 1 mL of mili Q water and 0.5 mL of this solution was added to the solution of zinc acetate and cadmium acetate. This solution was kept for stirring until the solids were precipitated out. The solids were then centrifuged and dried
in a vacuum desicator and later dispersed in CH$_3$CN in an ultra sonicator for spectral studies.

![Chemical structures and reactions](image)

Figure 2.1 Synthesis of ISBA
2.2 Characterization of the various components of sensor 1

2.2.1 $^1$H NMR, LC/MS characterization of DPBA

![Chemical structure of DPBA](image)

Figure 2.2 $^1$H NMR (400 MHz, DMSO-d$_6$) spectrum of DPBA
Figure 2.3 Mass spectrum of DPBA by LC/MS

2.2.2 $^1$H NMR, LC/MS and UV-Vis characterization of FSB

Figure 2.4 $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of FSB
Figure 2.5 Mass spectrum of FSB by LC/MS

Figures 2.6, 2.7 and 2.8 show the absorption spectrum, excitation spectrum and the emission spectrum of a solution of FSB in CH$_3$CN (2.4 \times 10^{-6} \text{ M}), respectively.

Figure 2.6 UV-Vis absorption spectrum of FSB in CH$_3$CN (2.4 \times 10^{-6} \text{ M})
Figure 2.7 Excitation spectrum of FSB in CH$_3$CN (2.4 x 10$^{-6}$ M), emission wavelength-428 nm

Figure 2.8 Emission spectrum of FSB in CH$_3$CN (2.4 x 10$^{-6}$ M), emission filter-345 nm, excitation wavelength-335 nm
2.2.3 UV-Vis characterization of 5-IA

Figures 2.9, 2.10 and 2.11 show the absorption spectrum, excitation spectrum and the emission spectrum of a solution of receptor, 5-IA in CH$_3$CN (3.1 × 10$^{-5}$ M), respectively. The spectral values of 5-IA in CH$_3$CN match the reported values in the literature [53, 54].

![UV-Vis absorption spectrum of 5-IA in CH$_3$CN (3.1 × 10$^{-5}$ M)](image)
Figure 2.10 Excitation spectrum of 5-IA in CH$_3$CN ($3.1 \times 10^{-5}$ M), emission wavelength-390 nm

Figure 2.11 Emission spectrum of 5-IA in CH$_3$CN ($3.1 \times 10^{-5}$ M), emission filter-345 nm, excitation wavelength-330 nm
2.2.4 $^1$H-NMR and LC/MS and UV-Vis characterization of ISBA

![Chemical Structure](image)

Figure 2.12 $^1$H-NMR (400 MHz, DMSO-d$_6$) spectrum of ISBA

![NMR Spectrum](image)

Figure 2.13 TOF (Time of Flight) mass spectrum of ISBA

![Mass Spectrum](image)
Figures 2.14, 2.15 and 2.16 show the absorption spectrum, excitation spectrum and the emission spectrum of a solution of ISBA \((1.6 \times 10^{-6} \text{ M})\) in CH\(_3\)CN respectively. The absorption, emission and the excitation maxima of ISBA did not show much difference compared to the receptor 5-IA, however when compared to FSB, which has emission maximum of 428 nm, the adduct ISBA showed a blue shift.

![Absorption Spectrum](image)

Figure 2.14 UV-Vis absorption spectrum of ISBA in CH\(_3\)CN \((1.6 \times 10^{-6} \text{ M})\)
Figure 2.15 Excitation spectrum of ISBA in CH$_3$CN (1.6 x 10$^{-6}$ M), emission filter 345 nm, excitation wavelength 335 nm.

Figure 2.16 Emission spectrum of ISBA in CH$_3$CN (1.6 x 10$^{-6}$ M), emission filter 345 nm, excitation wavelength 335 nm.
2.2.5 UV-Vis characterization of quantum dots-Zn$_{0.5}$Cd$_{0.5}$S

Alloy quantum dots, Zn$_{0.5}$Cd$_{0.5}$S were synthesized as explained in synthetic methods. Spectral properties of these quantum dots with varying compositions of Zn and Cd were extensively studied [55]. Figures 2.17, 2.18 and 2.19 show the absorption spectrum, excitation spectrum and the emission spectrum of Zn$_{0.5}$Cd$_{0.5}$S quantum dots, respectively. The absorption band edge was found to be 388 nm. They have an excitation maximum of 370 nm and show a very broad emission ranging from 450 nm to 650 nm, with an emission maximum of 540 nm.

![Absorption Spectrum](image)

Band edge 388 nm

Figure 2.17 UV-Vis absorption spectrum of Zn$_{0.5}$Cd$_{0.5}$S in CH$_3$CN
In order to make the full sensor, to a 4 mL solution of quantum dots with an absorbance of 0.35, 2 µL of (0.001 M) ISBA was added which gave a final concentration
of $5.0 \times 10^{-7}$ M of ISBA. Figures 2.20, 2.21 and 2.22 show the absorption spectrum, excitation spectrum, and the emission spectrum of full sensor I in CH$_3$CN, respectively. As shown in figure 2.20 the absorbance of full sensor (388 nm) did not show any difference compared to the absorbance of quantum dots alone (band edge 388 nm) shown in figure 2.17. Similarly as shown in figure 2.20, the excitation maxima (370 nm) has not changed when compared to the excitation maxima (370 nm) of quantum dots, shown in figure 2.18. However as shown in figure 2.22, after 2 minutes following the addition of ISBA the emission maxima of quantum dots has shifted to a shorter wavelength and also showed an emission peak corresponding to the emission of ISBA alone. Over a period of 30 minutes the emission maxima for ISBA as well quantum dots disappeared resulting in a single peak with an emission maximum of 470 nm. This shift in emission maxima indicates the adsorption of, ISBA on the surface of quantum dots.
2.2.6 UV-Vis characterization of full sensor I - Zn$_{0.5}$Cd$_{0.5}$S + ISBA

Figure 2.20 UV-Vis absorption spectrum of Zn$_{0.5}$Cd$_{0.5}$S + ISBA (0.5 x 10$^{-6}$ M) in CH$_3$CN

Figure 2.21 Excitation spectrum of Zn$_{0.5}$Cd$_{0.5}$S + ISBA (0.5 x 10$^{-6}$ M) in CH$_3$CN, emission wavelength - 470 nm
Figure 2.22 Emission spectrum of $\text{Zn}_{0.5}\text{Cd}_{0.5}\text{S} + \text{ISBA} \ (0.5 \times 10^{-6} \text{ M})$ in CH$_3$CN, emission filter-345 nm, excitation wavelength-370 nm
2.3 Study of components of Sensor I with DCP, DMMP and HCl

2.3.1 Interaction of 5-IA, FSB, ISBA with DCP

Figure 2.23 (a) Emission spectra of 5-IA ($3.1 \times 10^{-5}$ M) as a function of DCP concentration

Figure 2.23 (b) Emission spectra of FSB ($2.4 \times 10^{-6}$ M) as a function of DCP concentration
Figures 2.23 a, b and c show the emission change upon the interaction of different concentrations of the nerve gas analog, DCP with the receptor part of the Sensor I, 5-IA, the fluorescent monomer FSB and the Schiffbase adduct of 5-IA, FSB and ISBA, respectively. A solution of 5-IA (3.10 × 10⁻⁵ M) in CH₃CN was interacted with various concentrations of DCP ranging from 1.70 × 10⁻⁴ M to 1.70 × 10⁻³ M. As shown in Figure 2.23a, quenching of the fluorescence of 5-IA was observed with increasing concentrations of DCP. No change in the shape and band maxima of fluorescence spectra was observed. Similar studies on 5-IA with alkyl halides as quenchers resulted in quenching of emission intensity of 5-IA with out any changes in the shape and band maxima of its fluorescence spectra [56]. Different concentrations of DCP (4.25 × 10⁻⁵ M - 7.25 × 10⁻³ M) added to FSB (2.40 × 10⁻⁶ M), only exhibited a weak interaction as shown in figure 2.23 b. The addition of DCP (4.50 × 10⁻⁵ M to 2.45 × 10⁻⁴ M) to the Schiffbase
adduct of 5-IA and FSB, ISBA (1.6 × 10^{-6} M) resulted in quenching of the fluorescence of ISBA as displayed in figure 2.23 c. These results clearly indicate that DCP interacts more strongly with nitrogen atoms in a heterocyclic ring than it does with carboxylic acid.

2.3.2 Calculation of association constants

The interaction of the various components of Sensor I and the sensor were quantitated by assuming a 1:1 adduct between the component and the target compound as shown in equation 2.1. A 1:1 adduct is reasonable as there is one functional group (heterocyclic nitrogen or COOH) with which the target compound such as DCP could interact. The association equilibrium constants could be calculated from the fluorescence intensity change at different concentrations of the target employing the Stern-Volmer model which was developed for systems exhibiting fluorescence quenching upon the addition of a quencher. A more general model (C/I model) based only on the fluorescence intensity change with the concentration of the target compound was also developed to determine association constants from both fluorescence increase and decrease.

Each of these approaches have their strengths and weaknesses. The Stern-Volmer equation in its original and modified forms applies to fluorescence quenching and not enhancement. The traditional Stern-Volmer model assumes the quenching is due to the interaction of the excited state with the quencher and the modified Stern-Volmer model accounts for fluorescence quenching resulting from the interaction of the quencher both
with the ground and excited states of the substrate. The modified Stern-Volmer equation is employed when the simple Stern-Volmer equation results in nonlinear plots indicating a quenching mechanism where both ground and excited states are involved. The general model based only on the fluorescence intensity change with the concentration of the target compound does not differentiate between the quenching mechanisms but provides the association constant for both fluorescence increase and decrease. The association constants were calculated employing both Stern-Volmer and C/I models.

2.3.3 Stern-Volmer model

\[
\text{Target + Receptor} \overset{k_{\text{on}}}{\longrightarrow} [\text{Target-receptor}] \quad (2.1)
\]

When a fluorescent receptor interacts with the target molecule, the emission intensity of the receptor can be either quenched or enhanced. This phenomenon of quenching of emission intensity of a receptor molecule in presence of a target molecule can be explained by a traditional concept, called Stern-Volmer theory. According to this theory fluorescence intensity of a receptor molecule can be quenched through collisions between excited state receptor molecules and target molecules, which can be called as collisional quenching or dynamic quenching. In this case quenching constant, \( K \) can be calculated according the equation 2.2 which indicates a linear relationship between the ratio of the fluorescence intensity \( I_0 \) without quencher (T) and intensity with the quencher, \( I \) [57, 58].
\[
\frac{I_0}{I} = 1 + K_D [T]
\]  

(2.2)

\[
I_0 = \text{Fluorescence intensity of receptor in the absence of target}
\]

\[
I = \text{Fluorescence intensity of receptor in the presence of target at a given concentration}
\]

\[
K_D = \text{Quenching constant}
\]

\[
[T] = \text{Concentration of the target molecule}
\]

Quenching can also occur as a result of formation of a nonfluorescent ground-state complex between the receptor and target. When this complex absorbs light it returns to the ground state by losing energy nonradiatively, in which case the quenching constant \(K_{SV}\) can be calculated according to the equation (2.3)

\[
\frac{I_0}{I} = 1 + K_{SV} [T]
\]  

(2.3)

\[
I_0 = \text{Fluorescence intensity of receptor in the absence of target}
\]

\[
I = \text{Fluorescence intensity of receptor in the presence of target}
\]

\[
K_{SV} = \text{Association constant}
\]

\[
[T] = \text{Concentration of the target molecule.}
\]

It is important to note that the dependence of \(\frac{I_0}{I}\) on \([T]\) is linear both, in case of dynamic quenching and static quenching, except that in case of static quenching the quenching constant, \(K_{SV}\) is considered as the association constant.
2.3.4 Modified Stern-Volmer model

However, in many cases the emission intensity of receptor can be quenched both by collisions (dynamic quenching) and by complex formation (static quenching). In such cases the Stern-Volmer plots (equations 2.1 and 2.2) result in an upward curvature, which is concave towards the y-axis. Under these circumstances both $K_S$ and $K_D$ can be calculated according to the following equation.

\[
\frac{[I_0 / I-1]}{[T]} = (K_D + K_S) + K_D K_S [T] \tag{2.4}
\]

A plot of $[I_0 / I-1]/[T]$ versus $[T]$ results in a straight line with an intercept of $K_D+K_S$ and a slope of $K_S K_D$. The individual values for $K_S$ and $K_D$ can be calculated by solving equation 2.5.

\[
K_S = \text{Static association constants}
\]

\[
K_D = \text{Dynamic association constant}
\]

\[
K_S^2 - K_S I + S = 0 \tag{2.5}
\]

Following equation is obtained by solving the equation 2.5

\[
\alpha \pm \sqrt{\alpha^2 - 4\beta} \over 2 \tag{2.6}
\]

\[
\alpha = K_S + K_D, \beta = K_S K_D
\]
2.3.5 C/I plot

As shown above, Stern-Volmer theory deals with quenchers and calculation of quenching constants or association constants. However, with the help of C/I plot association constant can be calculated for both emission intensity enhancement and quenching of emission intensity.

2.3.6 Calculation of association constant, K in case of emission quenching

\[
\frac{I}{[R]} = \alpha + k[T]
\]  \hspace{1cm} (2.7)

I= Fluorescence intensity, [R] = Concentration of the receptor, [T] = concentration of the target, K= Association constant
By plotting fluorescence intensity divided by the concentration of the receptor $I/R$ as a function of the concentration of the target $[T]$, the association constant $K$ can be obtained by multiplying the slope and intercept [7].

2.3.7 Calculation of association constant, $K$ in case of emission enhancement

The relationship between the fluorescence intensity $I$, at a constant concentration of the receptor $R$ and the concentration of the target $T$ is given by equation 2.8.

$$\frac{[R]}{F} = \frac{1}{\alpha} + k[T]$$

Slope = $k$

$K = k/\alpha = \text{Association constant}$
By plotting concentration of the receptor divided by the fluorescence intensity \([R]/I\), as a function of the concentration of the target \([T]\), the association constant \(K\) can be calculated from the slopes and intercepts of the linear plot. Slope divided by the intercept yields the association constant.
2.3.8 Association constants for 5-IA with DCP

\[ K = (2.2 \pm 0.19) \times 10^3 \text{ M}^{-1} \]

Figure 2.26 (a) Association constant for 5-IA with DCP, C/I Plot

\[ K_{sv} = (2.1 \pm 0.19) \times 10^3 \text{ M}^{-1} \]

Figure 2.26 (b) Association constant for 5-IA with DCP, Stern-Volmer plot
$K_S = (7.92 \pm 0.40) \times 10^2 \text{ M}^{-1}$

$K_D = (9.68 \pm 0.50) \times 10^2 \text{ M}^{-1}$

Figure 2.26 (c) Association constant for 5-IA with DCP, modified Stern-Volmer Plot

2.3.9 Association constants for ISBA with DCP

$K = (2.1 \pm 0.20) \times 10^3 \text{ M}^{-1}$

Figure 2.26 (d) Association constant for ISBA with DCP, C/I Plot
$K_{SV} = (2.1 \pm 0.19) \times 10^3 \text{ M}^{-1}$

Figure 2.26 (e) Association constant for ISBA with DCP, Stern-Volmer Plot

$K_S = (1.6 \pm 0.08) \times 10^3 \text{ M}^{-1}$  
$K_D = (2.8 \pm 0.14) \times 10^3 \text{ M}^{-1}$

Figure 2.26 (f) Association constant for ISBA with DCP, modified Stern-Volmer Plot
The association constants have been calculated by three different methods [7, 57, 58]. Figures 2.26 a, b and c show the C/I, Stern-Volmer, and modified Stern-Volmer plots respectively from which the association constants for 5-IA with DCP were determined. Figures 2.26 d, e and f are the plots for the association of ISBA with DCP from which the association constants were determined. As can been seen the C/I and the normal Stern-Volmer plots for 5-IA with DCP, have an upward curvature indicating quenching occurring by the association of DCP with the ground and excited states of 5-IA. This was confirmed by constructing the modified Stern-Volmer equation which resulted in a linear plot [57, 58]. The ground state (K_S) and excited state (K_D) quenching constants were calculated from this plot. A similar behavior was observed for the association of ISBA with DCP with the K_S and K_D being larger than for the association of 5-IA with DCP. This observation clearly indicates that the interaction of 5-IA with the target molecule, DCP is amplified by fluorescent monomer, FSB which by itself has a very weak interaction with DCP (figure 2.23 b).
2.3.10 Interaction of 5-IA, FSB and ISBA with HCl

Figure 2.27 (a) Emission spectra of 5-IA ($3.1 \times 10^{-5}$ M) as a function of HCl concentration

Figure 2.27 (b) Emission spectra of FSB ($2.4 \times 10^{-6}$ M) as a function of HCl concentration
2.27 a, b and c show fluorescence spectral changes of 5-[IA], FSB and ISBA with HCl, respectively. A solution of 5-IA (3.10 × 10^{-5} M) in CH_{3}CN was interacted with various concentrations of HCl ranging from 3.30 × 10^{-6} M to 3.20 × 10^{-5} M. As shown in figure 2.27 a, upon successive additions of HCl the fluorescence intensity of 5-IA was been quenched. A solution of FSB (2.4 × 10^{-6} M) in CH_{3}CN when reacted with the same concentrations of HCl did not exhibit much change in the fluorescence intensity as shown in figure 2.27 b indicating a very weak interaction. When a solution of ISBA (1.6 × 10^{-6} M) in CH_{3}CN was reacted with various concentrations of HCl (3.30 × 10^{-6} M to 1.50 × 10^{-5} M), as shown in figure 2.27 c, a decrease in fluorescence intensity was observed. The figures 2.28 a, b and c display association constants for 5-IA with HCl as determined by C/I, Stern-Volmer, and modified Stern-Volmer plots, respectively. The figures 2.28 d, e and f display association constants for ISBA with HCl determined by the various plots.
is evident from the association constants that ISBA has a stronger interaction with HCl when compared to 5-IA. Figure 2.29 a, b and c show the interaction of 5-IA, FSB, and ISBA with DMMP. It is evident that the interaction is negligible in each case.

2.3.11 Association constants for 5-IA with HCl

\[ K = (6.4 \pm 0.61) \times 10^4 \text{ M}^{-1} \]

Figure 2.28 (a) Association constant for 5-IA with HCl, C/I plot
$K_S = (2.8 \pm 0.14) \times 10^4 \text{ M}^{-1}$

$K_D = (4.2 \pm 0.21) \times 10^4 \text{ M}^{-1}$

Figure 2.28 (b) Association constant for 5-IA with HCl, Stern-Volmer plot

$K = (6.1 \pm 0.52) \times 10^4 \text{ M}^{-1}$

Figure 2.28 (c) Association constant for 5-IA with HCl, modified Stern-Volmer plot
2.3.12 Association constants for ISBA with HCl

\[ K = (1.2 \pm 0.11) \times 10^5 \text{M}^{-1} \]

Figure 2.28 (d) Association constant for ISBA with HCl, C/I plot

\[ K = (1.4 \pm 0.12) \times 10^5 \text{M}^{-1} \]

Figure 2.28 (e) Association constant for ISBA with HCl, Stern-Volmer plot
Figure 2.28 (f) Association constant for ISBA with HCl, modified Stern-Volmer plot

\[ K_s = (3.4 \pm 0.17) \times 10^4 \text{ M}^{-1} \]
\[ K_D = (1.5 \pm 0.07) \times 10^5 \text{ M}^{-1} \]

2.3.13 Interaction of 5-IA, FSB and ISBA with DMMP

Figure 2.29 (a) Emission spectra of 5-IA (3.1\times10^{-5} \text{ M}) as a function of DMMP concentration
Figure 2.29 (b) Emission spectra of FSB (2.4 x10^-6 M) as a function of DMMP concentration

Figure 2.29 (c) Emission spectra of ISBA (1.6 x10^-6 M) as a function of DMMP concentration
2.3.14 Preparation of full sensor of sensor I

The quantum dot alloy Zn$_{0.5}$Cd$_{0.5}$S was chosen for Sensor I as it has emission maximum at 600 nm providing a emission spectral range of 400 – 700 nm for the sensor. Sensor I was reacted with DCP, DMMP and HCl by adding various concentrations of the target compounds to a solution of a Sensor I consisting of Zn$_{0.5}$Cd$_{0.5}$S and ISBA (5 ×10$^{-7}$ M). The Sensor I solution was generated in CH$_3$CN by adding enough concentration of ISBA to Zn$_{0.5}$Cd$_{0.5}$S quantum dots to match the emission intensities of ISBA and the quantum dot. Figure 2.30 a and b show the change in emission intensity of the quantum dot Zn$_{0.5}$Cd$_{0.5}$S and Sensor I with DCP. The quantum dot exhibited a decrease in emission intensity while Sensor I (quantum dot + ISBA) exhibited an increase in intensity. The Schiff base, ISBA as discussed before also exhibited a decrease in emission intensity with increasing concentration of DCP. Clearly Sensor I exhibits a “switch on” (emission intensity increase) mechanism while the individual components making up the sensor exhibit a “switch off” (emission intensity decrease) mechanism. The association constants for the quantum dot and Sensor I were determined from the emission intensity changes as shown in figure 2.31 a and b, respectively. The association constant for the quantum dot was determined from the Stern-Volmer plot employing only the emission intensities as the molar concentration cannot be defined. The association constant for Sensor I was determined from the C/I plot based on the concentration of ISBA. The Stern-Volmer plot that is normally employed for quenching and not emission intensity enhancement was not used for the determination of the association constant for
Sensor I. It is evident from figure 2.31 b that Sensor I has a better association constant compared to the quantum dot alone by about an order of magnitude and ISBA by a factor of 1.5 (see also Table 2.0).

Interactions of sensor I with various concentrations of HCl and DMMP were also performed as shown in figure 2.32 b and 2.34 b, respectively. The interaction with HCl was similar to the interaction with DCP with Sensor I exhibiting an increase in emission intensity with increasing concentrations of HCl. The association constants with HCl in general are larger than for DCP. The interaction with DMMP is much weaker as evident from figure 2.34 b. The association constants for the individual components and sensor I with DCP and HCl are summarized in Table 2.0

2.3.15 Interaction of quantum dots and full sensor (quantum dot + ISBA) with DCP

Figure 2.30 (a) Emission spectra of Zn$_{0.5}$Cd$_{0.5}$S as a function of DCP concentration
Figure 2.30 (b) Emission spectra of Zn$_{0.5}$Cd$_{0.5}$S + ISBA (0.5 \times 10^{-6} M) as a function of DCP concentration.

2.3.16 Association constants for quantum dots and full sensor (quantum dot + ISBA) with DCP

\[ K_{SV} = (8.2 \pm 0.46) \times 10^2 \text{ M}^{-1} \]

Figure 2.31 (a) Association constant for Zn$_{0.5}$Cd$_{0.5}$S with DCP, Stern-Volmer plot
Figure 2.31 (b) Association constant for Zn$_{0.5}$Cd$_{0.5}$S + ISBA with DCP, C/I plot

2.3.17 Interaction of quantum dots and full sensor (quantum dot + ISBA) with HCl

Figure 2.32 (a) Emission spectra of Zn$_{0.5}$Cd$_{0.5}$S as a function of HCl concentration
Figure 2.32 (b) Emission spectra of Zn$_{0.5}$Cd$_{0.5}$S$^+$ ISBA (0.5 \times 10^{-6} \text{ M}) as a function of HCl concentration

2.3.18 Association constants for quantum dots and full sensor with HCl

\[ K_{sv} = (3.9 \pm 0.30) \times 10^4 \text{ M}^{-1} \]

Figure 2.33 (a) Association constant for Zn$_{0.5}$Cd$_{0.5}$S with HCl, Stern-Volmer plot
Figure 2.33 (b) Association constant for $\text{Zn}_{0.5}\text{Cd}_{0.5}\text{S} + ISBA \ (0.5 \times 10^{-6} \text{ M})$ with HCl, C/I plot

2.3.19 Interaction of quantum dots and full sensor (quantum dot + ISBA) with DMMP

Figure 2.34 (a) Emission spectra of $\text{Zn}_{0.5}\text{Cd}_{0.5}\text{S}$ as a function of DMMP concentration
Figure 2.34 (b) Emission spectra of Zn$_{0.5}$Cd$_{0.5}$S+ ISBA (0.5 ×10$^{-6}$ M) as a function of DMMP concentration

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<th>DCP</th>
<th>HCl</th>
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<td>C/I (K, M$^{-1}$)</td>
<td>C/I (K, M$^{-1}$)</td>
</tr>
<tr>
<td></td>
<td>Stern-Volmer (K$_{SV}$, M$^{-1}$)</td>
<td>Stern-Volmer (K$_{SV}$, M$^{-1}$)</td>
</tr>
<tr>
<td>5-IA</td>
<td>(2.2 ± 0.19) ×10$^3$</td>
<td>(6.0 ± 0.61) ×10$^4$</td>
</tr>
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<td></td>
<td>(2.1 ± 0.19) ×10$^3$</td>
<td>(6.1 ± 0.52) ×10$^4$</td>
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<tr>
<td>ISBA</td>
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<td>(1.2 ± 0.11) ×10$^5$</td>
</tr>
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<td></td>
<td>(2.1 ± 0.22) ×10$^3$</td>
<td>(1.4 ± 0.12) ×10$^5$</td>
</tr>
<tr>
<td>Full sensor</td>
<td>(3.5 ± 0.21) ×10$^3$</td>
<td>(3.3 ± 0.18) ×10$^4$</td>
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<td>“SWITCH ON”</td>
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Table 2.1 Summary of association constants for sensor I
2.3.20 Emission lifetime data for sensor I and its components

Figure 2.35 Emission lifetime of ISBA (1.6 × 10⁻⁶ M) in CH₂CN, excitation wavelength-330 nm, emission wavelength-390 nm. The blue curve is the instrument response which is subtracted from the black emission intensity decay for ISBA.

Figure 2.36 Emission lifetime of Zn₀.5Cd₀.5S in CH₂CN, excitation wavelength-370 nm, and emission wavelength-540 nm.
Figure 2.37 Emission lifetime of Zn$_{0.5}$Cd$_{0.5}$S + ISBA ($0.5 \times 10^{-6}$ M) in CH$_3$CN, excitation wavelength-370 nm, emission wavelength-470 nm.

Figure 2.38 Emission lifetime of Zn$_{0.5}$Cd$_{0.5}$S + ISBA ($0.5 \times 10^{-6}$ M) with DCP ($4.25 \times 10^{-5}$ M) in CH$_3$CN, excitation wavelength-370 nm, emission wavelength-470 nm.
Figure 2.39 Emission lifetime of Zn$_{0.5}$Cd$_{0.5}$S + ISBA (0.5 $\times$ 10$^{-6}$ M) with DCP (1.0 $\times$ 10$^{-3}$ M) in CH$_3$CN, excitation wavelength-370 nm, emission wavelength-470 nm.

Figure 2.40 Graphical representation of summary of lifetimes for components of sensor.
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<th>Relative fluorescence intensity, f</th>
<th>Emission wavelength, nm</th>
<th>Excitation wavelength, nm</th>
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<td>QD</td>
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<td>7.805</td>
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<td>35.32</td>
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<td>ISBA</td>
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<td>390</td>
<td>330</td>
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<td>0.515</td>
<td>6.594</td>
<td></td>
<td></td>
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<td></td>
<td>4.077</td>
<td>78.20</td>
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<tr>
<td>QD+ISBA</td>
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<td>540</td>
<td>370</td>
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<td>22.01</td>
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</tr>
<tr>
<td>Sensor +DCP</td>
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<td>37.30</td>
<td>470</td>
<td>370</td>
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<tr>
<td>(4.25X10⁻⁵ M)</td>
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<td>39.41</td>
<td></td>
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<tr>
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<td>3.212</td>
<td>23.29</td>
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</tr>
<tr>
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<td>470</td>
<td>370</td>
</tr>
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<td>(1.0X10⁻³ M)</td>
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Table 2.2 Summary of lifetime decays of components for sensor I

The lifetimes for Sensor and its components and for Sensor I in the presence of DCP are summarized in Figure 2.40 and Table 2.1. Both the quantum dot and ISBA have multiple lifetimes resulting from exponential fitting of the emission decay curves. The shortest lifetimes 0.5 ns or less are not reliable as they fall within the rise time of the excitation source (hydrogen lamp and laser). These short lifetimes are mathematically required to fit the emission decay data and obtain reliable values for the longer lifetimes. The free quantum dots exhibit multiple lifetimes with the longest lifetime of about 36 ns. These lifetimes correspond to the radiative decay of the excitons generated and the multiple lifetimes are most likely due to the polydispersity of the sample. Quantum dots of diameter 10 nm or less have size dependent band gaps which also lead to size
dependent lifetimes. The Schiff base ISBA has 4.1 ns as the major component of the lifetime which corresponds to the $\pi-\pi^*$ excited state. The shorter lifetimes are difficult to interpret but may stem from partial aggregation of the Schiff base as it is an extended $\pi$ system of the stilbene family which are known to form aggregates.

When the quantum dots and the Schiff base are combined to make Sensor I the long exciton lifetime is no longer evident. A 0.7 ns (39%) and 3.1 ns (22%) lifetimes are observed for the Sensor I indicating that the Schiff base is attached to the quantum dots through the carboxylic acid groups. A simple mixture of the quantum dots and Schiff base would have still retained the long exciton lifetime. When Sensor I is interacted with a very low concentration of DCP ($4.25 \times 10^{-5}$ M) the lifetimes of Sensor I are not significantly altered but the contribution from the shorter lifetime (0.7 ns; 39%) is higher than that from the longer lifetime (3.2 ns; 23%). A much higher concentration of DCP ($1.00 \times 10^{-3}$ M) results in lifetimes of 1.16 ns (58%) and 2.13 ns (29%) indicating a much stronger interaction. The lifetimes also correspond to an increase in emission intensity with increasing concentrations of DCP. A possible reason is an increase in emission quantum yield which will need to be investigated further to fully understand the interaction between DCP and Sensor I. This is an important future scope of these studies.
CHAPTER III

SESNOR II

3.1 Experimental procedures

3.1.1 Materials and instruments

The compounds 1,10-phenanthroline-5, 6-Dione, dipyrido-[3, 2-a: 2’, 3’]phenazine and its zinc complex \([\text{Zn(dppz)}_2][\text{PF}_6]_2\) were synthesized according to a literature procedure [59]. The other compounds diethylchlorophosphate, dimethylmethylphosphonate and HCl employed in the studies and the instruments have been described in Chapter 2.

3.1.2 Synthetic methods

3.1.2.1 Synthesis of 1, 10-phenanthroline-5, 6-Dione (figure 3.1)

To a mixture of 1, 10-phenanthroline 0.5g (0.002 mol) and 2.35 g (0.02 mol) of KBr and H$_2$SO$_4$ (10 mL) was added drop wise followed by drop wise addition of HNO$_3$ (5 mL) and refluxed for 2-3 hrs at 80° C in an oil bath. After reflux the reaction mixture was cooled to room temperature and neutralized with NaHCO$_3$ followed by extraction with CHCl$_3$. Excess CHCl$_3$ was removed by evaporation which gave an orange-yellow solid (yield 71%).
3.1.2.2 Synthesis of the ligand, dipyrido-[3, 2-a: 2', 3'] phenazine (dppz) (figure 3.1)

A mixture of 1,10-phenanthroline-5,6-dione (0.2 g, 0.9 mmol) and 1,2-phenylenediamine (0.6 g, 1 mmol) in ethanol was stirred at 50°C for 2 hrs and then at room temperature overnight. The resulting solution was reduced in volume by rotary evaporation at 50°C to yield a cream colored solid. The crude product was left to stand for 8 hrs. Methanol-water (10:90) was then added and the product was filtered and recrystallized from methanol to give cream colored crystals (yield 63 %) [59]. Figure 3.2 shows the $^1$H NMR spectrum of DPPZ. The $^1$H NMR (400 MHz $d_3$-CHCl$_3$; TMS reference; $\delta$ ppm ) has these features: a) protons in phenanthroline ring 9.58 (dd, 2H, $J=1.8$ Hz); b) protons in phenanthroline ring 9.23 (dd, 2H, $J=1.8$Hz); c) protons in phenylamine ring 8.42 (dd, 2H, $J=3.28$); d) protons in phenylamine ring 8.08 (dd, 2H, $J=3.64$ Hz); e) protons in phenanthroline ring 7.97 (dd, 2H, $J=4.4$Hz).

3.1.2.3 Synthesis of Zn complex of dipyrido-[3, 2-a: 2', 3']phenazine (dppz) (figure 3.1)

To a solution of dppz (dipyrido-[3, 2-a: 2', 3'] phenazine) 0.075 g (0.2 mmol) in ethanol, 0.02 g (0.09 mmol) of Zn(CH$_3$COO)$_2$ was added and refluxed for 12 hrs. After reflux a clear pale yellow colored solution was obtained. To this 0.04 g (0.0002 moles) of ammonium hexafluoro phosphate was added and the Zn complex of dppz was precipitated out as hexafluoro phosphate salt (0.128 g, yield 71 %) which was filtered and dried in vacuum oven overnight (figure 3.1). Previously complexes of zn with
phenanthroline and its derivatives have been synthesized and procedures are available in the literature [60, 61]. Figure 3.3 shows the $^1$H NMR spectrum of [Zn(dppz)$_2$][PF$_6$]$_2$. $^1$H NMR (400 MHz d$_3$-CHCl$_3$; TMS reference; $\delta$ ppm) has these features: a protons of phenanthroline ring 9.78 (s, 2H); b protons of phenanthroline ring 9.14 (s, 2H); c protons in phenylamine ring 8.45(s, 2H); d protons of phenylamine ring 8.13(s, 2H); d and e protons of 8.13(d, 2H). More conclusive result for the structure of [Zn(DPPZ)$_2$][PF$_6$)$_2$ were obtained through mass spectral analysis. Figure 3.4 shows the mass spectrum of [Zn(DPPZ)$_2$][PF$_6$]$_2$ and as can be seen the peak at m/z = 628 is corresponding to [M$^+$-2PF$_6$] (Molecular weight=918), and the peak at m/z=779 is [M$^+$ –PF$_6$] and the peak at m/z=642 is [M$^+$+F].

3.1.2.4 Synthesis of ZnS: Mn/ZnS (0.01% Mn$^{2+}$, core/shell (1/4)) nanoparticles

The nanoparticles were synthesized by arrested precipitation in water, using Zn(DS)$_2$ precursor salt (DS = dodecyl sulfate). One mL (0.0151 MnSO$_4$. xH$_2$O dissolved in 500 mL of water) of Mn$^{2+}$ solution was added to 400 mL aqueous solution containing Na$_2$S (0.0156 g, 2mmol) at room temperature. This was followed by the addition of Zn(DS)$_2$ (1.19 g) to form the ZnS:Mn core solution. The core solution was stirred for another 10 min at room temperature. For the addition of 1:1/4 core to shell of ZnS, 0.0031 g of Na$_2$S was added to the stirred core solution followed by the addition of ZnSO$_4$ (0.144 g). This resulted in a cloudy white colored solution. After 15 to 20 min of stirring at room temperature white solids precipitated. The solution was centrifuged and the precipitates were isolated by decanting the supernatant solution. A small portion of this was kept for uv-vis and emission studies and the remaining sample was cooled to -20°C overnight and
brought back to room temperature. The solution was then centrifuged, the supernatant decanted, and the solids were separated. The obtained white solids were dried overnight in a vacuum desiccator over anhydrous CaCl$_2$.

Figure 3.1 Synthesis of [Zn(dppz)$_2$][PF$_6$]$_2$
3.2 Characterization of components of sensor II

3.2.1 $^1$H NMR and UV-Vis characterization of dppz

Figure 3.2 $^1$H- NMR (400 MHz, DMSO-d$_6$) spectrum of dppz

Figures 3.3 a, b and c show the absorption spectrum, excitation spectrum and emission spectrum of a solution of dppz in CH$_3$CN (2.5 $\times$ 10$^{-6}$ M), respectively.

![Figure 3.2](image)

![Figure 3.3](image)

Figure 3.3 (a) UV-Vis absorption spectrum of dppz (2.5 $\times$ 10$^{-6}$ M) in CH$_3$CN
Figure 3.3 (b) Excitation spectrum of dppz (2.5 \times 10^{-6} \text{ M}) in CH$_3$CN

Figure 3.3 (c) Emission spectrum of dppz (2.5 \times 10^{-6} \text{ M}) in CH$_3$CN
3.2.2 $^1$H NMR, mass and UV-Vis characterization of [Zn(dppz)$_2$][PF$_6$]$_2$

Figure 3.4 $^1$H NMR (400 MHz, DMSO-d$_6$) spectrum of Zn(dppz)$_2$[PF$_6$]$_2$

Figure 3.5 Mass spectrum of Zn(dppz)$_2$[PF$_6$]$_2$ by FAB
The Figures 3.6 a, b and c display the uv-vis absorption, excitation, and emission spectra of $1.6 \times 10^{-6}$ M Zn(dppz)$_2^{2+}$ in CH$_3$CN, respectively. These spectra are similar to that of dppz indicating that complexation with a d$^{10}$ metal ion does not perturb the electronic structure of the ligand.

Figure 3.6 (a) UV-Vis absorption spectrum of Zn (dppz)$_2^{2+}$ (1.6 x 10$^{-6}$ M) in CH$_3$CN

Figure 3.6 (b) Excitation spectrum of Zn (dppz)$_2^{2+}$ (1.6 x 10$^{-6}$ M) in CH$_3$CN, emission wavelength- 420 nm
Figure 3.6 (c) Emission spectrum of Zn(dppz)$_2^{2+}$ (1.6 $\times$ 10$^{-6}$ M) in CH$_3$CN, excitation wavelength- 270 nm, emission filter-345 nm

Figures 3.7 a, b and c display absorption spectrum, excitation spectrum and emission spectrum of ZnS:Mn/ZnS quantum dots in CH$_3$CN. As can be seen from the figure 3.7 c the peak at 590 nm corresponds to the emission from Mn$^{2+}$ indicating that ZnS quantum dots have been successfully doped with Mn$^{2+}$ ions [62]. Figures 3.8 a, b and c show the absorption, excitation and emission spectra of NNMR full sensor and as may be seen they do not show any change when compared the spectra of ZnS:Mn/ZnS quantum dots.
3.2.3 UV-Vis characterization of ZnS:Mn/ZnS quantum dots

Figure 3.7 (a) UV-Vis absorption spectrum of ZnS:Mn/ZnS in CH$_3$CN

Figure 3.7 (b) Excitation spectrum of ZnS:Mn/ZnS in CH$_3$CN, emission wavelength-590 nm
3.2.4 UV-Vis characterization of full sensor (ZnS:Mn/ZnS + MSA + Zn(dppz)$_2^{2+}$)

Figure 3.7 (c) Emission spectrum of ZnS:Mn/ZnS in CH$_3$CN, emission filter-445 nm, excitation wavelength-330 nm

Figure 3.8 (a) UV-Vis absorption spectrum of ZnS:Mn/ZnS + MSA + Zn(dppz)$_2^{2+}$ in CH$_3$CN
Figure 3.8 (b) Excitation spectrum of ZnS:Mn/ZnS + MSA + Zn(dppz)$_2^{2+}$ in CH$_3$CN

Figure 3.8 (c) Emission spectrum of ZnS:Mn/ZnS + MSA + Zn(dppz)$_2^{2+}$ in CH$_3$CN, excitation wavelength-330 nm, emission filter-445 nm
3.3 Studies of components of Sensor II with DCP, DMMP and HCl

3.3.1 Interaction of dppz and Zn(dppz)$_2^{2+}$ with DCP

Figure 3.9 (a) Emission spectra of dppz (2.5 × 10$^{-6}$ M) as a function of DCP concentration

Figure 3.9 (b) Emission spectra of Zn(dppz)$_2^{2+}$ (1.6 × 10$^{-6}$ M) as a function of DCP concentration
The figure 3.9 a shows the emission intensity decreases when dppz interacted with various concentrations of DCP ranging from $8.5 \times 10^{-5}$ M to $1.3 \times 10^{-3}$ M. Similarly as shown in figure 3.9 b a solution of Zn(dppz)$_2^{2+}$ in CH$_3$CN also exhibited an emission intensity decrease with increasing concentrations of DCP even though it exhibited an emission intensity increase at very low concentrations of DCP. The association constants $K$ and $K_{SV}$ for dppz with DCP were calculated using C/I method as well as Stern-Volmer method and are shown in figures 3.10 a and b respectively. The figures 3.10 c and d show C/I and Stern-Volmer plots of Zn(dppz)$_2^{2+}$ with DCP respectively. As can be seen from the values of $K$ or $K_{SV}$ Zn(dppz)$_2^{2+}$ appears to have a stronger association with DCP ($K = 1.0 \times 10^3$ M$^{-1}$) when compared to dppz with DCP ($K = 5.0 \times 10^2$ M$^{-1}$).

3.3.2 Association constants for dppz with DCP

![Figure 3.10 (a) Association constants for dppz with DCP, C/I plot](image)

$K = (5.0 \pm 0.91) \times 10^2$ M$^{-1}$
1.7

\[ K_{SV} = (5.2 \pm 0.72) \times 10^2 \text{ M}^{-1} \]

Figure 3.10 (b) Association constant for dppz with DCP, Stern-Volmer plot

3.3.3 Association constants for Zn(dppz)$_2^{2+}$ with DCP

\[ K = (1.0 \pm 0.08) \times 10^3 \text{ M}^{-1} \]

Figure 3.10 (c) Association constants for Zn (dppz)$_2^{2+}$ with DCP, C/I plot
Figure 3.10 (d) Association constant for Zn (dppz)$_2^{2+}$ with DCP, Stern-Volmer plot

$K_{SV} = (9.8 \pm 0.12) \times 10^2 \text{ M}^{-1}$

Figure 3.11 (a) Emission spectra of dppz ($2.5 \times 10^{-6} \text{ M}$) as a function of HCl concentration

3.3.4 Interaction of dppz and Zn (dppz)$_2^{2+}$ with HCl
Figure 3.11 (b) Emission spectra of Zn (dppz)$_2^{2+}$ (1.6 x 10$^{-6}$ M) as a function of HCl concentration

The emission intensity decrease upon the interaction of dppz (2.6 x 10$^{-6}$ M) in CH$_3$CN with various concentrations of HCl (7.5 x 10$^{-6}$ to 2.25 x 10$^{-5}$ M) is shown in figure 3.11 a. Similarly a solution of Zn(dppz)$_2^{2+}$ (1.6 x 10$^{-6}$ M) in CH$_3$CN with various concentrations of HCl (7.5 x 10$^{-6}$ to 3.25 x 10$^{-5}$ M) exhibited an irregular behavior with successive additions of increasing concentrations of HCl. The association constants, K and K$_{SV}$ for dppz with HCl calculated using C/I method and Stern-Volmer method and are shown in figures 3.12 a and b, respectively and the two methods yield similar values. The association constant K and K$_{SV}$ were also calculated using C/I and Stern-Volmer methods and are shown in figures 3.12 c and d respectively. Clearly from the values of K, dppz (K = 2.1 x 10$^{4}$ M$^{-1}$) appears to have better association with HCl when compared to Zn (dppz)$_2^{2+}$ (K = 1.0 x 10$^{4}$ M$^{-1}$).
3.3.5 Association constants for dppz with HCl

\[ K = (2.1 \pm 0.73) \times 10^4 \text{ M}^{-1} \]

Figure 3.12 (a) Association constant for dppz with HCl, C/I plot

\[ K_{SV} = (2.2 \pm 0.58) \times 10^4 \text{ M}^{-1} \]

Figure 3.12 (b) Association constant for dppz with HCl, Stern-Volmer plot
3.3.6 Association constants for Zn (dppz)$_2^{2+}$ with HCl

$$K = (1.0 \pm 0.19) \times 10^4 \text{M}^{-1}$$

![Graph](image)

Figure 3.12(c) Association constant for Zn (dppz)$_2^{2+}$ with HCl, C/I plot

$$K_{SV} = (1.0 \pm 0.18) \times 10^4 \text{M}^{-1}$$

![Graph](image)

Figure 3.12 (d) Association constant for Zn (dppz)$_2^{2+}$ with HCl, Stern-Volmer plot
3.3.7 Interaction of dppz and Zn\,(dppz)_{2}^{2+} with DMMP

The figures 3.13 a and b display the emission intensity change upon the interaction of dppz \((2.6 \times 10^{-6} \text{ M})\) and Zn\,(dppz)_{2}^{2+} \((1.6 \times 10^{-6} \text{ M})\) with various concentrations of DMMP \((5.75 \times 10^{-5} \text{ to } 1.90 \times 10^{-3} \text{ M})\). As can be seen from the figures that both dppz and Zn\,(dppz)_{2}^{2+} have very weak interaction with DMMP indicating that dppz and Zn\,(dppz)_{2}^{2+} are more selective and sensitive to DCP and HCl compared to DMMP.

![Figure 3.13 (a) Emission spectrum of dppz \((2.5\times10^{-6} \text{ M})\) with DMMP in CH_{3}CN](image-url)
Figure 3.13 (b) Emission spectrum of Zn (dppz)$_2^{2+}$ ($1.6 \times 10^{-6}$ M) with DMMP in CH$_3$CN

3.3.8 Interaction of ZnS:Mn/ZnS and full sensor ZnS:Mn/ZnS + MSA + Zn (dppz)$_2^{2+}$ with DCP

Figure 3.14 (a) Emission spectra of ZnS:Mn/ZnS as a function of DCP concentration
A complete sensor system of nanoparticle, monomer, nanomolecule and receptor (NMNR) was constructed from Zn(dppz)$_2^{2+}$ by ion pairing it with the carboxylate ions of mercaptosuccinic acid bound to the ZnS:Mn/ZnS. This NMNR sensor is displayed in figure 1.7, chapter I.

The emission changes upon addition of DCP to ZnS:Mn/ZnS is shown in figure 3.14a. ZnS:Mn/ZnS quantum dots were equilibrated with a small concentration (1.5 x 10$^{-7}$ M) of mercaptosuccinic acid in CH$_3$CN. The chemisorption of mercaptosuccinic acid through the thiol group on the quantum dots resulted in a decrease in emission intensity and reached a constant value within a few minutes indicating the equilibration to be complete. This was followed by the addition of various concentrations of DCP is shown.
in figure 3.14 b. A solution of Zn(dppz)$_2^{2+}$ to obtain a final concentration of $5 \times 10^{-7}$ M was added to the quantum dot - mercaptosuccinic acid pair and the emission of the quantum dot was monitored until it reached a constant value in about 30 minutes figure . The addition of the complex resulted in further reduction in the emission which was not due to dilution as a very small volume of the complex was added 3.15 a. The spectra only have the quantum dot emission as the strongest feature with the emission of the complex barely visible.

Figure 3.15(a) Emission spectra of ZnS:Mn/ZnS quantum dots, quantum dots with mercaptosuccinic acid, and quantum-mercaptosuccinic acid pair with Zn(dppz)$_2^{2+}$ complex (Sensor II)

The NNMR sensor (Sensor II) was interacted with different concentrations of DCP ($4.25 \times 10^{-5} - 6.37 \times 10^{-4}$ M) and the emission intensity recorded as shown in Figure
3.18. The emission spectra indicate that the interaction is weak. The weak interaction of Sensor II may stem from the ineffective coupling of the Zn complex with the quantum dots by mercaptosuccinic acid linker. L-cysteine could be a better linker and could provide a better Sensor II. This is a future scope of the project.

Figure 3.15 (b) Emission spectrum of ZnS:Mn/ZnS + MSA + Zn(DPPZ)$_2$ as a function of DCP concentration in CH$_3$CN
CHAPTER IV

CONCLUSIONS

Two types of nanosensors based on nitrogen heterocyclic compounds as receptors have been developed for the detection of nerve agent analog, DCP.

1) Sensor I (NMR- Nanoparticle, Monomer and Receptor)
- Receptor-5-IA
- Monomer-FSB
- Nanoparticle- Alloy quantum dots, Zn$_{0.5}$Cd$_{0.5}$S

2) Sensor II (NNMR- Nanoparticle, Nanomolecule and Receptor)
- Receptor- dppz
- Nanomolecule-Zn(dppz)$_2^{2+}$
- Nanoparticle- ZnS:Mn/ZnS (0.01% Mn$^{2+}$ and ¼ shell)

4.1 Sensor I

The receptor 5-IA was covalently linked to the fluorescent monomer, FSB through an imine bond resulting in a Schiffbase adduct, ISBA. ISBA was then attached to the alloy quantum dots, Zn$_{0.5}$Cd$_{0.5}$S through carboxylic end of ISBA to obtain Sensor I. This sensor was characterized by uv-vis absorption and luminescence spectroscopy and
lifetime studies and the results clearly indicate that ISBA has been associated with quantum dots. The components, 5-IA, FSB, ISBA, and Sensor I were analyzed for their interaction with DCP, DMMP and HCl.

1. The components of Sensor I (quantum dots, FSM and ISBA) were chemically linked as discerned from the emission spectra and lifetimes.

2. 5-IA and ISBA were found to be “SWITCH OFF” (emission decrease) sensors for DCP and HCl whereas Sensor I was found to be a “SWITCH ON” (emission increase) sensor.

3. The components 5-IA and ISBA and Sensor I exhibited a stronger interaction with HCl than with DCP and a very weak interaction with DMMP. This interaction was evident from changes in the emission intensities and lifetimes. The fluorescent monomer FSB on the other hand showed very weak interaction with all three target compounds. These observations support the central hypothesis that nanosensors constructed with systematic assembly of specific components for analyte of interest can provide sensitivity and selectivity through signal transduction mechanism.

4.2 Sensor II

1. For Sensor II, dppz was chosen as the receptor. The nanomolecule, \( \text{Zn(dppz)}_2^{2+} \) was obtained upon complexation of dppz with \( \text{Zn}^{2+} \). The Zn complex was
attached to the core-shell quantum dots, ZnS:Mn/ZnS (0.01% Mn$^{2+}$ and ¼ shell) through a thiol linker, MSA (mercapto succinic acid).

2. From the values of association constants it was evident that Zn(dppz)$_2^{2+}$ showed a stronger association with DCP when compared to dppz whereas in case of HCl, dppz was found to have a stronger interaction than Zn(dppz)$_2^{2+}$. Both dppz and Zn(dppz)$_2^{2+}$ showed very weak interaction with DMMP.

3. Sensor II did not show significant changes in its emission with various concentration of DCP. This may stem from mercapto succinic acid being a poor linker between the quantum dots and Zn(dppz)$_2^{2+}$ leading to poor signal transduction.

4. In general nitrogen heterocyclic compounds are found to be good receptors for nerve gas agent analogs. And they were all found to be more selective towards haloorganophosphates.

4.3 Future directions

1. Understand the mechanism of the interaction of Sensor I with DCP and HCl by conducting a systematic investigation of the lifetimes. This is necessary to gain a fundamental understanding of emission increase and the “switch on” mechanism and signal transduction.
2. Employ better monomer linkers such as L-cystein and a conjugated thiol such as 4-mercaptobenzoic acid in place of MSA for the generation of Sensor II and facilitate signal transduction.

3. Other metal complexes of dppz such as those of lanthanide metal ions Eu$^{3+}$ and Ho$^{3+}$ could have better sensitivities for DCP as these metal ions are also capable of interacting with organophosphates. Similarly electron donating and withdrawing groups could be introduced in dppz to fine tune the sensitivity and selectivity for nerve gas analogs.
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