Design, Synthesis and Characterization of Nanosensors for Nerve Gas Agents

Thushara Gunasinghe

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DESIGN, SYNTHESIS AND CHARACTERIZATION OF NANOSENSORS FOR NERVE GAS AGENTS

by

Thushara Gunasinghe

A Thesis
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Faculty of The Graduate College
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DESIGN, SYNTHESIS AND CHARACTERIZATION OF NANOSENSORS FOR NERVE GAS AGENTS

Thushara Gunasinghe, M.S.

Western Michigan University, 2005

This research is focused on the design synthesis and characterization of nanosensors to detect the nerve gas agents such as sarin at very low concentration levels (parts per billion and lower) which is very important to combat terrorism. Nanosensors constructed by the bottom up approach could provide the low sensitivity levels and high selectivity required for nerve gas detection. The sensing mechanism exploits the inherent properties of nanomaterials and signal amplification by signal transduction. The components of a nanoparticle-fluorescent monomer-nanomolecule-receptor (NMNR) sensor have been designed, synthesized, and characterized successfully. Interaction of the components of NMNR sensor with a model nerve gas agent diethylchlorophosphate (DCP) have been investigated by fluorescence spectroscopy. Arrays of NMNR composites where the nanoparticle is SiO$_2$ or TiO$_2$ have been obtained on quartz substrates and their interaction with DCP vapors have been studied by fluorescence. These NMNR arrays detect DCP in the gas phase at as low as 0.1 ppb concentration and the interaction is reversible.
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Thushara Gunasinghe
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INTRODUCTION

1.1 What are chemical warfare agents?

Chemical warfare agents are gases, liquids, or solids that are highly toxic to humans and animals [1]. Among these nerve gas agents shown in Figure 1 are the most dangerous [2]. They are stable and easily dispersed, highly toxic and effect rapidly both when inhaled and absorbed through the skin [3]. They can be manufactured by means of fairly simple chemical techniques. The raw materials are inexpensive and generally readily available.

In 1995 terrorists released sarin gas in a Tokyo subway killing 12 people and injuring 6000. Before that sarin was reportedly used in Iraq to kill thousands of Kurdish dissidents in 1998 [4]. It is critical to have an effective sensor to protect civilians and military personnel from attack by nerve gas agents. These sensors should have very good sensitivity, minimum fault signals, and should be highly selective [5].

1.2 Nerve agents

![Chemical structures of nerve agents]

Figure 1.1. Various nerve gas agents.
The highly toxic nerve gases or nerve agents are fluorine or cyanide containing organophosphates. They are very lethal and are hazardous by any method of exposure. The compound VX (O-ethyl S-(2-(diisopropyamino) ethyl) methylphosphonothioate is the most toxic nerve agent [7].

Nerve agents are generally grouped into two classes. G and V (Table 1.1). G agents are first generation and V agents belong to second generation. G agents are derivatives of phosphoramidocyanidic or methylphosphonofluoridic acid and include sarin (GB: isopropylmethylphosphonofluoridate), tabun (GA: ethyl N-dimethylphosphoramido cyanidate), and soman (GD: pinacolyl methylphosphonofluoridate). V agents are derivatives of methylphosphonothioic acid. VX is the primary V type agent and is the least volatile and the most potent [8]. Any liquid nerve agent splashed into eyes is potentially lethal. The nerve gas, sarin, is produced by mixing isopropyl alcohol with halogenated methyl phosphonates [9]. The synthetic methods for sarin, tabun and soman are available in the literature [10].

1.3 Tabun (GA)

Tabun (ethyl N, N-dimethyl phosphoramidocyanidate) is a pale to dark amber liquid which releases a colorless vapor. It has no odor in a pure state. It gives off rotting fruit odor.
as it oxidizes. Tabun was developed by Dr. Gerhard Scharder in 1937 that became poisoned from a single drop of the new chemical when it spilled onto his lab bench. Tabun was the first German nerve agent developed, and twelve thousand tons were produced for use as a weapon but it was never deployed.

Tabun forms a stable vapor cloud over its liquid and is rapidly absorbed via the skin as a liquid or as a vapor [11]. Between 1-1.5 grams of liquid solution (0.01 mg/kg concentration) will cause death in two minutes after dermal contact. Tabun presents the least vapor hazard of the nerve gases in terms of spread, whereas sarin presents the highest vapor hazard.

Tabun as a liquid can persist for two days in areas of shade in temperate climate conditions. Its vapor density is 5.6 times that of air and thus it will spill into lower elevations, confined spaces, and low lying areas in buildings and structures. Liquid skin exposure results in death in two minutes while vapor inhalation exposure results in death in ten minutes.

The manufacturing of tabun is by the interaction of dimethylamidophosphoryl dichloride and sodium cyanide in the presence of ethanol. Chlorine based decontaminants, which are recommended for organophosphate nerve gas skin decontamination, will release hydrogen cyanide on exposure to tabun residues. Microencapsulation of tabun would make it highly persistent in the environment.

1.4 Sarin (GB)

Sarin (isopropyl methyl phosphorofluoridate) is a colorless liquid that gives off a colorless vapor with no odor [12]. Sarin gives off a rotting fruit odor upon oxidation. Sarin
was the second organophosphate nerve gas developed by Dr. Gerhard Schrader in 1938. It worked 10 times as fast as tabun. Small scale sarin production began but the Soviet army captured the production facility in 1945 at the end of the World War II.

Sarin is the most toxic of the three German-developed nerve agents [13,14]. Death is rapid via skin or inhalation. Ingestion of 0.01 mg/kg causes death within one minute. Sarin is also the most volatile of the three original nerve agents and forms a vapor 36 times faster than tabun. It has a vapor concentration 2.9 times that of air and will flow downward into low lying areas and may be thickened by oils or petroleum products.

1.5 Soman (GD)

Soman (l-methyl-2,2-dimethyl propyl methyl phosphorofluoridate) is the third nerve gas produced. It is a colorless liquid, and gives off a colorless vapor [13]. It may have a camphor-like odor or odor of rotting fruit as it oxidizes. Soman has a vaporization rate between tabun and sarin. Its evaporation rate allows it to persist in an environment for about one day while producing a lethal vapor cloud, thus producing a hazard downwind. When the Soviet military captured the sarin and tabun production facilities in 1945, they also discovered the formula and plans for soman.

Soman is the second most toxic German nerve agent [14]. A dose of 0.01 mg/kg orally or 100 mg/min/m$^3$ causes death in one minute. It persists for one to two days in an environment which increases the likelihood of mass casualties and has a highly toxic affect on the brain and causes permanent damage. Soman is a clear, solvent looking poison with a vapor density six times that of air.
1.6 VX

VX (ethyl S-2-diisopropyl aminoethyl methyl phosphorothiolate) is the most toxic nerve agent known \[15,16\]. It is a pale amber liquid that looks like motor oil and gives off a colorless vapor. VX was developed in 1952 by the British who decided to pursue other nerve agents, so they sent the formula to the United States military. But it was intercepted by the Soviets in route. The U.S. produced 2.5 tons of VX. Production was halted in 1968 because 20 pounds of VX leaked from a spray tank in Skull Valley, Utah and killed 6,000 sheep. VX was designed to create casualties by skin absorption or from aerosolization by spraying and 0.001 grams is lethal. It has a low volatility and vapor pressure that minimizes inhalational routes of poisoning. However, VX is highly persistent in an environment and may last weeks \[17\]. Vapors are nine times heavier than air and therefore vapor hazards exist for continued low lying spaces.

1.7 Mechanism of action

A characteristic of nerve agents is that they are extremely toxic and that they have very rapid effect. The nerve agent either as a gas, aerosol, or liquid, enters to the body through inhalation or through the skin. Poisoning may also occur through the consumption of liquids or food contaminated with nerve agents \[18\].

Individuals exposed to nerve agents develop rapid onset of cholinesterase inhibition signs and symptoms. Depending on the agent, death may occur in 1-2 minutes. Nerve agents bind covalently to the enzyme acetylcholinesterase irreversibly inhibiting it and causing accumulation of acetylcholine, which is the mammalian neurotransmitter through
the synapse, at the neuroeffector junction in the peripheral and central nervous systems [7, 8, 19]. Also nerve agents inhibit the blood cholinesterase.

![Figure 1.2. Acetylcholine.](image)

Nerve gas agents disrupt the transmission of nerve impulses by attacking the enzyme acetylcholine esterase as shown in Figure 1.3. This enzyme regulates the concentration of acetylcholine shown in Figure 1.2. Regulation of acetylcholine is important to maintain the switch between depolarization and polarization states of the post synaptic membrane. Release of the acetylcholine causes depolarization of the post synaptic membrane. Subsequently to the excess acetylcholine rapidly hydrolyzed by acetylcholine esterase to choline and acetic acid [20]. When acetylcholine esterase is inactivated by a nerve gas, excess acetylcholine is not destroyed which will prevent the return of the postsynaptic membrane from the depolarized to the polarized state. This will prevent further neurotransmission, the disruption of nerve functions and eventually paralysis and death.

![Figure 1.3. Sarin binds to acetylcholine esterase binding site.](image)
1.8 Central hypothesis

The central hypothesis of the research described in this thesis is that nanosensors constructed by the “bottom up” approach will have the selectivity and sensitivity to detect nerve gas agents. The bottom up approach allows systematic layer by layer assembly of molecular moieties to achieve the desired selectivity and sensitivity for the target compound. This approach provides a means for signal amplification through transduction and optimization of the selectivity and sensitivity by varying the structure of the different moieties.

1.9 Rationale for the bottom up approach

Nanosensors with different moieties such as nanoparticles and molecules could through a synergistic interaction of the moieties be selective and sensitive for a target compound. The ability to achieve ordered nanostructures through a systematic assembly of these moieties is thus critical for the construction of nanosensors. A systematic assembly of moieties to obtain highly ordered nanostructures could be achieved through spontaneous and/or stimulated self-assembly. The bottom up approach is ideally suited for the self-assembly of the moieties to obtain ordered nanostructures for sensing. This approach provides the ability to achieve the best sensitivity and selectivity for the nanosensor by choosing the moieties that have the optimum synergy. An example of synergy is fluorescence resonance energy transfer by which a fluorescence change accompanying the interaction of the receptor moiety of a nanosensor with a target
molecule is amplified by other moieties that do not have a significant interaction with the target molecule. The bottom up approach is thus ideally suited for the construction of nanosensors capable of signal transduction through synergy between the different moieties.

1.10 Proposed research

The approach that has been investigated in this research to substantiate the central hypothesis is the nanoparticle-nanopolymer-nanomolecule-receptor (NNNR) shown in Figure 1.4 a and 1.4 b.

The fluorescent sulfonated monomer (FSM) in Figure 1.5 a and 1.5 b has been employed in place of the nanopolymer in a majority of the studies described here. A limited number of studies have been performed with the NNNR sensor. The nanoparticle-monomer-nanomolecule-receptor sensor (NMNR) is shown in Figure 1.6. The target molecule is sensed by a change in the fluorescence of the various moieties of NMNR.
Figure 1.4 (a). General Nanoparticle, Nanopolymer, Nanomolecule, Receptor (NNNR) Concept.

Figure 1.4 (b). NNNR sensor designed in this work.
Figure 1.5. (a). Fluorescent sulfonated monomer (FSM) attached to single SiO$_2$ nanoparticle.

Figure 1.5. (b). Fluorescent sulfonated monomer (FSM) attached to two SiO$_2$ nanoparticles.
The moieties of NMNR sensor are:

1. SiO$_2$ nanoparticle  
2. Fluorescent Sulfonated Monomer (FSM)  
3. Ruthenium (II) bipyridyl complex  
4. Isoquinoline.

Isoquinoline is the receptor end of NMNR which would sense the nerve gas analog, diethylchlorophosphate (DCP) as shown in Figure 1.7, based on previous reports in the literature [1, 4, 5].
Figure 1.7. Interaction of DCP with NMNR sensor.

1.11 Rationale for the proposed approach

The isoquinoline, Ru (II) complex, and FSM moieties are fluorescent in the NMNR sensor. The fluorescence of isoquinoline moiety in NMNR may be expected to change upon binding DCP. In addition the fluorescence of the Ru (II) complex and FSM may also change. In particular excitation by a 260 nm photon may lead to change in the emission of isoquinoline at 440 nm, FSM at 512 nm, and Ru (II) complex at 600 nm. It is also possible that fluorescence resonance energy transfer may occur by the photons emitted by the isoquinoline exciting FSM and the photons emitted by the FSM exciting the Ru (II) complex. A signal transduction from the isoquinoline receptor to the FSM and Ru (II) complex could occur and this would provide three types of emissions by the NMNR when it interacts with the target molecule. The integration of fluorescence intensity over a wide spectral range of the different moieties will provide a much lower detection limit for the target compound compared to the fluorescence change only for the receptor molecule.
The SiO$_2$ and TiO$_2$ nanoparticles attached to the both ends of the fluorescent sulfonated monomer through Schiff’s base provides several advantages.

1. Nanoparticles have high surface areas (surface to volume ratio of a nanometer size particle is 1000 times that of a macron size particle) leading to high local concentration of sensor and target molecules.

2. Many particles can be packed in a small compact area.

3. Self assembly of these particles to form an ordered array may provide high sensitivity.

4. Titanium nanoparticles may also be employed for the redox reactions to generate electrochemical signal and even destroy the target compound.

1.12 Project objectives

The objectives of the project towards the synthesis of NMNR sensor and characterization of their interaction with DCP are:

1. Synthesize and characterize 5,5’-dicarboxy-bis(5-aminoisoquinoline)(bpy-isq), Bis(bipyridine)-5,5’-dicarboxy-bis(5-aminoisoquinoline)-2,2’-bipyridineum ruthenium hexafluorophosphate(II)(Ru5ISQ) and fluorescent sulfonated monomer (FSM) moieties. (bpy: 2, 2’-bipyridyl; isq: isoquinoline) displayed in Figure 1.8.
Figure 1.8 (a) Ru \((\text{bpy})_2(\text{bpy-isq})(\text{PF}_6)_2\) complex.

(b) 4-{2, 5-Bis-[2-(4-formyl-phenyl)-vinyl]-4-methoxy-phenoxy} -butane-1-sulfonic acid sodium anion (4) (FSM).

2. Synthesize and characterize SiO$_2$ nanoparticles.

3. Synthesize of SiO$_2$- FSM- (Ru5ISQ) NMNR sensors.

4. Characterize NMNR by UV-Vis., NMR, IR, LC-MS, excitation, and emission spectra of isoquinoline, Ru5ISQ, FSM, and NMNR.

5. Characterize the interaction of the moieties of NMNR with DCP by UV-Vis and fluorescence spectroscopy.

6. Investigate by UV-Vis, and emission spectroscopy the interaction of NMNR in solution and as a thin film with DCP.

7. Investigate if signal transduction by fluorescence resonance energy transfer (FRET) occurs.
2.1 Materials

All starting compounds were obtained from Aldrich and used as received. Solvents were purchased from Fisher. Elemental analysis was performed by Desert Analytics, Tucson, Arizona.

2.2 Instruments for characterization

All target compounds and their intermediates were characterized by $^1$H NMR, $^{13}$C{$^1$H} NMR, FTIR, LC/MS, UV-Vis Spectroscopy and particulate species were examined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images. The IR spectra were obtained with a Bruker EQUINOX 55 FTIR Spectrometer and are reported in cm$^{-1}$; UV-Visible spectra were recorded on a Perkin-Elmer UV/Vis Lambda 20 Spectrometer and are reported as absorbance (A) vs λ (nm). Fluorescence spectra were obtained with a Perkin-Elmer Luminescence LB 50 spectrometer and a Edinburgh time resolved and steady state fluorescence spectrometer. The $^{13}$C{$^1$H} and $^1$H NMR spectra were obtained with an JEOL 400 MHz instrument and are reported in δ(ppm) values with TMS as an internal standard. LC/MS spectra were obtained on a Shimadzu LCMS-2010A Liquid Chromatograph Mass Spectrometer.
NMR: JEOL JNM-ECP 400 FT NMR SYSTEM.

Eclipse 400 FT-NMR Spectrometer with Delta NMR Software.

The solvents for NMR experiments were $d_3$-methanol, $d_6$-DMSO, $d$-chloroform and $D_2$O.

LC/MS

The LC/MS data were collected by Shimadzu LC/MS-2010EV High – Performance Liquid Chromatograph/Mass Spectrometer.

Software: LCMS Solution

Stationary phase: 5 µm, C$_{18}$ silica gel.

Sample introduction: Auto injection

Mobile phase: MeOH/H$_2$O (gradient)

Flow rate: 0.6 mL/min.

Ionization: APCI (low flow rate Atmospheric Pressure Chemical Ionization) ESI (Electro Spray ionization).

Detector: UV detector.

Figure 2.1. Liquid Chromatography/Mass spectrometer.
UV-Vis. Spectrometer

Perkin Elmer UV/Vis. Lambda20 Spectrometer.

Figure 2.2. UV-Vis. Spectrometer.

Luminescence Spectrometer

Perkin - Elmer Luminescence LS 50B Spectrometer.

Figure 2.3. Luminescence Spectrometer.
FTIR Spectrometer

Broker Equinox 55 FTIR Spectrometer

Figure 2.4. FTIR Spectrometer.

Spin Coating

KW-4A Spin Coater

Spinning

Stage 1: 500-2500 rpm

Stage 2: 1000-8000 rpm

Vacuum $>2.1$ CFM

Figure 2.5. Spin coater.
Luminescence and Life time Spectrometer.

Edinburgh time resolved and steady state fluorescence spectrometer

Software: F900 Data acquisition and analysis software for steady state and life time fluorescent measurements.

Light sources: 1.nF 900: The nanosecond Hydrogen flashlamp excitation source for lifetime studies.

2. Xe lamp 900: Continuous excitation source for steady state studies


Detector: PMT (in air cooled housing).

Figure 2.6. Luminescence and Life time Spectrometer.
2.3 Synthetic methods

Sodium 4-(4-methoxyphenoxy) butanesulfonate (MPS1) (1 in Figure 2.7)

To a stirred solution of 0.4 g (10 mmol) of NaOH in 10 mL of dry THF was added 1 g (8 mmol) of 4-methoxyphenol and 1 mL of 1,4 butanesultone were added under N₂ at room temperature for 5 hours. The white solid that formed was collected by filtration and was recrystalized from methanol: acetone (1:1) mixture to give 0.85 g of MPS1 (yield = 85%). Analysis calculated (%) for C₁₁H₁₅O₂: C: 67.7, H: 6.5, and O: 25.8. Found: C: 68.0, H: 6.45, O: 22.6. \(^1\)H NMR (in d₆-DMSO; TMS reference; δ ppm) δ: 1.2 (m, 4H): d and e protons in butane group; 2.1(t, 2H): f protons in butane group; 3.3(s, 3H): b protons in methoxy group; 3.5(t, 2H: e protons in butane group; 6.5(s, 4H): ortho and meta protons in aromatic ring. \(^1\)C \(^{1}\)H NMR (d₆-DMSO; δ ppm): 157: aromatic carbon directly bound to oxygen atoms; 115 ortho and meta aromatic carbon; 69: e protons in butane group; 56: b: protons in methoxy group; 50: f protons in butane group; 30: d protons in butane group and 24: e protons in butane group. IR, KBr pellet(cm⁻¹): 3050: aromatic C-H stretching vibrations: 2950: aliphatic C-H stretching; 2850: C-H stretching of methoxy group; 1520: C=C stretching vibrations; 1280: aromatic C-O stretching; 1130: aliphatic C-O stretching; 1150: ring hydrogen rocking vibrations; 910-665: ring substitution bands.
**Sodium; 4-(2, 5-bis-bromomethyl-4-methoxy-phenoxy)-butane-1-sulfonate (MPS2) (2 in Figure 2.7)**

To a stirred solution of 1g (3.5mmol) of MPS1 in 10 mL glacial acetic acid, 0.25 g (8 mmol) of paraformaldehyde and 1 mL of 30% HBr in 3.5 mL acetic acid were added. The resulting mixture was stirred at 70°C for 5 hours. The white solid that formed was collected by filtration and was recrystallized from benzene to give 70% yield. Analysis calculated for $C_{13}H_{17}O_5SBr_2Na$: C: 33.3, H 3.6, and O: 17.8. Found C: 34.0, H: 3.5, O: 17.50. $^1$H NMR (in d4-MeOD; TMS reference; δ ppm) δ: 7.0 (2s,2H) a aromatic protons in ortho and meta positions; δ: 4.5 (2s,2H) g protons in alkyl halide group; 4 (t,2H): c protons in butane group; 3.8(s,3H): b protons in methoxy group; 2.1(t,2H): f protons in butane group; 2 (m,4H): d and e protons in butane group. $^{13}$C {$^1$H} NMR (d4-MeOD, δ ppm): 157: aromatic carbon directly bound to oxygen atoms; 124: ortho and meta aromatic carbon; 72: g labeled carbon in; 56: b protons in methoxy group; 50: f protons in butane group; 30: d protons in butane group and 24: e protons in butane group. IR, KBr pellet(cm$^{-1}$): 3050: aromatic C=C stretching vibrations: 2950: aliphatic C-H stretching; 2850: C-H stretching of methoxy group; 1520: C=C stretching vibrations; 1280: aromatic C-O stretching; 1130: aliphatic C-O stretching; 1150: ring hydrogen rocking vibrations; 910-665: ring; 520: stretching vibrations of C-Br bond.
**Tetraethyl(2-butanesulfonate-5-methoxy) -1,4-phenylenebis (methylene)- biphosphonate. (MPS3) (3 in Figure 2.7)[21, 22, 23]**

A 1g (2.2 mmol) of MPS2 in 1mL (7 mmol) of triethylphosphite and heated in a seal tube at 180 °C for 5 hours. Given brownish oily compound was dispersed in cold ether and kept in refrigerator for 2 days to obtain 0.6 g of MPS3 white crystals (yield:60%). Analysis calculated: C: 43.4, H: 6.3, and P:10.2. Found C: 44.1, H: 6.5, P: 10.0. \(^1\)H NMR (in \(d\)-CDCl3 TMS reference; \(\delta\) ppm) 6.9-7.0(2s, 2H): a aromatic protons in ortho and meta positions; 4.0-4.2 (m,12H): h protons in molecule; 3.80(s,3H): b lprotons in methoxy group; 3.20 (t,2H): c protons in butane group; 1.80-2.00 (t,2H): f protons in butane group; 1.00-1.30 (m, 16H):i protons in methyl groups in ethoxy groups. \(^{13}\)C\{\(^1\)H\} NMR (\(d\)-CDCl3 ; \(\delta\) ppm); 150: aromatic carbon directly bound to oxygen atoms; 118: ortho and meta aromatic carbon connected to alkyl phosphate ester group; 115: free ortho and meta carbons in aromatic ring; 72: g carbon in alkyl halide group ; 68: h carbon in phosphate group; 56: b protons in methoxy group; 50: f protons in butane group; 30: d carbon in butane group; 22: methyl groups in phosphate ester group. IR, KBr pellet(cm\(^{-1}\)); 3050: aromatic C=C stretching vibrations; 2950: aliphatic C-H stretching; 2850:C-H stretching of methoxy group; 1520: C=C stretching vibrations; 1280:aromatic C-O stretching; 1130: aliphatic C-O stretching; 1150: ring hydrogen rocking vibrations; 910-665: ring bands.
4-{2, 5-Bis-[2-(4-formyl-phenyl)-vinyl]-4-methoxy-phenoxy}-butane-1-sulfonic acid sodium anion (FSM) (4 in Figure 2.7)[21]

Dry THF (10 mL) and 0.45 g (4 mmol) of t-BuOK were placed in a three neck round bottom flask. A connected dropping funnel was charged with terephthalaldehyde 0.7 g (3.5 mmol) and bis (phosphonate) 1g (1.7 mmol) dissolved in 12 mL of dry THF. After the solution had been purged with nitrogen, the mixture was added slowly (30 min.) to a mechanically stirred solution of the base at room temperature and stirring was continued for over night. The precipitate formed was diluted with \( \text{H}^+ \)/ cold water and collected by filtration and preparative TLC on silica plate with ethyl acetate in a solvent gave pure product (yield =45% and overall yield of reaction = 20%). \( ^1 \text{H} \) NMR (in \( d \)-CDCl\(_3\) TMS reference; \( \delta \) ppm); 10 (s 2H): \( j \) aldehyde protons; 7.8-8.0 (m 10H): aromatic protons in three benzene rings; 5.5 (d 2H) \( g \) : protons; 3.6 (m 7H; \( c, f \) protons; 1.2 (m 4H): \( d, e \) protons.

Poly (2-methoxy-5-propyloxy sulfonate phenylene vinylene [MPS-PPV] (5 in Figure 2.8)

2 g (4.4 mmol) of MPS2 in 15 mL of dry THF was refluxed with 1.0 g (10 mmol) of potassium tertiary butoxide for 12 hours. The deep red solution of MPS-PPV was purified by dialysis (10,000 molecular weight cut off films). THF was removed by evaporation and deep red shiny polymer was obtained and was stored in desicater. (Yield=50%) Analysis calculated for \( C_{13}H_{15}O_5SNa \): C: 50.32, H: 5.17, and O: 25.78. Found C: 49.52, H: 5.03, O: 26.0. IR, KBr pellet (cm\(^{-1}\)); 3400: aromatic C-H stretching
vibrations; 1500: aromatic C=C stretching vibrations; 1400: aromatic C-O stretching vibrations.

Figure 2.7. Synthesis of Fluorescent Sulfonated Monomer (FSM).
Figure 2.8. Synthesis of Poly (2-methoxy-5-propyloxy sulfonate phenylene
vinylene)[MPS-PPV (5)].

**5, 5’-Dimethyl-2, 2’-bipyridine (6 in Figure 2.8) [23]**

3-picoline (115 mL) (1.2 mol) was refluxed with 5 g of 10% palladium on charcol for three days. After the addition of 40 mL of hot benzene the reflux was continued for one hour. The hot mixture was filtered to remove the catalyst and concentrated to 40 mL. Colorless crystals precipitated and were recrystallized from ethyl acetate. NMR (in $d$-CDCl$_3$ TMS reference; δ ppm): 9.20: a proton; 8.25: b proton; 7.5: c protons; 2.3: d proton in compound number 6. $^{13}$C{$_1$H} NMR (CDCl$_3$, δ ppm): 155: e carbon; 150: a carbon; 138: b carbon; 133: f carbon; 120: c carbon; 18: g carbon in compound number 6.
5, 5’-Dicarboxy-2, 2’-bipyridine (7 in Figure 2.9)[23]

1 g of 5, 5’-Dimethyl-2, 2’-bipyridine (5 mmol) and 3g (17 mmol) of potassium permanganate were refluxed in 40 mL of water for 15 hours. Yellowish solution obtained upon filtration was extracted with ether to remove unreacted starting materials. The addition of concentrated hydrochloric acid resulted in white crystals which were filtered and washed several times with water. NMR (in $d_2$-D$_2$O, TMS reference; δ ppm) 9.20: i protons in compound number 7; 8.55: j protons in compound number 7; 8.45: k proton. IR, KBr pellet (cm$^{-1}$): 1650: carbonyl stretching vibrations.

Cis- dichlorobis (bipyridine) ruthenium (8 in Figure 2.10)[23]

Ruthenium trichloride 5 g (23 mmol) and 7.5g (48 mmol) of 2, 2’-bipyridine were heated at reflux in 200 mL of N, N’-dimethylformamide for four hours. More than half of the solvent was removed by rotary evaporation and the remaining solution was cooled to room temperature. It was treated with 150 mL of acetone and kept at 0°C overnight. Dark red crystals formed were collected by filtration and washed several times with water. These crystals were refluxed with 250 mL of water: ethanol (1:1) for 2 hours, filtered to remove insoluble solids and the filtrate was treated with 50 g (1.2 mol) of lithium chloride. The ethanol was removed by rotary evaporation and the resulting water solution was cooled in an ice bath. Dark red crystals precipitated (yield = 80%). $^1$HNMR (in $d$-CDCl$_3$ TMS reference; δ ppm): 9.9(d,2H): a protons; 8.6(d,2H): e protons; 8.5(d,2H): d
protons; 8.1(t,2H): b protons; 7.4(d,2H): h protons; 7.79(t,2H): f protons; 7.66(t,2H): c protons; 7.1(t,2H): g protons.

**Bis (bipyridine)-5, 5’-dicarboxy-2, 2’-bipyridineum ruthenium(9 in Figure 2.10)[23]**

1 g (2 mmol) of cis- dichlorobis(bipyridine)ruthenium, 0.7 g (2.6 mmol) of 5,5’-dicarboxy-2,2’-bipyridine and 0.7 g (10 mmol) sodium bicarbonate were heated in a mixture of 30 mL of water and 20 mL of methanol for 3 hours at reflux temperature. Then an aqueous solution of ammonium hexafluorophosphate (1g in 5 mL water) was added and the resulting solution refrigerated overnight. Deep red crystals of product precipitated (Yield = 70%).

**Bis(bipyridine)-5,5’-dicarboxy-bis(5-aminoisoqunoline)-2,2’-bipyridineum ruthenium.(Ru 5 ISQ) (10 in Figure 2.10)[23, 24]**

Bis(bipyridine)-5,5’-dicarboxy-2,2’-bipyridineum ruthenium, 2 g (4 mmol), was reacted with 15 mL of fresh thionyl chloride overnight and unreacted thionyl chloride was removed by rotary evaporation. The dark brown product was dissolved in 15 mL of dichloromethane, 1.2 g (9 mmol) of 5-aminoisoquinoline was added and this mixture was degassed with dry N\textsubscript{2}. The mixture was kept at room temperature for one day and the solvent removed by rotary evaporation. The dark red product was recrystallized from acetone: methanol (1:1) mixture and was purified by an alumina column with ethyl acetate eluent.
Figure 2.9. Synthesis of 5,5-Dicarboxy-2,2'-bipyridine(7).

Figure 2.10. Synthesis of Bis (bipyridine)-5, 5'-dicarboxy-bis (5aminoisoquinoline)-2, 2'-bipyridinium ruthenium hexafluorophosphate(Ru5ISQ) (10).
Synthesis of 40 nm Silica nanoparticles. [25]

Tetraethylorthosilicate (1.0mL), 1.5 mL 30% ammonia and 1.0 mL water in 500 mL of methanol were reacted at 32°C for 6h. 1.0 mL of ammonia and 1.0 mL of tetraethylorthosilicate were added and reacted for another hour. The mixture was diluted with water and centrifuged at 5000 RPM for 30 minutes to isolate the SiO$_2$ nanoparticles. They were air dried for 2 days. TEM images revealed the size to be 40±15 nm.

FSM-Schiff’s base (1 in Figure 2.11)

The compound, FSM (1 g; 2 mmol) in 20 mL of acetonitrile was mixed with 1.2 mL of 3-aminopropyltriethoxysilane at room temperature for 30 minutes.

SiO$_2$-FSM-Schiff’s base (2 in Figure 2.11)

A 20 mg quantity of 40 nm silica nanoparticles were reacted with 20 mg of FSM-Schiff’s base overnight in 20 mL acetonitrile solvent at reflux temperature overnight. The SiO$_2$ nanoparticles were obtained by centrifugation at 5000 RPM for 30 minutes and dried for 2 days.

NMNR sensor (in Figure 1.4 a and b)

A 50 mg quantity of SiO$_2$ nanoparticles derivatized with FSM was equilibrated with 30 mg of bis(bipyridine)-5,5’-dicarboxy-bis(5aminoisoquinoline)-2,2’-bipyridinium ruthenium (10 in Figure 2.10) in CH$_3$CN for 5 hours at room temperature. The solvent was removed by rotary evaporation and dried in a vacuum.
Figure 2.11. Synthesis of SiO$_2$-FSM-Schiff’s base.
CHARACTERIZATION OF THE MOIETIES OF NMNR SENSOR

The spectral and microscopic imaging characterizations of the moieties of the NMNR sensor and the intermediate compounds synthesized to obtain these moieties are described in this chapter. The assignment of peaks in characterization by $^1$H and $^{13}$C{1H} NMR and IR spectra have been listed in chapter 2.

3.1 NMR, IR and LC/MS characterization of MPS1 (2 in Figure 2.7)

![NMR Spectrum of MPS1](image)

Figure 3.1. $^1$H NMR spectrum of MPS$_1$ (in $d_6$-DMSO).
Figure 3.2. $^{13}$C{$^1$H} spectrum of MPS1 (in d6-DMSO).

Figure 3.3. FTIR spectrum of MPS1 (KBr pellet).
Figure 3.4 (a). Chromatogram of MPS1 by LC/MS.

Figure 3.4 (b). Mass spectral fragmentation of MPS1.
3.2 NMR, IR and LC/MS characterization of MPS2 (3 in Figure 2.7)

Figure 3.5. $^1$H NMR spectrum of MPS2 (in $d_4$-MeOD$_3$).

Figure 3.6. $^{13}$C{$^1$H} spectrum of MPS2 (in $d_4$-MeOD$_3$).
Figure 3.7. FTIR spectrum of MPS2 (KBr pellet).

Figure 3.8 (a). Chromatogram of MPS2 by LC/MS.
3.3. NMR, IR and LC/MS characterization of MPS3 (4 in Figure 2.7)

Figure 3.9. $^1$H NMR spectrum of MPS3 (in $d$-CDCl$_3$).
Figure 3.10. $^{13}$C$\{^1$H$\}$ spectrum of MPS3 (in $d$-CDCl$_3$).

Figure 3.11. FTIR spectrum of MPS3 (KBr pellet).
The chromatogram of MPS3 by LC/MS indicates the compound to be pure. The molecular ion peak corresponding to m/z value of 561 is a minor species which quickly loses 2 hydrogen to yield the species with a m/z value of 559 as the major peak.

Figure 3.12 (a). Chromatogram of MPS3 by LC/MS.

Figure 3.12 (b). Mass spectral fragmentation of MPS3 by LC/MS.
3.4 Characterization of FSM by NMR, IR, LC/MS, UV-Vis and Fluorescence

Figure 3.13. \(^1\)H NMR spectrum of FSM (in \(d\)-CDCl\(_3\)).

Figure 3.14 (a). Chromatogram of FSM by LC/MS.
Figure 3.14 (b). Mass spectrum of FSM by LC/MS.

UV-Vis spectrum of FSM has three absorbance peaks 412 nm, 340 nm, and 254 nm in acetonitrile solvent. The excitation spectrum in Figure 3.17 for 520 nm emission is in agreement with the absorbance spectrum by Edinburgh and Perkin-Elmer instruments are similar and the spectrum from Edinburgh instrument has better resolution.
Figure 3.15. FTIR spectrum of FSM (KBr pellet).

Figure 3.16. UV-Vis spectrum of FSM in CH$_3$CN (10$^{-5}$ M).
Figure 3.17(a). Excitation spectrum of FSM in CH$_3$CN (emission wavelength: 550 nm, emission filter: 495 nm) ($10^{-5}$ M)[Edinburgh instrument].

Figure 3.17(b). Excitation spectrum of FSM in CH$_3$CN emission wavelength: 550 nm, emission filter: 495 nm) ($10^{-5}$ M)[Perkin-Elmer instrument].
Figure 3.18(a). Emission spectrum of FSM in CH$_3$CN (Excitation wavelength: 254 nm; emission cut off filter: 340 nm) (10$^{-5}$ M)[Edinburgh instrument].

Figure 3.18(b). Emission spectrum of FSM in CH$_3$CN (Excitation wavelength: 254 nm; emission cut off filter: 340 nm) (10$^{-5}$ M)[Perkin-Elmer instrument].
The same emission spectrum was obtained by exciting at 254, 340, and 412 nm indicating that the emission occurs from the same excited state irrespective of the excitation wavelength.

3.5 Characterization of the FSM Schiff’s base with trimethoxyaminopropylsilane by IR, UV-Vis and emission studies

The UV-Vis spectrum of the Schiff’s base of FSM with triethoxyaminopropylsilane in acetonitrile has two absorption maxima at 325 and 400 nm which resemble the spectrum of FSM in Figure 3.20. The emission spectrum has a maximum at 470 nm with a shoulder at 500 nm in contrast to the emission spectrum of FSM. The same emission spectrum with different intensities were obtained with 335 and 400 nm excitations.

![Figure 3.19. UV-Vis spectrum of FSM-Schiff’s base in CH₃CN (10⁻⁵ M).](image)
Figure 3.20(a). Excitation spectrum of FSM-Schiff’s base in CH$_3$CN ($10^{-5}$ M) (emission wavelength: 470 nm, emission cut off filter: 455 nm) [Edinburgh instrument].

Figure 3.20(b). Excitation spectrum of FSM-Schiff’s base in CH$_3$CN ($10^{-5}$ M) (emission wavelength: 470 nm, emission cut off filter: 455 nm) [Perkin-Elmer instrument].
Figure 3.21(a). Emission spectrum of FSM-Schiff’s base in CH$_3$CN (Excitation wavelength: 335 nm; emission cut off filter: 390 nm) ($10^{-5}$ M) [Edinburgh instrument].

Figure 3.21(b). Emission spectrum of FSM-Schiff’s base in CH$_3$CN (Excitation wavelength: 335 nm; emission cut off filter: 390 nm) ($10^{-5}$ M) [Perkin-Elmer instrument].
3.6 Characterization of SiO$_2$–FSM Schiff’s base by TEM and UV-Vis and emission spectroscopy

The UV-Vis spectrum of SiO$_2$-FSM Schiff’s base in acetonitrile has three shoulders at 390, 250 and 230 nm and the excitation peaks at 405, 330 and 300 nm. The emission spectra shown in Figure 3.26(a) and 3.26(b) is similar to that of the FSM Schiff’s base.

Figure 3.22(a). TEM image of unreacted SiO$_2$ nanoparticles.

Figure 3.22(b). TEM image of unreacted SiO$_2$ nanoparticles at different magnifications 500 nm.
Figure 3.23. TEM images of SiO$_2$–FSM Schiff’s base at 100 nm magnification at different regions of the grid.

Figure 3.24. UV-Vis spectrum of FSM Schiff’s base- SiO$_2$ in CH$_3$CN (10$^{-5}$ M).
Figure 3.25(a). Excitation spectrum of FSM-Schiff’s base- SiO$_2$ in CH$_3$CN (emission wavelength: 496 nm, excitation cut off filter: 455 nm) ($10^{-5}$ M) [Edinburgh instrument].

Figure 3.25(b). Excitation spectrum of FSM-Schiff’s base- SiO$_2$ in CH$_3$CN (emission wavelength: 496 nm, excitation cut off filter: 455 nm) ($10^{-5}$ M) [Perkin-Elmer instrument].
Figure 3.26(a). Emission spectrum of SiO$_2$-FSM-Schiff’s base in CH$_3$CN Excitation wavelength: 300 nm, emission cut off filter: 395 nm $10^{-5}$ M) [Edinburgh instrument].

Figure 3.26(b). Emission spectrum of SiO$_2$-FSM-Schiff’s base in CH$_3$CN excitation wavelength: 300 nm, emission cut off filter: 395 nm($10^{-5}$ M)[ Perkin-Elmer instrument].
The TEM images, Figure 3.22 a and 3.22 b of unreacted silica nanoparticles, indicates the average size to be \(40 \pm 15\) nm and it has almost mono disperse. These SiO\(_2\) nanoparticles when dip coated on to silicon wafer self assembled to yield hexagonal closed packed arrays as displayed in Figure 3.27.

![SEM images of self assembled SiO\(_2\) nanoparticles.](image)

When FSM is attached to SiO\(_2\) and TiO\(_2\) nanoparticles, it may be anchored on a single nanoparticle or bridge to two nanoparticles through the formation of as Schiff’s base. This bridging can lead to aggregation of nanoparticles as shown in Figure 3.23.

The IR spectra of SiO\(_2\) in Figure 3.28 and SiO\(_2\) derivatized with FSM in Figure 3.29 clearly indicate that FSM is attached to SiO\(_2\).
Figure 3.28. FTIR spectrum of SiO$_2$ nanoparticles. (KBr pellet)

Figure 3.29. FTIR spectrum of SiO$_2$-FSM nanoparticles. (KBr pellet)
SEM images of SiO$_2$-FSM particles coated on quartz plate are shown in Figure 3.30. Different sections of plate are displayed in a, b, c and d.

Figure 3.30 (a,b,c,d). SEM images of sensor coated on quartz.

The SiO$_2$-FSM nanoparticles were deposited by spin coating on quartz plates. The plates were initially coated with the film of trimethoxyaminopropyl silane followed by layer of nanoparticles dispersed in CH$_3$CN. The SEM images in 3.30 a, b, c and d indicate that coating is not uniform. Some areas of quartz is not covered with nanoparticles. The areas that are covered by the nanoparticles have multiple layers of
nanoparticles compared to the uniform hexagonal close packing achieved for underivatized SiO₂ nanoparticles on silicon wafers.

3.7. Characterization of MPS-PPV by UV-Vis and emission spectroscopy

MPS-PPV((Poly(2-methoxy-5-propyloxy sulfonate phenylene vinylene) is a highly conjugated, negatively charged light emitting polymer which dissolves very easily only in water [26]. The photophysical properties of fluorescent, water soluble polyanionic conjugated polymer [poly(2-methoxy-5-propyloxy sulfonate phenylene vinylene(MPS-PPV) one of a larger class of related semi conducting organic polymers [poly phenylene vinylene(PPV)] that have been the subject of recent interest [27, 28, 29]. Although much attention has focused on the well known potential for use of PPV derivatives as electronic materials [e.g. electrochemical sensors, light-emitting diodes, and integrated circuits], the highly charged back bone of MPS-PPV (with charge density approximating that of polynucleic acids such as DNA and RNA), also makes it a model polymer for understanding the interactions and self-assembly properties of charged biopolymers [30].

The polymer MPS-PPV was characterized by UV-Vis, excitation, emission and IR spectroscopy. These spectra are displayed in Figures 3.31, 3.32, 3.33 and 3.34. The absorbance and excitation spectra are similar. The absorbance spectrum is dominated by the peak with an absorbance maximum at 470 nm which is different from that of FSM where this peak is not dominant. The emission spectra of FSM and MPS-PPV are similar with λ_max being 510 nm and 575 nm, respectively. The value has red
shifted by 65nm in MPS-PPV compared to FSM. The aggregation of MPS-PPV in water has been characterized by TEM and is discussed in section 3.10.

Figure 3.31. UV-Vis spectrum of MPS-PPV in H₂O (10⁻⁶ M).

Figure 3.32. Excitation spectrum of MPS-PPV in H₂O (Excitation wavelength: 470 nm, excitation cut off filter: 545 nm) (10⁻⁶ M).
Figure 3.33. Emission spectrum of MPS-PPV (Excitation wavelength: 470 nm, emission cut off filter: 495nm) \((10^{-6} \text{ M})\).

Figure 3.34. IR spectrum of MPS-PPV. (in KBr pellet)
3.8. Characterization of Bis(bipyridine)-5,5'-dicarboxy-bis(5-aminoisoquinoline)-
2,2'-bipyridinium ruthenium hexafluorophosphate (Ru5ISQ)

Figure 3.35. $^1$H NMR spectrum of Ru(bpy)$_2$Cl$_2$ (in $d_3$-CDCN).

Figure 3.36. UV-Vis spectrum of Ru(bpy)$_2$Cl$_2$ (in CH$_3$CN).
The target (Ru5ISQ) complex with the isoquonoline receptor was synthesized by the procedure shown in Figure 2.3. The final complex and the various intermediates were characterized by $^1$H NMR, UV-Vis and fluorescence spectroscopy. These are discussed in this section.

The $^1$H NMR and UV-Vis spectra of Ru(bpy)$_2$Cl$_2$ are shown in Figure 3.36 and 3.37. The $^1$H NMR as indicated by the peaks assigned indicates the correct structure. The UV-Vis spectrum is in agreement with the published spectra [21]. The 360 nm absorption corresponds to π-π* transition of the bipyridyl ring and the 550 nm absorption is the metal to ligand charge transfer (MLCT) absorption. The excitation and emission spectra are displayed in Figures 3.38 and 3.39. The excitation spectrum for 760 nm emission is similar to the absorption spectrum in Figure 3.36. The emission spectra...
obtained with 365 nm excitation has a maximum at 760 nm corresponding to ligand to metal charge transfer (LMCT) deactivation of the MLCT excited state. This indicates that \(\pi-\pi^*\) excited state generated by 365 nm excitation quickly transitions to MLCT excited state from which photon emission occurs.

![Emission spectrum of Ru(bpy)_2Cl_2 in CH_3CN (excitation wavelength: 550 nm, emission cut off filter: 595 nm) (10^{-5} M).]

The \(^1\)H NMR of 5,5'-bipyridyl dicarboxylic acid shown in Figure 3.39 clearly indicates the various protons and their chemical shifts. The IR spectrum of this compound in KBr pellet in Figure 3.40 indicates the OH stretch at 3000 cm\(^{-1}\), C-H stretch at 2600 cm\(^{-1}\), C=O stretch at 1600 cm\(^{-1}\) and the aromatic stretch at 900-1500 cm\(^{-1}\).
The complex Ru(bpy)$_2$(5,5'-bpy-dicarboxilic acid) was characterized by $^1$H NMR (Figure 3.41), UV-Vis (Figure 3.42), excitation(Figure 3.43) and emission (Figure 3.44) spectroscopy. The $^1$H NMR as indicated by the labels in agreement with the structure of the complex. The UV-Vis spectrum is different from that for Ru(bpy)$_2$Cl$_2$ with the MLCT charge transfer transition at 450 nm compared to 550 nm for the former.

The additional bipyridyl ring considerably increases the molar absorptivity for $\pi$-$\pi^*$ transition in the compound and as a result the MLCT absorption peak. The excitation spectrum for 675 nm emission in Figure 3.43 resembles the UV-Vis absorption spectrum in Figure 3.42 indicating the emission to be due to LMCT transition. The LMCT emission spectrum is obtained with 475 nm excitation is displayed in Figure 3.44.

Figure 3.39. $^1$H NMR spectrum of 2,2’ Bipyridine-5,5’-dicarboxylic acid (in d6-DMSO).
Figure 3.40. IR spectrum of 2,2'-Bipyridine-5,5'-dicarboxylic acid. (KBr pellet)

Figure 3.41. $^1$H NMR spectrum of Bis(bipyridine)-5,5'-dicarboxy-2,2'-bipyridineium ruthenium (in d3-CDCN)].
Figure 3.42. UV-Vis spectrum of Bis(bipyridine)-5,5’-dicarboxy-2,2’-bipyridinium ruthenium (in CH$_3$CN).

Figure 3.43. Excitation spectrum of Bis(bipyridine)-5,5’-dicarboxy-2,2’-bipyridinium ruthenium in CD$_3$CN (Emission wavelength: 675 nm, excitation cut off filter: 595 nm)(10$^{-5}$ M).
Figure 3.44. Emission spectrum of Bis(bipyridine)-5,5'-dicarboxy-2,2'-bipyridineum ruthenium in CH$_3$CN (Excitation wavelength: 475 nm, emission cut off filter: 545 nm).

The complex with the receptor molecule isoquinoline, Ru5ISQ has the $^1$H NMR spectrum in Figure 3.45, UV-Vis spectrum in Figure 3.46, the excitation spectrum in Figure 3.47 and the emission spectrum in Figure 3.48. The UV-Vis spectrum has two prominent maxima, one at 440 nm due to MLCT absorption and 287 nm which is absent in the spectrum of the bis(bipyridine)-5,5'-dicarboxy-2,2'-bipyridinium ruthenium. The 287 nm absorption is likely due to the isoquinoline receptor. The excitation spectrum is shown in Figure 3.47. The emission spectrum in Figure 3.48 obtained with 287 nm excitation has two emission maxima at 440, and 660 nm. The 660 nm emission is due to the LMCT transition [22]. Also 440 nm most is likely due to the isoquinoline receptor which has an emission maximum at 380 nm by itself in CH$_3$CN. The emission maximum
for this moiety has red shifted by 60 nm. The UV-Vis and the emission spectra clearly indicate that the π electron density of isoquonoline is coupled to the π electron density of the bipyridyl rings through the amide bond.

Figure 3.45. $^1$H NMR spectrum of Ru5ISQ (in $d_3$-CDCN).

Figure 3.46. UV-Vis spectrum of Ru5ISQ in CH$_3$CN (10$^{-5}$ M).
Excitation at 287 nm leads to Ru(II) centered emission at 660 nm and isoquinoline emission at 440 nm indicating that π-π* excited state convert to MLCT excited state in addition to the 287 nm photon directly populating the MLCT excited state. The only emission observed in the case of Ru(bpy)$_2$Cl$_2$ and Ru(bpy)$_2$(5,5'-bipyridine dicarboxylic acid) are the emission due to the MLCT excited state corresponding to LMCT transition. The coupling of the π electron density of isoquinoline with those of the bipyridyl rings is important for signal transduction by fluorescence resonance energy transfer.
Figure 3.48.(a) Emission spectrum of Ru5ISQ in CH$_3$CN (excitation wavelength: 300 nm, emission cut off filter: 345nm) (10$^{-5}$ M)[Edinburgh instrument].

Figure 3.48.(b) Emission spectrum of Ru5ISQ in CH$_3$CN (excitation wavelength: 300 nm, emission cut off filter: 345nm) (10$^{-5}$ M)[Perkin-Elmer instrument].
Figure 3.49. UV-Vis spectrum of NMNR sensor in CH$_3$CN (10 mg in 10 mL).

Figure 3.50. Excitation spectrum of NMNR sensor in CH$_3$CN (emission wavelength: 700 nm, excitation cut off filter: 595 nm) (10 mg/mL).
The UV-Vis spectrum of NMNR sensor shown in Figure 3.49 clearly has contribution from the different moieties. The excitation spectrum in Figure 3.50 resembles the UV-Vis spectrum and the emission spectrum in Figure 3.51 has emission due to isoquinoline, FSM, and Ru(II) complex.

3.9 Quantum yields of emission

The emission quantum yields of the moieties of NMNR sensor were determined and are listed in table 3.1. Rhodamine B (quantum yield: 0.91, in acetonitrile) [31] was used as the reference compound for the determination of the quantum yields of isoquinoline and FSM and Ru(bpy)$_3$Cl$_2$.6H$_2$O (quantum yield: 0.12) [32] was used as
reference for the Ru(bpy)$_2$(bpy-isq) and FSM- Ru(bpy)$_2$(bpy-isq). The quantum yields were determined by integrating the spectra of the reference compound and the NMNR moiety over the entire spectral region indicated in Table 3.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Spectral range</th>
<th>Quantum yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>isoquinoline</td>
<td>300-675 nm</td>
<td>0.12</td>
</tr>
<tr>
<td>FSM</td>
<td>400-700 nm</td>
<td>0.48</td>
</tr>
<tr>
<td>Ru(bpy)$_2$(bpy-isq)</td>
<td>400-800 nm</td>
<td>0.38</td>
</tr>
<tr>
<td>FSM- Ru(bpy)$_2$(bpy-isq)</td>
<td>400-800 nm</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 3.1. Quantum yields of emission for NMNR moieties in acetonitrile.

3.10 Aggregation properties of FSM and MPS-PPV

The compound FSM was dissolved in methanol and deposited on TEM grids to determine the nature of it’s aggregation. The TEM images are displayed in Figure 3.52 a and 3.52 b. The FSM compound forms spherical structures of very small (5 nm) to very large (10 µm) in size. The large spherical structures encapsulate small spheres resembling multivesicle vesicle structures encountered with phospholipids. Some of the smaller spheres melt under the electron beam and can be seen as white spheres in Figure 3.31 b. The extended π electron density of FSM lead to π – π* electrostatic and van der Waal’s interactions leading to spherical self assemblies in methanol. The aggregations have been investigated by light scattering as discussed in this section.
Figure 3.52. (a and b) TEM images of FSM in methanol.

The TEM image of the polymer MPS-PPV (Poly(2-methoxy-5-propyloxy sulfonate phenylene vinylene) dissolved in water and deposited in TEM grids was also investigated. As shown in Figure 3.53 this polymer form dense spherical structures which extensively aggregate [33]. This polymer similar to DNA has a backbone from which propyl group with terminating sulfonate group branch. Self assembly of this polymer through electrostatic, $\pi - \pi^*$ and van der Waal’s force can result in spherical and linear structures. The TEM images reveal that spherical structures are indeed formed but most likely the linear structures bundle into spherical structures as the Watson-Crick type DNA structure of complementary pairs through hydrogen bonding.
The aggregation of monomer FSM in methanol and MPS-PPV polymer in water have been characterized by static multi angle light scattering (MALS) and dynamic or queri elastic light scattering (QELS). The technique provide their aggregate molecular weight, hydrodynamic radii($R_g$), and the radius of gyration($R_h$).

In multiple light scattering employing a incident laser beam at 690 nm, the intensity of scattered light $I(\theta)$ as a function of scattering angle is given by equation 3.1.

$$I(\theta) \propto R(\theta) = K^* M C P(\theta) [1-2A_2 MC P(\theta)] \quad (3.1)$$

Here

M= Molar mass

C= Concentration in g/mL
\[ K^* = \frac{4n^2 n_0^2}{N\lambda_0^4} \left( \frac{dn}{dc} \right)^2 \]  \hspace{1cm} (3.2)

Where

\( n_0 \) = solvent refractive index

\( N \) = Avogadro’s number

\( \lambda_0 \) = wave length of incident light (690 nm)

\( dn/dc \) = specific refractive index increment as a function of solute concentration.

\( P(\theta) \) = scattering function or the excess Rayleigh ratio. It is the ratio of the scattered and incident light intensities that relates the angular variation in scattering intensity to the mean square radius or radius of gyration, \( R_g \) of the particle.

\( A_2 \) = second virial coefficient which is a measure of solute-solvent interaction. If \( A_2 = 0 \) the solvent is ideal. If it is positive it is a good solvent for the solute and if it is negative it is poor solvent for the solute. It enters the light scattering equation as a correction factor for concentration effects.

The MALS experiments are conducted by measuring the angular and concentration dependent light intensity and fit to equation 3.1. In principle a three dimensional plot of intensity of scattering light as a function of scattering angle \( \theta \) and concentration \( C \) can be constructed. A two dimensional slice of the three dimensional plot is called a Zimm plot where \( KxC / R(\theta) \) is plotted as a function of \( \sin^2 (\theta/2) + kC \), where \( k \) being a stretch factor used to make \( kC \) and \( \sin^2(\theta/2) \) in the same numerical range. Zimm plot typically has lines for each concentration of solute employed. It resembles a grid with each horizontal line corresponding light scattering as a function of scattering angle for a given concentration an each vertical line corresponding to light
scattering at a given angle as a function of concentration. Slope of the horizontal line provides the radius of gyration $R_g$ and slope of the vertical line provides the second virial coefficient $A_2$. Extrapolation of the values to zero concentration provides the molecular weight $M$.

Debye plot is the plot of $R(\theta)/KxC$ as a function of $\sin^2(\theta/2)$. Unlike Zimm plot Debye plot can be constructed for a single concentration. The intercept of the Debye plot gives the reciprocal of the molecular weight and the individual slope gives the radius of gyration $R_g$.

In a QELS experiment the sample is illuminated with light and the scattered light is observed as a function of time at a fixed angle. This measurement is performed at only scattering angle and provides the diffusion coefficient $D_T$ and the hydrodynamic radius $R_h$. The ratio $R_h/R_g$ is the polydispersity factor. The autocorrelation function $g(\lambda)$ is plotted as a function of time. The function $g(\lambda)$ is given by

$$g(\lambda) = 1 + \alpha e^{-\lambda/\kappa} \quad (3.3)$$

$$\kappa = R_h \frac{3\eta \lambda^2}{16\pi k_B T \sin^2(\theta/2)}$$

$R_h$ = hydrodynamic radius

$\eta$ = viscosity

$\lambda = \lambda_0/n$ = wavelength in vacuum/refractive index

$k_B$ = Boltzmann constant

$T$ = temperature in K

$\theta$ = scattering angle at which light intensity is measured (usually $90^0$).
The Zimm plot for FSM at concentrations $3.12 \times 10^{-5}$ g/mL and $6.25 \times 10^{-5}$ g/mL is shown in Figure 3.54. The Debye plot for $3.12 \times 10^{-5}$ g/mL is shown in Figure 3.55 and the autocorrelation factor as a function of time is shown in Figure 3.56. The molecular weight $M_w$, radius of gyration $R_g$, hydrodynamic radius $R_h$, second virial coefficient $A_2$ and diffusion coefficient $D_T$ are listed in table 3.2.

<table>
<thead>
<tr>
<th>M (Zimm)</th>
<th>M (Debye)</th>
<th>$R_g$ (Zimm) (nm)</th>
<th>$R_g$ (Debye) (nm)</th>
<th>$A_2$ (mol/mL/g)²</th>
<th>$R_h$ (nm)</th>
<th>$D_T$ (Cm²/sec)</th>
<th>$R_h/R_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(5.8 \pm 0.2) \times 10^4$</td>
<td>$(1.92 \pm 0.08) \times 10^5$</td>
<td>$63.6 \pm 16.5$</td>
<td>$79.9 \pm 12.8$</td>
<td>$-0.088 \pm 0.028$</td>
<td>$40.1 \pm 2.$</td>
<td>$5.31 \pm 0.3$</td>
<td>$4 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

Table 3.2. The $M$, $R_g$, $R_h$, $A_2$ and $D_T$ values for FSM.

Figure 3.54. Zimm plot for FSM at $3.12 \times 10^{-5}$ g/mL and $6.2 \times 10^{-5}$ g/mL in methanol.
Figure 3.55. Debye plot for FSM at $3.12 \times 10^{-5}$ g/mL in methanol.

Figure 3.56. Correlation function vs. time for FSM at $3.12 \times 10^{-5}$ g/mL in methanol.
The molecular weight of FSM determined by the mass spectrometry is 519. It is evident from light scattering data that it aggregates extensively in methanol solution as indicated by the molecular weight and the hydrodynamic radius. Light scattering measures an average molecular weight and size. The TEM images indicate aggregate larger and smaller than the $R_h$ value from light scattering measurements. The TEM measurements are performed by evaporating a methanol solution on a carbon grid which could lead to further aggregation and result in larger sizes observed. The molecular weight from MALS experiment indicates that approximately 1000 FSM monomers aggregate to spherical structures.

The molecular weight ($M_w$), $R_g$, $R_h$, $R_g/R_h$, $D_T$ and $A_2$ of MPS-PPV were determined as a function of NaCl concentration. It is well established that a polyelectrolyte like MPS-PPV will have different conformations and aggregation properties at different ionic strengths. The Zimm plot for MPS-PPV concentration at 0.3 M NaCl is shown in Figure 3.57. The Debye plot for MPS-PPV at $1.25 \times 10^{-4}$ g/mL in 0.3 M NaCl is shown in Figure 3.58 and the correlation function dependence on time is shown in figure 3.59. The different molecular parameters are summarized in Table 3.3.

![Zimm plot](image)

**Figure 3.57.** The Zimm plot for MPS-PPV for the concentrations of $3.12 \times 10^{-5}$, $6.25 \times 10^{-5}$, and $1.25 \times 10^{-4}$ g/mL in 0.3 M NaCl in water.
Figure 3.58. The Debye plot for MPS-PPV at $1.25 \times 10^{-4}$ g/mL in water.

Figure 3.59. Correlation function as a function of time for MPS-PPV at $1.25 \times 10^{-4}$ M in 0.3 M NaCl.
<table>
<thead>
<tr>
<th>NaCl</th>
<th>M (Zimm)</th>
<th>M (Debye)</th>
<th>R_\text{g (zimm))} (nm)</th>
<th>R_\text{g (Debye)} (nm)</th>
<th>A_2 (mol mL/\text{g}^2)</th>
<th>R_\text{h} (nm)</th>
<th>D_T (cm^2/sec)</th>
<th>R_\text{h}/R_\text{g}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 M</td>
<td>3.5 \times 10^5</td>
<td>2.9 \times 10^5</td>
<td>40 \pm 2</td>
<td>33 \pm 2</td>
<td>(1.44 \pm 0.05) \times 10^5</td>
<td>14.5 \pm 0.5</td>
<td>(1.29 \pm 0.05) \times 10^{-7}</td>
<td>0.43</td>
</tr>
<tr>
<td>10 mM</td>
<td>4.4 \times 10^5</td>
<td>4.4 \times 10^5</td>
<td>80 \pm 10</td>
<td>58 \pm 1</td>
<td>1 \times 10^{-3}</td>
<td>18 \pm 1</td>
<td>(1.14 \pm 0.1) \times 10^{-7}</td>
<td>0.31</td>
</tr>
<tr>
<td>50 mM</td>
<td>7.5 \times 10^5</td>
<td>(6.9 \pm 0.3) \times 10^5</td>
<td>65 \pm 3</td>
<td>60 \pm 3</td>
<td>3.02 \times 10^{-3}</td>
<td>22 \pm 1</td>
<td>(1.05 \pm 0.03) \times 10^{-7}</td>
<td>0.36</td>
</tr>
<tr>
<td>300 mM</td>
<td>1.18 \times 10^6</td>
<td>1.26 \times 10^{-6}</td>
<td>65 \pm 2</td>
<td>70 \pm 5</td>
<td>(3.5 \pm 0.6) \times 10^{-3}</td>
<td>42 \pm 1</td>
<td>(1.34 \pm 0.04) \times 10^{-7}</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Table 3.3. The M, R_\text{g}, R_\text{h}, A_2 and D_T values for MPS-PPV polymer at different NaCl concentrations.

The agreement between Zimm and Debye determination of M and R_\text{g} is generally good. It is evident from Table 3.3 that the molecular weight, radius of gyration and hydrodynamic radius are dependent on the concentrations of NaCl. The molecular weight increases with concentrations of NaCl. The M, R_\text{g} and R_\text{h} values indicate that the polymer is aggregated in water and at all concentrations of NaCl. The molecular weight changes by a factor of 4 from water to 0.3 M NaCl. The aggregation of MPS-PPV has also been observed with TEM, even though the nature of aggregation is not as clear as in the case of FSM. The polydispersity ratio is very small at low NaCl concentrations and gets larger at 0.3 M NaCl. The polymer is close to a spherical shape at higher NaCl concentrations.
CHAPTER- 4

INTERACTION OF DCP WITH THE MOIETIES OF NMNR SENSOR

The interactions of the model compound for nerve gas agents DCP (Chapter 1, Figure 1.7) with the moieties of the NMNR sensor have been characterized by UV-Vis and emission spectroscopy. These studies are described in this chapter.

4.1 Interaction of DCP with isoquinoline

The UV-Vis spectrum of $10^{-5}$ M isoquinoline in CH$_3$CN was recorded as a function of DCP concentration as shown in Figure 4.1. The absorbance decreases upon the formation of the 1:1 adduct between isoquinoline and DCP. The adduct formation constant was calculated from the UV-Vis spectra and found to be $(1.98\pm 0.18) \times 10^4$ M$^{-1}$. This was determined by considering the interaction as follows, where IQ = isoquinoline.

\[
\text{IQ} + \text{DCP} \rightleftharpoons \text{IQ–DCP} \quad (4.1)
\]

\[
K = \frac{[\text{IQ–DCP}]}{[\text{IQ}] [\text{DCP}]} \quad ; \quad [\text{IQDCP}] = K[\text{IQ}]_r[\text{DCP}]_r \quad (4.2)
\]

\[
[\text{IQ}]_t = [\text{IQ}]_r + [\text{IQ-DCP}]_r = [\text{IQ}]_r + K[\text{IQ}]_r[\text{DCP}]_r = [\text{IQ}]_r (1 + K[\text{DCP}]_t)
\]
\[ [IQ]_f = \frac{[IQ]_t}{1+K[DCP]_f} \quad (4.3) \]

\[ A_t = \varepsilon_{IQ}[IQ]_t + \varepsilon_{IQDCP}[IQDCP]_t \quad (4.4) \]

\[ [IQ]_t = \frac{\varepsilon_{IQ}}{1+K[DCP]_f} + \varepsilon_{IQDCP}[IQ]_t[DCP]_f \]

\[ = \frac{[IQ]_t}{1+K[DCP]_f} + \frac{\varepsilon_{IQDCP}[IQ]_t}{1+K[DCP]_f} \]

\[ A_t = \frac{[IQ]_t}{1+K[DCP]_f} \left\{ \varepsilon_{IQ} + \varepsilon_{IQDCP}[DCP] \right\} \quad (4.5) \]

\[ \frac{A_t}{[IQ]_t} = \frac{\varepsilon_{app} = \varepsilon_{IQ} + \varepsilon_{IQDCP}[DCP]_f}{1+K[DCP]_f} \quad (4.6) \]

\[ 1+K[DCP]_f = \frac{\varepsilon_{IQ}}{\varepsilon_{app}} - \frac{\varepsilon_{IQDCP}}{\varepsilon_{IQ}} \]

\[ K = \frac{1}{[DCP]_f} \left\{ \frac{\varepsilon_{app}}{\varepsilon_{IQ}} \left( 1 + \frac{\varepsilon_{IQDCP}}{[DCP]_t} \right) - 1 \right\} \quad (4.7) \]
The $K$ value was determined from the plot of the apparent molar absorptivity, $\varepsilon_{\text{app}}$ as a function of DCP concentration. This plot is shown in Figure 4.2.

Figure 4.1. UV-Vis spectra of $10^{-5}$ M isoquinoline as a function of DCP concentration.
Figure 4.2. Plot of apparent molar absorbtivity vs. DCP concentration.

Figure 4.3. Emission spectra of $10^{-5}$ M isoquinoline as a function of DCP concentration.
The emission spectrum of $10^{-5}$ M isoquinoline in CH$_3$CN as a function of DCP concentration is displayed in Figure 4.3. It is interesting to note that the emission intensity increases with increasing concentration of DCP. The association constant $K$ for adduct formation can be calculated from the following equations.

$$
\text{IQ} + \text{DCP} \xleftrightarrow{K} \text{IQ-DCP} \quad \text{(Add)}
$$

$$
[\text{IQ}] = [\text{IQ}]_r + [\text{Add}] = [\text{IQ}]_r + K[\text{IQ}]_r [\text{DCP}]_r
$$

$$
[\text{IQ}]_f = \frac{[\text{IQ}]_r}{1 + K[\text{DCP}]_f}
$$

Emission intensity is $I$

$$
I_t = \text{Total intensity from experiment.}
$$

$$
I_t = \alpha[\text{IQ}]_r + \beta [\text{Add}]
$$

$\alpha$ and $\beta$ are proportionality constants.

$$
I_t = \alpha[\text{IQ}]_r + \beta K[\text{IQ}]_r [\text{DCP}]_r
$$

$$
I_t = [\text{IQ}]_r \left\{ \alpha + \beta K[\text{DCP}]_f \right\}
$$

$$
I_t = \frac{[\text{IQ}]_r \left\{ \alpha + \beta K[\text{DCP}]_f \right\}}{1 + K[\text{DCP}]_f}
$$

$$
\frac{I_t}{[\text{IQ}]_f} = \frac{\alpha + \beta K[\text{DCP}]_f}{1 + K[\text{DCP}]_f}
$$
There are two limiting cases

1. When $K_{[DCP]} f << 1$

$$\frac{I_r}{[IQ]_r} \simeq \alpha + \beta K_{[DCP]} f \quad (4.12)$$

This will result in the following linear plot from which the association constant $K$ can be calculated if $\alpha = \beta$. 

\[ \text{slope} = \beta K = K' \]

$K'$ = apparent association constant
The apparent association constant \( K'(\beta K) \) is \( 1.48 \times 10^{13} \text{ M}^{-1} \) calculated from the plot of \( \frac{I_t}{[IQ]_t} \) vs. \([DCP]\) shown in Figure 4.4. The proportionality constant \( \alpha \) for isoquinoline from the intercept is \( 5.58 \times 10^7 \). If we assume that \( \alpha \) and \( \beta \) are similar, dividing \( K' \) by \( \alpha \) yields a \( K \) value of \( 2.6 \times 10^5 \). This value is approximately 10 times the value derived from the UV-Vis spectral change. This indicates that \( \beta \) is approximately 10 times larger than \( \alpha \).

2. When \( K[DCP] >> 1 \)

\[
\frac{I_t}{[IQ]_t} = \frac{\alpha + \beta K [DCP]_t}{K[DCP]_t}
\]
\[
\frac{\alpha}{K[DCP]} + \beta = \frac{\alpha}{K}
\]

\[\text{slope} = \frac{\alpha}{K}\]

\[\frac{1}{[IQ]_t} = \frac{K}{\alpha} = K' = \text{apparent association constant}\]

Have also we assumed that \(\beta\) and \(\alpha\) are reasonably constant.

This limiting case does not occur under the conditions of the experiments performed here.
4.2 Interaction of FSM with DCP

The interaction of FSM with DCP was studied by UV-Vis and emission spectroscopy as a function of DCP concentration at a constant FSM concentration of $10^{-5}$ M. The UV-Vis spectra are shown in Figure 4.3 and the fluorescence spectra in Figure 4.4. It is evident from these figures that the interaction between FSM and DCP in the same concentration range studied for isoquinoline is very weak and negligible.

![Figure 4.5. UV-Vis spectra of $10^{-5}$ M FSM vs. DCP concentration.](image-url)
4.3 Interaction of Ru5ISQ with DCP

The UV-Vis spectral change when $10^{-5}$ M Ru5ISQ is interacted with various concentrations of DCP is shown in Figure 4.7. The spectral changes are not as large as with the case of free isoquinoline (Figure 4.1). The emission spectral change when the complex interacts with DCP is displayed in Figure 4.8. Only the region of the emission spectrum corresponding to the isoquinoline moiety was examined as the Ru(II) centered emission is very weak. Further the DCP directly interacts with the isoquinoline moiety. The emission due to the isoquinoline moiety decreases with increasing concentration of DCP.
Figure 4.7. UV-Vis spectrum of Ru5ISQ amide vs. DCP concentration.

Figure 4.8. Emission spectrum of Ru5ISQ vs. DCP concentration.
The interaction between the complex and DCP may be treated as a conventional quenching mechanism indicated in the following equations.

\[ I_t = \alpha [IQ]_t \] (4.12)

\[ I_t = \frac{\alpha [IQ]_t}{1 + K [DCP]_t} \] (4.13)

\[ \frac{I_t}{[IQ]_t} = \frac{\alpha}{1 + K [DCP]_t} \] (4.14)

\[ \frac{[IQ]_t}{I_t} = \frac{1}{\alpha} \frac{1}{1 + K [DCP]_t} \] (4.15)

Equation 4.15 leads to the following linear plot, the slope of which gives the association constant \( K \).

\[ \text{Slope} = \frac{K}{\alpha} \]
This model indicates that the plot of $[\text{IQ}] / I_1$ vs. [DCP] should be linear as indicated in Figure 4.9. The slope of this plot provides the association constant $K$ to be $(1.04 \pm 0.2) \times 10^6 \text{ M}^{-1}$. The adduct formed between DCP and complex does not have a measurable fluorescence in contrast to the isoquinoline adduct which has a higher fluorescent intensity than isoquinoline itself. The association constant between DCP and the complex is almost two orders of magnitude larger than the association constant for the isoquinoline. From UV-Vis measurements $(6.4 \times 10^4 \text{ M}^{-1})$. 

Figure 4.9. Plot of $[\text{IQ}] / I_1$ vs. DCP concentration.
4.4 Interaction of the FSM-Ru5ISQ ion-pair with DCP

The ion pair between FSM and Ru5ISQ was generated in a CH$_3$CN in a 2:1 molar ratio. The UV-Vis spectrum of $10^{-5}$ M of the ion pair at various DCP concentrations is displayed in Figure 4.10. The corresponding fluorescence spectra of the isoquinoline moiety are displayed in Figure 4.11. The fluorescence behavior of the ion pair in fluorescence change upon adduct formation is analogous to free isoquinoline. This change can be used to determine the adduct formation constant between the ion pair and DCP by plotting $I_t / [IQ]_t$ vs. [DCP]. This plot is shown in figure 4.12. The slope and the intercept of the plot yield an association constant $K$ of $1.65 \times 10^5$ M$^{-1}$.

![Figure 4.10 UV-Vis spectra of FSM-Ru5ISQ vs. DCP concentration.](image)
Figure 4.11 Emission spectra of FSM-Ru5ISQ vs. DCP concentration.

Figure 4.12. Plot of $[\text{IQ}]_t / I_t$ vs. DCP concentration.
4.5 Interaction of nanoparticle sensor with DCP

The ion-pair were reacted with SiO$_2$ nanoparticles to generate the ion pair bound to the nanoparticles by the formation of a Schiff’s base. These nanoparticles were suspended in CH$_3$CN for both UV-Vis and fluorescence measurements. The absorbance value at 287 nm corresponding to isoquinoline indicated the concentration of the nanoparticles bound Schiff’s base to be about $10^{-5}$ M. This assumes that the molar absorptivity of isoquinoline in the nanoparticles bound Schiff’s base in the same as it’s value in solution. The UV-Vis spectral change when the nanoparticle dispersion is interacted with various concentration of DCP is shown in Figure 4.13 and the corresponding fluorescence spectral change in the 350-600 nm region is shown in Figure 4.14. This spectral region shown contains emission from the FSM and isoquinoline moieties. However we have shown that FSM interacts very weakly with DCP. The emission spectral change which is analogous to the changes observed for Ru5ISQ + DCP indicates the emission to be strongly quenched by DCP. The association constant can be calculated from the slope of the [IQ]t / It vs. [DCP] plot which is shown in Figure 4.15. The association constant for DCP with the various moieties of the NMNR sensor and the sensor is summarized in Table 4.2.
<table>
<thead>
<tr>
<th>Moiety</th>
<th>Association constant(K), M(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoquinoline(Absorbance)</td>
<td>((1.98\pm0.2)\times10^4)</td>
</tr>
<tr>
<td>Isoquinoline(emission)</td>
<td>((2.6\pm0.3)\times10^5)</td>
</tr>
<tr>
<td>Ru(bpy)_2(bpy-isq)</td>
<td>((1.04\pm0.2)\times10^6)</td>
</tr>
<tr>
<td>FSM</td>
<td>0</td>
</tr>
<tr>
<td>FSM-Ru(bpy)_2(bpy-isq)</td>
<td>((3.17\pm0.1)\times10^6)</td>
</tr>
<tr>
<td>NMNR sensor</td>
<td>((4.7\pm0.2)\times10^6)</td>
</tr>
<tr>
<td>SiO(_2) NMNR sensor(film)</td>
<td>((5.26\pm0.4)\times10^7)</td>
</tr>
<tr>
<td>TiO(_2) NMNR sensor(film)</td>
<td>((3.27\pm0.4)\times10^8)</td>
</tr>
</tbody>
</table>

Table 4.2. Association constant for DCP with the moieties of the NMNR.

The association constant for DCP with SiO\(_2\) and TiO\(_2\) NMNR sensor films are also indicated in this table. These are discussed in detail in chapter 5. The association constants are almost 2 order of magnitude higher for the films compared to the sensor particles dispersed in solution.
Figure 4.13. UV-Vis spectra of sensor vs. DCP concentration.

Figure 4.14. Emission spectra of sensor vs. DCP concentration.
4.6 Signal transduction

The emission spectra of the different moieties and the nanosensor in the presence of DCP provide evidence for signal transduction. The isoquinoline moiety is the only molecular fragment that directly interacts with the DCP. The FSM moiety does not interact with DCP as indicated by its fluorescence being insensitive to the presence of various concentrations of DCP. The Ru(II) complex with two isoquinoline receptor fragments exhibits changes in fluorescence in the spectral region of isoquinoline. Both FSM –Ru(II) complex and SiO$_2$ –FSM-Ru(II) complex exhibit a decrease in fluorescence
in the isoquinoline and FSM spectral region. Clearly the FSM moiety in combination with Ru(II) complex and isoquinoline exhibit a change in its fluorescence. This is the result of synergistic interaction between FSM, Ru(II), bipyridyl, and isoquinoline. The total emission change in the 350-800 nm range is useful for the detection of DCP. The photon intensity can be integrated over the spectral range as the intensity decreases with increasing concentration of DCP. This integration will provide a much lower detection limit for DCP in comparison to monitoring the emission intensity over a limited wavelength range if only the isoquinoline moiety is sensitive to the interaction with DCP. The emission spectral change of the SiO$_2$-FSM-Ru(II) complex assembly upon interaction with DCP is due to signal transduction. The signal transduction is also enhanced when the film of SiO$_2$-FSM-Ru5ISQ complexes are formed on quartz substrates by the top down and bottom up approaches as described in chapter 5.

The DCP interacts more strongly SiO$_2$-FSM-Ru5ISQ nanoparticles self-assembled on a quartz substrate compared to the dispersion in solution as indicated in Table 4.2. This is discussed in chapter 5.
5.1 Top down fabrication of nanosensor arrays on quartz

The SiO$_2$ nanoparticles derivatized with FSM and ion-paired to Ru5ISQ complex were self-assembled on a quartz slide by spin coating. The quartz plate was covered with a thin layer of 3-aminopropyltrimethoxysilane by spin coating and then the nanoparticles were adhered to this silane layer. The coated quartz plate was cured at 50 °C for 10 minutes. It was then used for spectral measurements and interaction with DCP vapor. This process of self-assembling the SiO$_2$ sensor nanoparticles on quartz could be considered a top down fabrication of nanosensor array as shown in Figure 5.1. The self assembly of sensor nanoparticles by this method is not uniform as can be seen from the SEM images in Figures 5.2 (a) and 5.2 (b). As can be seen portions of the quartz plate are not covered with nanoparticles and regions covered by nanoparticles have multiple layers of particles stacked on each other. This nonuniform coverage and self-assembly of the nanoparticles may be due to the FSM- Ru5ISQ complex ion-pairs that hinder an ordered self-assembly and lead to random coverage.

In order to examine the interaction of the self assembled nanoparticles with DCP vapor a calibration curve was constructed by measuring the absorbance of
DCP in CH$_3$CN at 280 nm as a function of concentration. This calibration displayed in Figure 5.3 conforms to Beer’s law behavior.

Figure 5.1. Top down fabrication of nanosensor array.
Figure 5.2 (a and b). SEM images of self-assembled sensor nanoparticles on quartz.

Figure 5.3. Calibration curve, absorbance vs. DCP concentration in CH$_3$CN.
Table 5.1. Absorbance (280 nm) vs. concentration of DCP.

<table>
<thead>
<tr>
<th>[DCP] x 10^-8, M</th>
<th>Absorbance x(10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>2.1</td>
</tr>
<tr>
<td>6.67</td>
<td>4.5</td>
</tr>
<tr>
<td>10</td>
<td>6.9</td>
</tr>
<tr>
<td>13.34</td>
<td>8.3</td>
</tr>
<tr>
<td>16.67</td>
<td>11.125</td>
</tr>
</tbody>
</table>

A solution of 10^-5 M DCP in CH$_3$CN was prepared. A 100 mL of this solution was placed in 200 mL round bottom flask for two hours to saturate the flask with DCP vapor. Various volumes of DCP vapor were withdrawn from the flask through the rubber septum with a gastight syringe. The vapor was dissolved in 3 mL of CH$_3$CN and its absorbance at 287 nm was determined. The values are given in Table 5.2 and a linear correlation is found between the volume of DCP vapor and DCP concentration as shown in Figure 5.4.

The self assembled SiO$_2$ sensor particles were exported to DCP vapor for different lengths of time. This was performed by placing the quartz plate coated with sensor particles above the DCP solution in a Petri dish. The quartz plate was removed after exposure for a desired time and its emission spectrum was obtained by excitation at 280 nm. This is shown in Figure 5.5 which indicates that the emission due to isoquinoline at 400 nm, FSM at 500 nm and the Ru5ISQ at 650 nm decreased. Sharp spikes due to lines from the xenon lamp are also seen in this spectra due to low emission intensities. Successive two minute exposure of the quartz plate to DCP vapor reduced the
emission intensity from 400-700 nm similar to the observation when the sensor nanoparticles dispersed in CH$_3$CN where interacted with DCP. Upon exposure of the quartz plate to air to remove the DCP, this emission intensity recovered as indicated in Figure 5.5. This indicates reversibility of the binding of DCP by the SiO$_2$ sensor nanoparticles.

![Figure 5.4](image)

**Figure 5.4.** Plot of Concentration of DCP in various volumes of vapor.

<table>
<thead>
<tr>
<th>DCP vapor/ mL</th>
<th>Absorbance $\times 10^{-3}$</th>
<th>[DCP]$\times 10^{-8}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.5652</td>
<td>3.86</td>
</tr>
<tr>
<td>10</td>
<td>5.2592</td>
<td>7.89</td>
</tr>
<tr>
<td>20</td>
<td>8.98</td>
<td>13.53</td>
</tr>
<tr>
<td>30</td>
<td>12.314</td>
<td>18.72</td>
</tr>
</tbody>
</table>

**Table 5.2.** Concentration of DCP in various volumes of DCP vapor from $10^{-5}$ M solution in CH$_3$CN.
Similar experiments were conducted with TiO$_2$ sensor nanoparticles coated on a quartz substrate. The dependence of the emission from the sensor nanoparticles as a function of DCP concentration was studied here. The results are displayed in Figure 5.6. The quartz plate coated with the sensor nanoparticles was kept in a covered beaker to which various volumes of DCP vapor from $10^{-5}$ M DCP in CH$_3$CN was injected. The emission spectra were recorded after a 3 minute equilibration time. Again the emission due to the isoquinoline, FSM, and Ru5ISQ complex moieties can be observed at 400, 500 and 650 nm respectively. The emission intensity decreases when interacted with
DCP as observed for SiO$_2$ nanosensor particles. The quartz plates after the measurements were exposed to air to remove DCP as indicated by the recovery of fluorescence intensity. It was then exposed to a different concentrations of DCP by injecting an appropriate volume of the vapor. As may be seen from Figure 5.6 the fluorescence intensity decreases in the increasing DCP concentration. The association constant was determined from the plot of $I_0/[IQ]_0$ vs [DCP] displayed in Figure 5.7.

![Figure 5.6. Emission of TiO$_2$ sensor on quartz vs. DCP vapor concentration.](image)

The effect of HCl vapor on the TiO$_2$ sensor nanoparticles was also investigated in a manner analogous to interaction of DCP. Quartz plate coated with TiO$_2$ sensor particles was kept in a beaker to which various volumes of HCl vapor from a $10^{-5}$ M HCl
in CH$_3$CN was injected. Figure 5.8 shows the emission intensity as a function of HCl vapor concentration. The decrease in fluorescence intensity with HCl is much smaller than that with DCP vapor.

Figure 5.7. Plot of $[IQ]_t / I_t \times 10^8$ vs. DCP concentration.

Figure 5.7. Plot of $[IQ]_t / I_t$ vs. DCP concentration.
Figure 5.8. Emission intensity vs. HCl vapor concentration.
Figure 5.9. Bottom up fabrication of NMNR sensor array.
5.2 Bottom up approach of nanosensor array on quartz

The bottom up fabrication of nanosensor array on quartz is indicated in Figure 5.9. A thin layer of 50 nm SiO$_2$ nanoparticle was formed on a quartz plate by spin coating after a thin layer of trimethoxyaminopropylsilane had been formed on the plate. The SEM images of this film of SiO$_2$ is shown in Figure 5.10 (a) and (b) which indicate that the self-assembly of SiO$_2$ nanoparticle is not totally uniform. Region of the quartz plate are not covered with SiO$_2$, but in contrast to the top down fabrication multilayer formation of SiO$_2$ nanoparticles has been avoided.

![Figure 5.10 (a and b) Self-assembly of SiO$_2$ nanoparticles on quartz.](image)

This was cured at 100 °C for 18 hours after which the SiO$_2$ nanoparticles had adhered strongly to quartz. Even ultrasonication for extended periods in methanol and acetonitrile did not dislodge the nanoparticles. To prevent silanization of the plate on the bare side, a second quartz plate was placed on this side and secured with a polypropylene string at both ends. This was placed in 70 mL of methanol or toluene solution containing 7% 3-aminopropyltriethoxysilane and stirred at room temperature for 6 hours. The plates were removed and repeatedly washed with methanol and air dried.
The FSM molecule was attached to the silanized nanoparticles by immersing the quartz plate pair in a 100 mL of $10^{-5}$ M FSM in CH$_3$CN and refluxing overnight. The plates were removed and washed three times in CH$_3$CN. They were immersed in CH$_3$CN and ultrasonicated for several minutes when no FSM leached from the plates. This indicated the formation of Schiff’s base between the NH$_2$ group of the silanized nanoparticles and CHO group of the FSM.

Figure 5.11. AFM images of SiO$_2$ derivatized with FSM on quartz.
The attached pair of quartz plates was then immersed in a 100 mL CH$_3$CN solution containing 10$^{-5}$ M Ru$_5$ISQ for 12 hours at room temperature with stirring. The plates were removed and repeatedly rinsed with CH$_3$CN and air dried. The polypropylene string was removed to separate the plates. The plates with NMNR sensor array was characterized by UV-Vis, excitation, and emission spectroscopy.

The UV-Vis absorption spectrum of NMNR sensor array attached by the bottom up approach is displayed in Figure 5.11. The excitation and fluorescence spectra are displayed in Figure 5.12 and 5.13 respectively. The sensor array was then exposed to vapors of DCP from solution with varying concentrations of DCP.

The sensor array was exposed to DCP vapor for 2 minutes in every case. Figure 5.14 shows that the fluorescence intensity decrease with concentration of DCP. The plot of $I_r/ [IQ]_t$ with DCP concentration in vapor is shown in Figure 5.15.

Figure 5.12. UV-Vis spectrum of NMNR sensor of film by bottom up approach.
Figure 5.13. Excitation spectrum of NMNR sensor of film by bottom up approach (emission wave length: 650 nm; excitation cut off filter: 595 nm).

Figure 5.14. Emission spectrum of NMNR sensor of film by bottom up approach (excitation wave length: 287 nm; emission cut off filter: 345 nm).
Figure 5.15. Emission spectrum of NMNR sensor by bottom up approach vs. DCP concentration.

Figure 5.16 Plot of $\frac{[IQ]}{I_t} \times 10^{10}$ with DCP concentration in vapor.
As discussed in chapter 4, the NMNR consisting of FSM, Ru(II) complex, and isoquinoline moieties exhibit a change in the fluorescence of all its moieties even though only the isoquinoline moiety binds to DCP. This indicates the synergy among the moieties and signal transduction due to the synergy.

The detectable limit from Figure 5.14 for DCP is about 30 nM (nanomolar) if only the emission intensity at the wavelength corresponding to isoquinoline is considered. Integration of the intensity through the entire spectral region (350-800 nm) where the emission decreases with increasing DCP concentration lowers the detection limit to 30 pM (picomolar). The fluorescence intensity of isoquinoline, FSM and charge transfer transition due to the Ru(II) metal ion all change when isoquinoline binds DCP. This allows an integration of all photon intensities and as a result an improvement of 3 orders of magnitude is detectable limit. This clearly is the result of synergy among the moieties of NMNR and signal transduction.

The NMNR sensor obtained by the self-assembly of nanoparticle, monomer, complex, and receptor moieties is a useful approach for sensing target compounds. The moieties interact synergistically and provide signal amplification by transduction. This approach is amenable to the construction of sensor arrays by the top down and the bottom up fabrication methods. The bottom up approach provides a greater control of the fabrication process, results in a monolayer of NMNR sensor particles, and higher sensitivity (lower detection limit) for the detection of DCP than the top down approach.
CHAPTER- 6

CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Conclusions

Nanosensors for the model nerve gas agent diethoxychlorophosphate (DCP) consisting of nanoparticle, fluorescent molecule, Ru(II) complex, and isoquinoline receptor have been fabricated by step-by-step assembly of the moieties. The NMNR sensor for DCP exhibits changes in the emissions of the fluorescent molecule and the Ru(II) complex in addition to that of isoquinolone even though DCP is only associated with isoquinolone. This signal transduction results in fluorescence change over a large spectral window leading to a low detection limit (picomolar). Arrays of the DCP sensors have been obtained both by the top down and bottom up approaches through self-assembly. Sensor arrays obtained by bottom up fabrication are more ordered, exhibit better spectral characteristics, larger fluorescence change upon binding DCP, and lower detection limit compared to those obtained by top down fabrication. The central hypothesis that nanosensors obtained by systematic assembly of moieties can provide high sensitivity for a target molecule has been substantiated.

The significant results obtained in the design and fabrication of the NMNR sensors for DCP are:

1. New highly conjugated fluorescent molecule FSM has been synthesized as a fluorescent moiety of the NMNR sensor. This molecule can be readily self-
assembled on SiO₂ nanoparticles through Schiff’s base formation and provides the platform to self-assemble the Ru5ISQ complex with the isoquinoline receptor. The FSM does not directly interact with DCP but exhibits a change in its fluorescence through signal transduction when the isoquinoline of NMNR sensor binds DCP.

2. A new bipyridyl ligand attached to two isoquinoline receptors in the 5 and 5’ positions through amide bonds has been synthesized and characterized in terms of its fluorescence in the absence and presence of DCP. The DCP molecule binds reversibly to isoquinoline nitrogen through the P center.

3. A new complex Ru5ISQ containing 2 bipyridyl ligands and one bipyridyl with isoquinoline has been synthesized and characterized by fluorescence spectroscopy to discern its interaction with DCP. The binding of DCP is similar to the bipyridyl-isoquinoline ligand with isoquinoline.

4. The Ru5ISQ ligand has been spontaneously self-assembled on SiO₂ nanoparticles derivatized with FSM through electrostatic interaction with the SO₃⁻ groups to obtain the NMNR sensors.

5. Arrays of NMNR sensors on quartz substrates have been obtained by the top down and bottom up approaches. In the top down approach the NMNR nanoparticles are self-assembled on a quartz plate by spin coating. In the bottom up approach the SiO₂ particles are self-assembled on a quartz plate through hexagonal close packing. The SiO₂ particles are silanized with trimethoxysilylpropylsilane and the FSM
molecules are attached to the silane by Schiff’s base formation. The Ru5ISQ complex is self-assembled on the monolayer of FSM molecules through electrostatic interaction. Both the top down and bottom up fabrications employ a systematic self-assembly of the moieties of NMNR from the receptor to the substrate or vice versa.

6. The bottom up approach yields a more uniform self-assembly of the NMNR sensor nanoparticles on quartz than the top down approach. As a result the fluorescence spectra of the sensor arrays from the bottom up approach have higher intensity and are of better quality compared to the arrays from the top down approach.

7. The DCP molecule reversibly binds to the sensor arrays resulting in a fluorescence change in the spectral range 350-800 nm. The integration of the change over this spectral range provides a detection limit of about 1 ppb. The binding constant for DCP with Ru5ISQ in CH$_3$CN is $1.04 \pm 0.2 \times 10^6$ M$^{-1}$, with (FSM)$_2$-Ru5ISQ is $3.17 \pm 0.1 \times 10^6$ M$^{-1}$ and the binding constant with the NMNR arrays is $5.26 \pm 0.4 \times 10^7$ M$^{-1}$. The binding constant for DCP on the self-assembled NMNR arrays is about two orders of magnitude larger than the binding with the complex in solution and three orders of magnitude larger than the (FSM)$_2$-Ru5ISQ ion-pair. Self-assembled sensor arrays compared to individual molecules bind the target much more strongly providing a low detection limit.

8. The reversible binding of DCP to the sensor arrays allows their repeated use for detection of this molecule.
6.2 Future directions

The following studies would provide a better understanding of the mechanism of sensing and improvements to the NMNR sensor:

1. Measurement of the lifetimes of the excited states of the various moieties and the NMNR sensor in the absence and presence of different concentrations of DCP.

2. The selectivity of the NMNR sensor for DCP by examining its interaction with other more commonly occurring organophosphates such as pesticides and herbicides and their analogs.

3. Changing the position of the isoquinoline receptor to 4,4’ on the bipyridyl ring which will change the electronic structure and hence the fluorescence change when DCP is bound.

4. Changing the metal ion from Ru(II) to Eu(III) to eliminate the quenching of the metal centered emission by O₂.

5. Attaching the FSM through only one end of the molecule to SiO₂ nanoparticles instead of both ends to minimize aggregation of nanoparticles and obtain a more ordered self-assembly of this moiety on nanoparticles.

6. Employing core: shell nanoparticles in which the core consists of nanoparticles such as ZnS doped with Mn(II) (quantum dots) which have luminescence in the
visible and the shell is SiO$_2$ for the generation of NMNR sensor particles. All the moieties in such sensors have luminescence and signal transduction in such systems could lead to even lower detection limits for DCP.

7. Self-assembling NMNR sensors with quantum dots encapsulated in a thin SiO$_2$ shell on conducting indium tinoxide (ITO) glass and applying a potential to the sensor array simultaneously with photon excitation during DCP binding. The pumping of electrons while the excitations of the quantum dots are generated by photons would be fundamentally interesting and could lead to an additional sensing mechanism.
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