INVESTIGATIONS ON SELECTED REAGENTS LEADING TO DEVELOPMENT OF OPTICAL CHEMICAL SENSOR FOR ORGANIC VAPOUR POLLUTANTS

A thesis submitted to the University of Manchester for the Degree of Master of Philosophy in the Faculty of Chemical Engineering and Physical Science.

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By

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<tr>
<td>ANTH</td>
<td>Anthracene</td>
</tr>
<tr>
<td>ADC</td>
<td>Analogue-to-Digital Converter</td>
</tr>
<tr>
<td>AMR</td>
<td>Analytical Method Recovery</td>
</tr>
<tr>
<td>ATSDR</td>
<td>Agency for Toxic Substances and Disease Registry</td>
</tr>
<tr>
<td>BB</td>
<td>Base Block</td>
</tr>
<tr>
<td>BaP</td>
<td>Benzo(a)pyrene</td>
</tr>
<tr>
<td>BPT</td>
<td>Benzo(a)pyrene tetrol</td>
</tr>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>CFD</td>
<td>Constant Fraction Discriminators</td>
</tr>
<tr>
<td>CT</td>
<td>Charge Transfer</td>
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<td>CoP(ph-OMe)&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Cobalt tetra-(methoxy-phenyl) porphyrin</td>
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<tr>
<td>COSHH</td>
<td>Control of substances hazardous to health</td>
</tr>
<tr>
<td>Del</td>
<td>Electrical Delays</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DQ</td>
<td>Dynamic Quenching</td>
</tr>
<tr>
<td>ε</td>
<td>Molar absorptivity</td>
</tr>
<tr>
<td>EE</td>
<td>Extraction Efficiency</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Di-aminotetraacetic Acid</td>
</tr>
<tr>
<td>EMS</td>
<td>Electromagnetic Spectrum</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>ETEOS</td>
<td>Ethyltriethoxysilane</td>
</tr>
<tr>
<td>ETV</td>
<td>Environmental technology verification</td>
</tr>
<tr>
<td>FBFOCS</td>
<td>Fluorescence Based Fibre Optical Chemical Sensing</td>
</tr>
<tr>
<td>FIS</td>
<td>Flow Injection System</td>
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<tr>
<td>FLXN</td>
<td>Bis [N,N-bis(carboxymethyl)aminomethyl] fluorescein</td>
</tr>
<tr>
<td>FQ</td>
<td>Fluorescence Quenching</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IC</td>
<td>Intersystem Conversion</td>
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<td>IR1</td>
<td>Instrument response factor curve (for scatter solution)</td>
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<td>ISC</td>
<td>Intersystem Crossing</td>
</tr>
<tr>
<td>K&lt;sub&gt;sv&lt;/sub&gt;</td>
<td>Stern-Volmer quenching constant</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
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<tr>
<td>LDR</td>
<td>Linear Dynamic Range</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit Of Detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit Of Quantification</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
</tr>
<tr>
<td>Mem</td>
<td>Digital memory</td>
</tr>
<tr>
<td>MDL</td>
<td>Maximum Detection Limit</td>
</tr>
<tr>
<td>MDC</td>
<td>Minimum Detectable Concentration</td>
</tr>
<tr>
<td>MIP</td>
<td>Molecularly Imprinted Polymer</td>
</tr>
<tr>
<td>MLOD</td>
<td>Method Limit Of Detection</td>
</tr>
<tr>
<td>MP</td>
<td>Metallo-Porphyrin</td>
</tr>
<tr>
<td>MTEOS</td>
<td>Mehtyltriethoxysilane</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical Aperture</td>
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<tr>
<td>NIOSH</td>
<td>National Institute for Occupational Safety and Health</td>
</tr>
<tr>
<td>NMAM</td>
<td>Manual for Analytical Methods</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density or Absorbance</td>
</tr>
<tr>
<td>OPs</td>
<td>Organic Pollutants</td>
</tr>
<tr>
<td>OSHA</td>
<td>Occupational Safety and Health Administration</td>
</tr>
<tr>
<td>PAHs</td>
<td>Polycyclic Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic Aromatic Hydrocarbon</td>
</tr>
<tr>
<td>PDMS</td>
<td>Poly-Di-Methyl Siloxane</td>
</tr>
<tr>
<td>PEL</td>
<td>Permissible Exposure limits</td>
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<td>PMTs</td>
<td>Photomultipliers</td>
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<tr>
<td>PRN</td>
<td>Pyrene</td>
</tr>
<tr>
<td>Pt</td>
<td>Platinum</td>
</tr>
<tr>
<td>PVC</td>
<td>Poly-Vinyl Chloride</td>
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<tr>
<td>Py</td>
<td>Pyridine</td>
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<tr>
<td>QCM</td>
<td>Quartz Crystal Microbalance</td>
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<td>QF</td>
<td>Fluorescence Quantum yield</td>
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<tr>
<td>RG</td>
<td>Rubber Gasket</td>
</tr>
<tr>
<td>RLOD</td>
<td>Relative Limit Of Detection</td>
</tr>
<tr>
<td>RQL</td>
<td>Reliable Quantification Limit</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
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<td>---------</td>
<td>------------</td>
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<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
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<td>R(t)</td>
<td>Sample decay model</td>
</tr>
<tr>
<td>SAW</td>
<td>Surface Acoustic Wave Device</td>
</tr>
<tr>
<td>SEM</td>
<td>Surface Emission Microscopy</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface Enhanced Raman Spectroscopy</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface Plasmon Resonator</td>
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<td>SRE</td>
<td>Surface Removal Efficiency</td>
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<td>STD</td>
<td>Standard</td>
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<td>SVOCs</td>
<td>Semi-volatile organic compounds</td>
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<td>SQ</td>
<td>Static Quenching</td>
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<td>SVS</td>
<td>Syringe Vapour Sucking</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Tau (Fluorescence Decay life time)</td>
</tr>
<tr>
<td>TAC</td>
<td>Time-to-Amplitude Converter</td>
</tr>
<tr>
<td>TBT</td>
<td>Tri-butyl phosphate</td>
</tr>
<tr>
<td>TCSPC</td>
<td>Time Correlated Single Photon Counting</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIR</td>
<td>Total internal reflection</td>
</tr>
<tr>
<td>TWA</td>
<td>Time Weighted Average</td>
</tr>
<tr>
<td>UB</td>
<td>Upper Block</td>
</tr>
<tr>
<td>VAM</td>
<td>Validated Analytical Measurements</td>
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<tr>
<td>vs</td>
<td>Versus</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ZnPc</td>
<td>Zinc Phthalocyanine</td>
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<td>ZPP</td>
<td>Zinc Porphyrin</td>
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Abstract

This thesis presents investigations carried out in order to evaluate the suitability of Cobalt tetra-methoxyphenyl porphyrin (CoP(ph-OMe)₄) and Bis[N,N-bis(carboxymethyl)]amino methyl]fluorescein (FLXN) as sensing reagents for the organic pollutants namely; anthracene (ANTH), benzo(a)pyrene (BaP), pyrene (PRN) and pyridine (Py). Analyses leading towards development of a fluorescence based fibre optical chemical sensing system (FBFOCS) has been carried out. The mechanism is based on monitoring the fluorescence properties of thin solid film of a chemical transducer with changing concentration of the organic pollutants selected for this research. Immobilization of thin films of these sensing macromolecules in Poly-dimethyl Siloxane (PDMS) matrix has been successfully achieved. These solid thin films are prepared for the purpose of using them as optical chemical sensing systems for the mentioned organic pollutants. A Flow cell with compartment (sensing platform) for holding the sensing films and enabling pumping in and out of the organic pollutant vapours have been fabricated for this research. Absorption and fluorescence measurements performed on these films when subjected to Polycyclic-Aromatic Hydrocarbons (PAHs) and also to pyridine (Py) vapours have illustrated that, these organic pollutants change the way the sensing molecules emit and absorb radiant energy from the electromagnetic spectrum.

Evaluated LOQ and MDL for both sensing molecules with the pollutants, range from 0.002 to 171.32 μg/L (LOQ) and 0.0001 to 6.0 μg/L (MDL). These values are lower than Permissible Exposure Limits (PEL), which range from 0.1 to 0.2 mg/L, set by international environmental pollution monitoring agencies. Therefore these reagents have been evaluated and can be recommended for development as viable sensors for organic pollutants under this study.
Declaration

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- Lastly personnel who did the binding of this work.
Dedication

To my beloved family,
Ben, Eric, Vicky, Sue, Mark,
Edwin, Billy, Grace, Dad and my siblings. [To mum, I wish you lived to witness my success].
Chapter 1: Introduction and thesis structure.

1.1 Preamble.

The use of sensors will make it easy to monitor hazardous pollutants in the environment. Chemicals which are toxic and carcinogenic (cancer-causing agents) including heavy metals, gases, solid organic molecules and their vapours can be monitored using sensing devices that are able to detect their presence and the information relayed to the detector and hence graphical representation for their quantification.

As mentioned in the first paragraph, there are various toxic and carcinogenic elements and compounds, but the scope of this thesis is limited to four carcinogenic compounds namely; anthracene, benzo(a)pyrene, pyrene and pyridine. The first three are among a class of chemicals which are derivatives of two or more fused benzene molecule rings known as Polycyclic Aromatic Hydrocarbons (PAHs) and these three are abbreviated in this thesis as ANTH, BaP and PRN respectively. Pyridine abbreviated in this thesis as ‘Py’ is a heteroatom which has properties similar to a group of compounds called tertiary amines. In this research we utilise Cobalt tetra phenyl-methoxy porphyrin and Bis[N,N-bis(carboxy-methyl)amino-methyl]fluorescein as chemical sensors for the selected pollutants and these sensor molecules are abbreviated in this research as CoP(ph-OMe)₄ and FLXN respectively.

Polycyclic aromatic hydrocarbons occur naturally in coal, crude oil and gasoline and they are also created by the incomplete combustion of coal, oil, gas, garbage and tobacco. Many products including roofing tar, certain medicines, dyes and pesticides contain PAHs. They therefore find their way into the atmosphere from vehicle exhaust, emissions from residential and industrial furnaces, tobacco smoke, volcanoes, and forest fires. These pollutants may attach to particles produced during emission and in the air and by so doing, contaminate surface and groundwater. Pyridine is also found in mixtures of these PAHs.
Considerable research has previously been carried out on optical fibre based gas and vapour sensing which utilised porphyrin films for detecting gases and organic vapours [Yusoff et al 2008], but FLXN has not been used before for gas or vapour sensing.

1.2 The need for monitoring environmental levels for ANTH, BaP, PRN and Py.

According to the International Agency for Research on Cancer, the National Toxicology Program, and the U.S. Environmental Protection Agency, these pollutants have been classified as definite carcinogens. Some people who have breathed or touched mixtures of PAHs for long periods have developed cancer. In laboratory animals, some PAHs have caused lung, stomach, or skin cancer. PAHs are lipophilic and therefore they easily form adducts with DNA in animal tissues [Chard et al 2001]. They are therefore harmful compounds to humans and animals hence least desired in the environment.

Since these organic chemicals are toxic, Environmental Protection Agency (EPA), World Health Organisation (WHO), Occupational Safety and Health Administration (OSHA), Agency for Toxic Substances and Disease Registry (ATSDR) and National Institute for Occupational Safety and Health (NIOSH) among many environmental monitoring agencies have set Permissible Exposure limits (PEL) dissolved in drinking water and airborne which they deem less harmful through research. Permissible exposure limits are kept in control by monitoring the air at workplace and drinking water and this is done over a given length of time referred to as Time Weighted Average (TWA); which should be 8 hours per day and 40 hours per week to which nearly all workers maybe repeatedly exposed.

Table 1.1 presents PEL data for ATSDR, EPA, NIOSH and OSHA for PAHs, which also encompass those selected for this research namely; ANTH, BaP, PRN and for Py.
Table 1.1: PEL for ANTH, BaP, PRN and Py

<table>
<thead>
<tr>
<th>AGENCY</th>
<th>PEL</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parts per billion (ppb)</td>
</tr>
<tr>
<td></td>
<td>Air mg/m³</td>
<td>Water mg/L</td>
</tr>
<tr>
<td>ATSDR</td>
<td>0.2</td>
<td><em>0.0002</em></td>
</tr>
<tr>
<td>EPA</td>
<td>0.2</td>
<td><em>0.0002</em></td>
</tr>
<tr>
<td>NIOSH</td>
<td>0.1</td>
<td><em>0.0001</em></td>
</tr>
<tr>
<td>OSHA</td>
<td>0.2</td>
<td><em>0.0002</em></td>
</tr>
</tbody>
</table>

PEL for pyridine 5 ppm and 15 mg/m³. Asterisks show same concentration converted from mg/L to ppb.

The data in Table 1.1 from the listed agencies’ websites.

Though PEL are set as guidelines shown in Table 1.1, it is highly recommended by the same agencies that exposure limits should be slightly below those guidelines. With growing awareness of such exposure limits there is need to monitor the concentrations of these pollutants in the environment. Therefore need to develop suitable optical chemical sensors for such toxic chemicals.

### 1.3 Objective and scope of this research.

The aim of this thesis is to produce solid thin films of identified chemical reagents that selectively interact with the pollutants selected for this research. The purpose of producing thin films of these reagents is to evaluate their suitability as optical chemical sensors for the selected pollutants. These reagents are to be evaluated through preliminary solution study to monitor the way they interact with the pollutants using spectroscopic methods of analysis. Finally they will be immobilised into thin films in suitable polymeric matrices and evaluated for their suitability as sensors using fluorescence based fibre optical chemical sensing (FBFOCS) analytical technique. A miniaturised sensor platform (flow cell) will be fabricated which can be interfaced to the fluorimeter using an optical fibre as a means of transmitting radiant energy to the sensing film placed inside the sensor platform. The purpose of miniaturising the sensor and platform is to facilitate remote sensing.
1.4 Thesis organisation.

This thesis has been organised in such a way as to follow the logical steps of evaluating chemicals as viable sensors. It starts with initial spectroscopic investigations of interaction of solutions of CoP(ph-OMe)$_4$ and FLXN as sensor molecules with the solutions of organic pollutants selected for this research. These investigations are based on monitoring changes caused to the optical signals of the sensing molecules when they interact with the pollutant molecules. Once the interaction patterns of the sensing reagents with the pollutant molecules are established, and then finally progress is made towards a suitable optical system with the necessary electronic components for interrogating immobilised solid thin films of these sensing reagents when they interact with the organic pollutants selected for this research. The organisation of this thesis is summarised schematically as in figure 1.1.

Chapter 1: Introduction
Chapter 2: Sensors and pollutants for this research and fibre optical chemical sensor technique
Chapter 3: Analytical techniques
Chapter 4: Solutions study
Chapter 5: Solid thin films study
Chapter 6: Discussion, conclusion and appraisal of future work

Figure 1.1: Schematic diagram of thesis organisation
Assessing the environmental impact of organic pollutants requires analytical tools that can screen them rapidly with minimal analyte handling. Screening can even be done continuously at their source thereby facilitating remote online sensing. In this regard, immobilised chemical sensors with miniaturised sensor platforms that are interfaced to offsite bulk analytical instruments are expected to play a leading role in environmental pollution monitoring. Recent technological advances are sure to facilitate application of sensing devices that are highly selective, sensitive to the analytes of interest, cost effective, stable and robust. An advance towards development of PAHs’ sensors is a growing area of interest that is reflected by many publications and some of which are cited in this thesis.

In this thesis, chapter 2 gives a brief literature review of the sensing molecules and pollutant molecules selected for this research and also survey of some of the work that has been undertaken in developing sensors for detection of PAHs and Py in their vapour and liquid phases. Miniaturisation of sensing systems as mentioned earlier is desirable and this can be achieved by immobilising sensing molecules into thin films. Therefore common methods of immobilisation have also been highlighted in chapter 2. To achieve remote sensing, radiant energy has to be transmitted to the sensor platform hence, there is need to use radiant energy transmitters like optical fibres. Optical fibres and sensor platforms configurations have also been covered in chapter 2. Chapter 3 continues with a highlight of analytical techniques employed in this research and a brief description of interfacing of the sensor platform with the analytical instruments using the optical fibre. Chapter 4 discusses methodology and results for preliminaries studies achieved in solutions of the sensing molecules and the pollutants. Chapter 5 discusses thin films study including immobilisation method used here and construction of sensor platform and how this can be interfaced to the fluorimeter. The method of feeding the sensor film inside the sensor platform with pollutant vapours is also discussed. Finally chapter 6 contains discussion, conclusion and future work appraisal.
Chapter 2: Sensors and pollutants of this research and fibre optical chemical sensing technique.

2.1 Introduction.

Advancement in technology has in turn given rise to industrial development and industries are a contributory factor to environmental pollution. Environmental pollution monitoring therefore, becomes a vital necessity globally. In earlier times, pollution monitoring involved sampling of pollutants, transportation to analytical laboratories, pre-treatment and quantification. This approach is more tedious and may be of little help when it comes to immediate danger through pollution.

To be more practical in pollution monitoring, research scientists across the globe are carrying out research in the quest to develop sensors for various environmental pollutants. Among the innovations are optical chemical sensors, which have attracted interest in the field of sensor technology. In this type of sensing systems, chemical reagents that react with target pollutants are used either in solution or solid thin films. This is because chemicals may react and the products change colour or may interact to give specific changes when monitored using spectroscopic methods of analysis.

In this study, we investigate selected reagents (section 2.2 – 2.3) for their suitability as optical chemical sensors for organic pollutants; anthracene (ANTH), benzo(a)pyrene (BaP), pyrene (PRN) and pyridine (Py) (section 2.4). Methodology and results for this investigation are reported in chapters 4 and 5.
2.2 Sensor molecules for this research.

As already highlighted in chapter 1, the two sensor molecules selected for this research are; Cobalt tetra-(phenyl-methoxy)porphyrin abbreviated as CoP(ph-OMe)$_4$ and Bis[N,N-bis(carboxy-methyl)amino-methyl]fluorescein, abbreviated in this thesis as FLXN. Other names for FLXN are calcein and fluorexon. These molecular systems are presented in this research as sensor system 1 and sensor system 2 denoting CoP(ph-OMe)$_4$ and FLXN respectively.

2.3 Sensor system 1: CoP(ph-OMe)$_4$.

This sensor system is a metalloporphyrin which is a derivative of the mother ligand called the porphine (appendix 1). As observed in Figure 2.1, it is a highly conjugated π-system and as cited in literature, its π-electrons are delocalised in the macrocyclic ring system. The CoP(ph-OMe)$_4$ exists as bright purple crystals and in this study it was highly soluble in dichloromethane (CH$_2$Cl$_2$).

![Figure 2.1. Cobalt tetra-(phenyl-methoxy) porphyrin (MW. 791.77 g/mol; C$_{48}$H$_{36}$CoN$_4$O$_4$)](image)

The four phenyl-methoxy constituents at the periphery are similarly highly conjugated and they also have oxygens that have lone pair or n-electrons. These characteristics make the metalloporphyrin versatile in applications.
2.4 Characteristic properties for CoP(ph-OMe)$_4$.

Cobalt tetra (phenyl-methoxy)porphyrin, is thought to be planar with four phenyl methoxy substituent groups at the periphery of the ring system. The methoxy groups have in total four oxygens, (-O-), which have in total 16 non-bonding n-electrons. Thus there are two pairs of n-electrons on each of the oxygens constituent to this molecule.

This sensor molecule has Co$^{2+}$ as the central metal ion. The cobalt has a high co-ordination number of six. In the porphyrin complex, cobalt ion has formed four co-ordinate bonds with the four azine nitrogens and therefore satisfying a co-ordination number of four. The transition metal ion Co$^{2+}$ therefore has two extra vacant d-orbitals available for accepting electrons to be donated by ligand(s) in order to satisfy its maximum co-ordination number six. The Co$^{2+}$ ion in the macromolecule has an electronic configuration of [Ar] 3d$^7$ and if this is represented diagrammatically using boxes to symbolise orbitals, then it is hypothesised as in Figure 2.2.

![Figure 2.2. The 3d$^7$ electronic configuration for divalent cobalt ion.](image)

The arrows indicate the electrons and their respective spins. In order to facilitate co-ordination to tetra-(phenyl-methoxy) porphyrin, the transition metal cobalt must provide four empty d-orbitals for the n-electrons of the azine nitrogens of this powerful ligand. The hypothesised empty orbitals are 4s and 4p, which are illustrated using boxes to symbolise empty orbitals as in Figure 2.3.

![Figure 2.3. The hypothesised empty orbitals are 4s and 4p](image)
The empty 4s and three 4p (xyz) orbitals are available for the four lone pair electrons from the four azine nitrogens in the porphyrin ring. The four nitrogens will individually donate two electrons to each sub-shell (box). In the co-ordinated complex, the electronic configuration may be represented as in Figure 2.4.

\[
\begin{array}{c|c|c|c}
3d & 4s & 4p \\
\hline
\uparrow & \uparrow & & \uparrow \\
\hline
\end{array}
\]

Figure. 2.4. Representation of electronic configuration in a co-ordinated complex

The small circles indicate the electrons donated by the nitrogen atoms of the porphyrin molecule. Both electrons have to be provided by individual nitrogen atoms. According to Hund’s rule and the spin quantum number (m_s), each orbital has to carry only two electrons with opposite spins. It follows that the electrons have to be distributed as shown and the individual colours indicate opposite spins in order to satisfy a stable configuration as explained by the magnetic spin quantum number. The hypothesised hybridisation is sp^3 in the cobalt tetra-(phenyl-methoxy) porphyrin complex which indicates that it is a tetrahedral complex of co-ordination number (4). However, cobalt is capable of forming an octahedral complex with a co-ordination number 6. It is therefore envisaged that given an analyte(s) that has a non-bonding pair(s) to donate, the full six co-ordinate bonds can be formed. Thus Co^{2+} can still interact with hetero atomic aromatic analytes like pyridine with N-atom(s). In literature, [Antina et al 2001], reported the formation of CoP(ph)_4-C_5H_5N, thus only one pyridine molecule was coordinated to the tetra phenyl porphyrin macromolecule.

In their study they used a 1:1 ratio of the Cobalt porphyrinato complex to pyridine. In their research, [Medek et al 1997], using NMR study, reported the co-ordination of two pyridine molecules to CoP(ph)_4. In this study, CoP(ph)_4 has four methoxy groups, –OCH_3, at the periphery of the ring system instead of only four phenyl substituent groups as in the case study by [Antina et al 2001] and [Medek et al 1997]. Hypothesised sp^3d^2 hybridisation for octahedral complexation, where
4s, 4p and 4d orbitals are utilised for the co-ordination, may be as indicated in Figure 2.5.

![Figure 2.5. Hypothesised sp³d² hybridisation for octahedral complexation](image)

The two electron pairs in two of the total five 4d sub-orbitals are thought to have been donated by two analytes/ligand molecules or a bidentate ligand with n-electrons that can complete the six co-ordination bonds to form octahedral complex. Prior to arrangement, Co²⁺ having utilised 4s and 4p orbitals, for co-ordination, a tetrahedral complex with the sensor molecule existed. In this study pyridine is the candidate organic pollutant for this kind of interaction. Also the 3d orbitals of the Co²⁺ have three unpaired electrons as indicated in orbital diagrams earlier. Theoretically these unpaired electrons may give rise to a phenomenon called charge transfer, so that an electron during excitation may be promoted from the donor atom to these orbitals associated with the Co²⁺ metal ion. This may result in changes in the way the sensing molecule interact with the radiant energy of the electromagnetic spectrum. These changes are specific to particular analytes that will interact with the sensing molecule.

### 2.5 Reaction centres for CoP(ph-OMe)₄

This metalloporphyrinato complex has multiple reaction centres and they can be specific to various analytes. Such reaction centres are highlighted as follows:

- The central transition metal ion, Co²⁺ of the porphyrin complex which has vacant dπ-orbitals (4dπ-subshell) available to facilitate co-ordination interactions with the analytes which possess non-bonding, n-electrons. Thus central metal ion Co²⁺ of the porphyrinato complex exhibit 4, 5 to 6 co-ordination numbers. After forming four co-ordinate bonds with the nitrogens of the ring system of CoP(ph-OMe)₄, can also form two more co-
ordinate bonds with pyridine as highlighted in section 2.4. Pyridine has N-atom with the lone-pair; n-electrons to donate to the vacant dπ-orbitals of the central cobalt ion of porphyrin complex. The non-bonding electrons on N-atom of pyridine are not delocalised in the aromatic ring system but they are instead directed towards the N-atom making this end of pyridine molecule more polar to facilitate co-ordination to Co²⁺ more easily. Transition metal ions also show a characteristic called back bonding which can also cause noticeable features in their spectra on interactions with the analytes.

- Highly conjugated π-electron system delocalised in the ring system of this macromolecule which in most cases are easily excited to higher energy levels that can facilitate certain interaction that can take place at these excited levels such as orbital or exciton couplings.

- Four peripheral substituents phenyl-methoxy rings which have in total four oxygen atoms, 4(ph−O−CH₃), with two lone or non-bonding pair of electrons each for interaction with electron acceptors or π-orbital donors for conjugation in order to form π-complex systems or adducts like those described by Diels-Alder.

Envisioned Diels-Alder adduct (Scheme 2.1) formation between CoP(ph-OMe)₄ is as exemplified using anthracene as in Scheme 2.2 while Schemes 2.3 and 2.4 exemplifies single and dimeric sensor molecule charge transfer interaction respectively.

![Scheme 2.1. Representation of the chemistry of adducts formation (cf Diels-Alder reaction).](image-url)
Scheme 2.2. Envisioned Diels'-Alder adduct formation of anthracene with one of the phenyl-methoxy constituent of CoP(ph-OMe)₄.

Cycloaddition reactions which are regioselective have been reported between PAHs and other π-conjugated systems [Koganti et al 2001]. In these type of interactions, the Diels-Alder adducts have been formed [Margetice et al 2003]. Adducts formed are characteristic of the sensor and PAHs in question.

When it applies to metalloporphyrins, in most cases adducts so formed are between their pyrole ring and the conjugated system of the PAHs. The metalloporphyrin is behaving like a diene in this case and the PAHs as a dienophile. Physical or chemical changes can be monitored which are characteristic of interaction of a sensing layer with a particular PAH.
Scheme 2.3. Envisaged single-sensor molecule CT complexation for anthracene with the central metal ion of sensing molecule.

Scheme 2.4. Envisaged dimeric CT complexation for anthracene with the central metal ion of sensing molecule.

Such charge transfer interactions are in most cases formed when the molecules are in the excited state having been excited with radiant energy from certain
wavelengths at which they absorb \[\text{Eichhorn 2000}\]. Charge transfer occurs when, on absorption of a photon an electron is promoted from the orbitals of the electron donor species to those of the electron acceptor species.

It is also envisaged that CoP(ph-OMe)\(_4\) through its central metal ion can facilitate axial-ligation with two pyridine molecules as represented in Scheme 2.5. Pyridine can co-ordinate to the central transition metal ions of the metalloporphyrins due to the presence of n-electrons on the N-atom in the ring system of pyridine molecule. Such complexes formed by two pyridine ligands co-ordinating to the central metal ion have been reported \[\text{Wojaczynski et al 2002}\].

Scheme 2.5. A representation of axial ligation of pyridine to the central metal ion Co\(^{2+}\) in the sensing molecule CoP(ph-OMe)\(_4\).

The co-ordination of pyridine molecule to the central metal ion of porphyrin has also been demonstrated by \[\text{Cammidge et al 2000}\]. Their synthesis of zinc pyridyl-porphyrin complex using zinc porphyrin (ZPP) in solution of CH\(_2\)Cl\(_2\) revealed through \(^1\)H NMR study that pyridine molecule got co-ordinated to the \(\text{Zn}^{2+}\) metal ion at the centre of porphyrin ring. Their absorption studies revealed absorbance \(\lambda_{\text{max}}\) 419 nm for ZnPP alone and at \(\lambda_{\text{max}}\) 428 nm with pyridine. In this study, absorption measurements revealed \(\lambda_{\text{max}}\) 414 nm for the solution of CoP(ph-OMe)\(_4\) in CH\(_2\)Cl\(_2\) alone and at \(\lambda_{\text{max}}\) 438 nm and other two broader peaks of low intensity towards the red end of the electromagnetic spectrum with pyridine while absorbance for solid thin films in poly-dimethyl siloxane were at \(\lambda_{\text{max}}\) 420 nm with CoP(ph-OMe)\(_4\) alone and \(\lambda_{\text{max}}\) 440 nm and a broad absorption band of lower intensity towards the red end of the spectrum with pyridine vapour. Pyridine can also form adducts with metalloporphyrins.
Due to these interaction sites therefore, the shapes of metalloporphyrins spectra after interaction with the analytes are characteristic to the particular analyte. Charge transfer and back bonding spectra have low energy and therefore transition bands arising from this kind of interaction are weak and hence resonate towards the red end of the electromagnetic spectrum (EMS). Absorption bands arising from dπ-dπ* transitions are forbidden theoretically basing on the laws of quantum mechanics, since they arise from unpaired electron spins (Scheme 2.6). It follows therefore that this transitions populate the triplet states (T) other than the singlet states (S) due to longer times created during flipping of the electronic spins. Wave frequencies for triplet states of electrons resonate towards the red end (longer wavelength) of the electromagnetic spectrum and are of low intensity because of their low energy.

Scheme 2.6. Triplet (T) and singlet (S) states.

The Metalloporphyrin may also interact through adsorption forming stacks or dimmers with analytes selected for this study including PAHs which can be useful.
for qualitative and quantitative measurements during sensing. In-case of such interaction, they may form excimers or exciplexes. These exciplexes may emit at specific longer wavelengths [Lakowicz et al 1994], a phenomenon called Stoke’s shift \((h\nu_{(ex)}-h\nu_{(em)})\). The excimers or exciplexes formed can be monitored spectroscopically and interpreted accordingly, and the information extracted can be used as a basis for sensing. Metalloporphyrins may also form secondary orbital overlap with these polluting compounds at certain interaction centres that can result in bond formation in suitable electron environment conditions. Even without bond formation, the fact that interaction is at particular sites on the sensor and the analyte, specific electronic transitions take place. These electronic transitions give characteristic spectroscopic information for specific molecules. If strong bonds have not been established, the sensor can be regenerated and reused.

2.6 Absorption and fluorescence modes for CoP(ph-OMe)$_4$

The CoP(ph-OMe)$_4$ has intense absorption band at 414 nm called soret band which is thought to be originating from \(\pi-\pi^*\) transition since it is thought of as belonging to \((0,2\text{ thus } S_0\rightarrow S_2)\) vibrational energy level of the molecule which is higher in energy and intensity compared to weaker bands of lower energy at 338 nm and 530 nm which might be originating from \(n-\pi^*\) and \(d\pi-d\pi^*\) \((S_0\rightarrow S_1)\) electronic transitions respectively. This macromolecule gets excited by the radiant energy from the near UV-visible end of the EMS (between 350 nm and 500 nm) and the valence electrons get promoted to higher excited vibrational energy level states \((S_0\rightarrow S_2)\). From the absorption spectra of the solution of CoP(ph-OMe)$_4$ in CH$_2$Cl$_2$, three distinct absorption bands are observed as enlisted above in this paragraph. These vibrational levels may be illustrated as in (Scheme 2.7).
As illustrated in Scheme 2.7, a (0,0) at 530 nm wavelength electronic transition has lower energy and therefore usually shifted to the red end of the electromagnetic spectrum. This kind of transitions may originate from theoretically forbidden transitions such as dπ-dπ*. The next excited state for a transition of (0,2) at 414 nm has a higher energy than the latter and this may originate from π-π*. The transition (0,3) at 350 nm is higher in energy than the last two and is likely to originate from n-π*. These n-π* are usually less intense bands in the spectrum of a compound and resonate at the blue end of the electromagnetic spectrum.

In most cases absorption spectra appear as broad bands. This indicates that there are more vibrational levels that have been smoothed out due to interactions with polar solvent molecules and spin couplings from neighbouring orbitals.

This sensing molecule also highly fluoresces and has two fluorescence bands with a major one at 659 nm and a smaller one at 723 nm. The excitation wavelength is at 423 nm the energy of which this sensing molecule absorb and the valence electrons are promoted to molecular orbital associated with excited state. The molecules then return to the ground state by loosing this excess energy, e.g. in form of heat exchange to the neighbouring molecules and or fluorescence. These transitions may be represented as in Scheme 2.8.
2.7 Sensor system 2: FLXN.

Bis[N,N-bis(carboxy-methyl)amino-methyl]fluorescein is a tetra-dentate ligand with \([-\{(\text{Carboxy-methyl})\text{amino-methyl}\}\)] constituents. Figure 2.6 represents the structure of FLXN.

The FLXN, exists as orange crystals and it is highly fluorescent. It dissolves in water resulting in solution that appears to have multiple of colours; greenish, yellowish and bluish tinges. The solution appears to change-colour when observed at different angles in ambient light indicating that this reagent may be highly polarisable.
2.8 Reaction centres for FLXN.

Fluorescein has reaction centres which include:

- Conjugated π-system
- Hydroxyl groups; -OH
- Carboxyl moieties; -COO⁻
- Carbonyl moieties; -CO
- Then two N-atoms
- Oxygen

Fluorexon (FLXN) is used as a complexometric indicator for calcium ions (Ca²⁺) titration with ethylene di-aminotetraacetic acid (EDTA) and as a labelling agent for cells in tissues of living organisms to facilitate screening [Oncel et al 1990]. Research has also been carried out using immobilized FLXN for fluorimetric determination of Co²⁺, Cu²⁺ and Ni²⁺ ions [Saari et al 1984]. FLXN has absorption maximum at 494 nm and highly fluoresces at 514 nm.

It is envisioned that due to high conjugated system that may avail dienes and dienophiles, FLXN can form Diels-Alder adducts with the organic pollutants selected for this research. The labile hydrogens present in the structure of FLXN having the –OH on the carbonyl moiety is a proton donor and may form hydrogen bonds with the analytes selected especially protonation of the N-atom on the pyridine molecule. The N-atoms and O-atoms present in FLXN are n-electron donors and therefore can react with electron acceptors.

Envisaged hydrogen bonding between pyridine and FLXN is as presented in Scheme 2.9.
Scheme 2.9. Hydrogen bonding of pyridine to periphery hydrogens in sensing molecule FLXN; R stands for the FLXN core.

It is therefore envisaged that, when the sensing molecules, after irradiation and subsequent interact with the pollutant molecules as illustrated in the schemes above, there will be changes in the sensing molecules’ physical or chemical properties and these can be monitored spectroscopically. Adsorption of the pollutant molecules onto the sensing molecules is also envisioned as another mode of interaction, though not illustrated schematically. These changes are required for these sensing molecules to qualify as optical chemical sensors for the selected organic pollutants.

### 2.9 Basis for choosing these sensor molecules and general desired properties of sensing molecules.

The basis for choosing these dyes is due to:

- Rigidity and planarity [Linstead 1934] of the ring systems of the metalloporphyrins, which avails interaction sites to be easily accessible to the analytes and form stable products with the analytes.

- Highly conjugated systems that strongly absorb radiation in the UV-VIS regions of the electromagnetic spectrum and highly fluorescence properties for both FLXN and CoP(ph-OMe)₄.

- Versatile reaction centres for selected organic pollutants.
2.9.1 General desired properties of sensing molecules.

Generally the sensor molecule is expected to exhibit the following characteristics:

1.) **Selectivity**: The ability of the sensor molecule to choose between interfering similar species that may produce the same sensor signal and the true analyte. Selectivity is matrix and concentration dependent. At the limit of detection (LOD) of the analyte, the interfering species can be a problem than at high analyte concentration. Selectivity parameter is a very important property to a chemical sensor because it determines reliability of the sensor molecule.

2.) **Limit of detection (LOD) to analyte**: As cited in literature, LOD is the detection level where the signal of the analyte is only three times the instrumental noise \( (3 \times \frac{S}{N}) \), with error > 5%. This is a very low concentration which is difficult to achieve by any average analytical measurements. For any reasonable measurable signal from the analyte, the signal should be ten times the instrument noise \( (10 \times \frac{S}{N}) \), error <5% (Figure 2.7).

3.) **Accuracy or reproducibility**: This is the ability of the sensor molecule to give replicate measurements even if these measurements are done at different times and environmental conditions. In most cases errors ranging between 1-5% are expected and acceptable. This has been established through inter- and intra-laboratory testing globally and it is christened as Validated Analytical Measurements [VAM].

4.) **Sensitivity (slope of calibration curve; signal/concentration)**: It is the ability of the sensor molecule to recognise a specific analyte and subsequent generation of a quantifiable signal. Sensitivity depends on the concentration of the sample, sample matrix, temperature, pressure and humidity.

5.) **Dynamic response range**: This is seen as the linear calibration range of the calibration curve of analyte concentration versus signal as illustrated in (Figure 2.7).
The linearity in the calibration curve can be described by a single mathematical equation as 2.1.

\[ y = mx + C \]  \hspace{1cm} 2.1

In this formula, \( y \) is the signal \( x \) the concentration, \( m \) is sensitivity and \( c \) is a background constant (y intercept). The linearity of the dynamic response range (LDR) is limited by the minimum detectable concentration (MDC), in reference to lowest concentration of analyte, and maximum detectable limit (MDL), where saturation may occur at highest concentration of the analyte.

6.) Stability: The chemical sensor is stable if there are no drifts in the signal measurements and therefore reproducibility. Although some signal drifts can be as a result of the drift in the electronic system of the instrument concerned. Therefore, calibration of the instrument is necessary before the measurements are done. If the drift in the measurement results in changes in the slope of the calibration curve, then there is a change in the sensor sensitivity. However if it is
due to the aging of the sensor, then it is expected that the sensitivity, selectivity and stability of the sensor reduce with time.

7.) **Response time:** This is the expected time interval where at least the signal reaches 90-99% of its final value after tenfold increase in concentration of analyte \((10 \times \frac{S}{N})\) which is the threshold for any quantifiable signal. Response time for good sensors ranges from milliseconds to a few minutes. In general the response time for a sensor depends on the analyte concentration and the matrix, especially for immobilised sensors. Longer response times are observed at low concentration of the analyte. The sensor responds faster at high concentration of the analyte.

Surface texture of the sensor molecule has influence on the response time of the sensor. A rough surface has a longer response time due to diffusion hindrances.

8.) **Reliability:** *This is interpreted as maintenance free working time for the sensor.* Reliability depends on the time period over which the chemical sensor performs to its maximum, without replenishes or maintenance. The sensor also should be specific for a long period towards the analyte in question without responding for other analytes with time. Thus, it should not be modified easily by changes in the environmental conditions.

9.) **Life-time:** A good sensor should have a longer life span. Stability, sensitivity and selectivity of good solid-chemical sensors take several years to diminish.

### 2.10 Organic pollutants selected for this research.

As mentioned in chapter 1, the pollutant molecules selected for this study include: ANTH, BaP, PRN and Py. The chemical structures of these pollutants are as presented in Figure 2.8.
These molecules are highly conjugated π-systems exhibiting both dienes and dienophiles as illustrated in a scheme in section 2.4. Pyridine, in addition to the conjugated π-system possesses a nitrogen atom which has localised n-electrons. These are chemical properties that may facilitate envisaged interactions with the sensing molecules for this research. These molecules especially PAHs are generally hydrophobic though to little extent soluble in water. Pyridine is slightly water soluble as well. The PAHs and pyridine are completely soluble in CH$_2$Cl$_2$ solvent used in this study. Pyridine is non-fluorescent while PAHs are highly fluorescent.

Anthracene has three-fused benzene rings and exists in solid phase as yellowish crystals. It has a high absorption coefficient of about 7 x 10$^6$ L mol$^{-1}$ cm$^{-1}$, with intense absorption between 290 nm and 390 nm with its absorption spectrum prominently showing five vibrational energy bands. Benzo(a)pyrene has five-fused benzene rings and exists in form of yellow solid crystals. It absorbs strongly in the UV-region of the electromagnetic spectrum exhibiting an absorption spectrum with eight vibrational energy bands and it also has a high molar absorptivity; 4.3 x10$^5$ L mol$^{-1}$ cm$^{-1}$. Pyrene also has four-fused benzene rings.

It has a high absorption coefficient of 7.5 x 10$^6$ L mol$^{-1}$ cm$^{-1}$ too, absorbing strongly in the UV-region of the electromagnetic spectrum with seven heavily pronounced vibrational energy structures.
2.11 Optical chemical sensors.

Optical chemical sensors are devices containing reagent molecules that are used in detecting target analytes when the spectroscopic properties of the sensor molecules are modified and monitored with appropriate analytical instruments. These molecules may be used in solution or they may be immobilised in solid thin films on substrates such as glass, metals or entrapped in gels or elastomers; adsorbed or bound covalently onto polymeric materials (Scheme 2.10).
Scheme 2.10. Illustration of Methods of immobilisation of sensing reagent.
The sensing molecules can also be immobilised on optical fibres as mentioned earlier (section 2.15). Solid film sensing systems surpasses solution sensing systems since immobilisation of thin films on smaller surface areas ensures miniaturisation and therefore easy handling. On the other hand, the disadvantage for solution sensing is that it is more bulky and more likely prone to more interferences and contaminations through numerous apparatus used before these solutions are eventually transferred to sample holders for instrumental analysis. Errors due to losses through evaporation of volatile solvents can also be a problem in solution based sensing systems. Solid surface optical chemical sensing stands out to be more modern because it is less prone to unnecessary contamination through apparatus as used for solutions. The solid films can also be stored for longer periods if the sensing molecule used does not suffer photo-bleaching and other reactions that shorten its lifespan. The sensor based on solid surface if regenerated, may also be available for reuse unlike solution sensing where the processes involved in regeneration of the sensing reagent from the bulk solvent(s) and matrices can be tedious and time consuming. Analytical scientists have found it more appropriate to develop reagent molecules that can be regenerated and re-used as sensors. This approach is economical and time saving. Exposure of these films to radiant energy can be done by fabricating e.g. flow cells for analyte vapours (gases and volatiles) or solutions (for metals and other non-volatile compounds).

2.12 Immobilisation.

2.12.1 Introduction.

In this research, solution studies that will enable observation of how the pollutants selected interact with the sensing molecules will be carried out first. After solution studies, the main aim is to miniaturise the sensor platform. Therefore, the sensing molecules will be made into solid thin films. This will be possible by immobilising them onto clean suitable surfaces embedded in polymeric matrices. In this regard, literature review on immobilisations done by other researchers is outlined in the later paragraphs of this section.
2.12.2 Substrates or supports.

These are solid surfaces onto which reagents can be immobilised and these include:

- Glass cover slips
- Glass slides and glass discs
- Graphite rods
- Metal plates
- Metal rods
- Fibre glass wafers
- Optical fibre
- Polymeric material

Immobilisation may be referred to as fixing a reagent onto a surface (substrate) by embedding in a matrix or covalently bonding with another molecule to make planar in order to avail reaction centres readily to the analytes and also make it easy to handle (see section 2.11).

2.12.3 Methods of immobilisation.

There are two methods of immobilisation and they are:

- Physical methods
- Chemical methods

2.12.3.1 Physical immobilisation methods.

This type of immobilisation involves non-bonding methods such as:

- Adsorption
- Encapsulation
2.12.3.1.1 Adsorption.

This method involves deposition of sensor molecules’ thin films as layers on substrates. The pioneers of this method were [Langmuir et al 1935] which yielded what is referred to as Langmuir- Blodgett films. These films are achieved by spraying, smear coating, sublimation, spin coating and dipping of known amounts of slurries or solutions for sensing molecules on suitable substrates. Once adsorbed, various curing processes have been employed and these include leaving at room temperature to set slowly or heat-setting in ovens or other heaters. Ultra-thin films have a quick response time as demonstrated by [Zhou et al, 2002].

Langmuir-Blodgett films on solid glass support like quartz glass slides or discs have been achieved by coating the glass surface with ultra-thin films and even several layers of films of dissolved sensor molecules using Langmuir-trough which is made to rotate at a uniform frequency.

Transducers like Surface Plasmon resonance (SPR) used by [Granito et al 1996], [Spedaveccha et al 2004], [Feresenbet et al 2001] and [Lloyd et al 1987]. Surface Acoustic Wave devices (SAW), used by [Katrizky et al 1991] and Quartz Crystal Microbalances (QCM) used by [Zhang et al 1998], in these devices, these researchers have employed Langmuir Blodgett immobilisation method for producing films.

These devices exploit the changes in the mass of the sensing layer. The surface Plasmon Resonators (SPR) takes advantage of polarisable piezoelectric crystals at the interface of the sensing layer and the substrate.

When piezoelectric crystals get polarised, a resonating plasmon or sea of electrons results at the interface with the sensing layer and when organic vapours are introduced to the surface, it results in mass change of the sensing layer and the resonating frequency of the plasmon also changes and this is recorded as a shift in the resonating frequency of the system. The SAW device also record the change in the mass of the sensing layer due to interaction of the analyte with the
sensor molecules and this is recorded as a shift in the wave frequency. They are similar in principle to QCM, which are also based on piezoelectricity of solids; the only difference being that the electric field is applied only at the surface of the crystal while for QCM; it is applied across the substrate. Quartz (Glass) is a piezoelectric material and quartz is stimulated to vibrate where the vibration is achieved by using an oscillating or an alternating electric current (AC). Deformation of the crystal lattice results due to inter-conversion of mechanical and electrical vibrations. In this case the quartz material is then converted into a device called a resonator or oscillator. When the sensor molecule layer immobilised on this resonator interacts with organic vapours, a change in the mass of the sensing layer alters the resonance frequency of the resonator and it is recorded as a change in resonance frequency.

2.12.3.1.2 Encapsulation and entrapment.

Immobilisation by encapsulation or entrapment can be achieved by some of the following methods:

- Introducing the sensing molecule into an inert matrix and subsequently coating the matrix containing the sensor with a semi-permeable membrane that can allow diffusion of the target analyte onto the sensing region.

- Incorporate in porous sol-gels like:
  - Hydro-gels; polymer chains with ~99 % water, flexible due to water content.
  - Organo-gels; contain liquid (oil, organic solvent) organic phase entrapped in three dimensional cross-linked network. They are elastic and firm.
  - Xerogels they are solid formed from a gel by drying (removal of solvent). They are porous and the degree of porosity depends on the method of drying, whether under supercritical or normal conditions.
Incorporation in nano-engineered shells [L’vov et al 2003]; drug nano encapsulation.

- Entrapment in poly-acryl amide matrix.
- Encapsulated in zeolites and coated with semi-permeable membrane.
- Entrapment in carbon nano-tubes.

### 2.12.3.2 Chemical immobilisation methods.

Simple molecules or other surface functionalised polymeric materials have been bound chemically on substrates as sensor macromolecules. Thus they involve bonding mechanism such as:

- Covalent bonding on organic polymeric material or simple molecules.
- Ionic bonding on inorganic materials.

Chemical immobilisation works have been achieved by several research scientists including; [Yang et al 2005], [Panchagnula et al 2005], [Wöhrle et al 2001] and [Kuswandi et al 2001]. Selectivity of these thin films towards certain analyte molecules depends on the central metal ion and substituent groups or moieties at the periphery and in the ring system as demonstrated by [Zhou et al 2002]. Sensitivity of a sensor dye towards the analytes of interest depends on the film thickness, concentration of sensor in the matrix and analyte concentration. Ultra-thin films have a quick response time [Zhou et al 2002]. These films are either made from single crystal dyes or their polymers. Also cited in literature [Granito et al 1996], such films of up to 120 nm thickness of copper and nickel phthalocyanine for toluene gas detection have produced.

Ultra-thin films of Zn, Cu, and Ru-phthalocyanines with electron withdrawing groups have been produced by [Spadaveccha et al 2004]. In their study [Zhang et al in 1998], have also produced ultra-thin films of polymers on the Quartz Crystal Microbalances (QCM) as sensor molecules. Vapours of pollutant molecules can be introduced onto these thin films by pumping to the sensing area [Armstrong 2000]. Vapours and gases interact with these films either reversibly
or irreversibly. Reversible interaction occurs when simple processes such as charge transfer or absorption and adsorption of the analyte onto receptor molecules in these films take place. Irreversible interaction involves formation and breaking of bonds of analyte under study with the sensing reagent. Chemical methods of immobilisation involve reaction of the sensing or receptor molecule at the surface of the support so long as they do not interfere with specific binding sites of the sensor molecule. Co-immobilisation of thin films of a given molecule can be done with other different molecules having functional moieties of interest [Wolfbeis et al 1988]. Such a technique has also been applied by [Zhu et al 2007] and [Yuan-Hong et al 2009]. The films are then held for analysis onto sensor platforms.

### 2.13 Sensor platforms.

To facilitate interaction of pollutants or target analytes with sensing reagents, analytical scientists have developed varieties of sensor platforms. Sensor platforms are gadgets that hold substrates onto which the sensor molecule films are immobilised. Among these sensor platforms are flow injection systems (FIS) which have been designed such that sensing molecule(s) immobilised on a substrate as mentioned earlier can be placed in compartmentalised flow cell as exemplified in Figure 2.9.

![Figure 2.9. Model of a sensor flow cell](image)
This flow cell is for illustration only and therefore, a similar flow cell for this research has been fabricated and it is presented in chapter 5.

The size and shape of the flow cell may depend on the size of the substrate onto which the sensor molecule films are immobilised and area where the sensor is to be installed. Such sensor platforms enable interface to the analytical instrument of the sensing molecule films using the optical fibre. The optical fibre is used for transmission of radiant energy to the sensing film. When an optical fibre is used in this case, then these sensors are called fibre optical chemical sensors. Fibre optical chemical sensing technique is the one employed for the solid thin films study in this research.

2.14 The optical fibre.

By definition, an optical fibre is a device either made from; glass, gemstone such as ruby or plastic designed to guide light along its length by confining as much light waves as possible in a propagating form. Confinement of light in optical fibres depends on their core diameter. While in optical fibres with a large core diameter, confinement is based on total internal reflection (section 2.14.1) and in small core diameter fibres confinement of radiant energy is done by establishing a waveguide (See appendix 2 for waveguides).

2.14.1 Design of optical fibre.

The optical fibre is designed such that the phenomenon of total internal reflection is applied. The total internal reflection phenomenon is defined by Snell’s law. According to Snell’s law, an incident ray of light from a low dense medium of low refractive index $\eta_a$ striking the interface of the medium with a high density which has a high refractive index will get bent away from the normal at an angle of refraction $\theta_b$ in relation to the angle of incidence $\theta_a$. This phenomenon of bending of light is referred to as refraction of light. The behaviour of light at the interface of these materials is defined according to equation 2.2.
\[ \sin \theta_a \times \eta_a = \sin \theta_b \times \eta_b \]  

2.2

Subscripts (a) and (b) represents media from which the light emerges from and where it enters respectively.

Refraction of light occurs until a certain incident angle known as critical angle \( \theta_c \) where the angle of refraction \( \theta_b \) is equal to 90° to the normal (Figure 2.10). At the angle of refraction 90°, corresponding to the critical angle \( \theta_c \) of the incident ray, no more refraction of light will take place. At angle(s) \( \theta_c+ \), which greater than the critical angle \( \theta_c \), a total internal reflection (TIR) will occur at the interface of these two media, and confined in the medium with a high refractive index (Figure 2.10). The critical angle is therefore defined by equation 2.3.

\[ \theta_c = \sin^{-1}\frac{\eta_b}{\eta_a} \]  

2.3

Total internal reflection is the principle applied to guide light in an optical-fibre.

Figure 2.10. Diagram illustrating critical angle \( (\theta_c) \) above which total internal reflection within the denser medium occurs.
As illustrated in Figure 2.10, the incident ray on entering a second medium of higher density and hence high refractive index, its velocity is altered and refraction occurs. The angle of refraction, on reaching 90\(^0\) and thus parallel to the interface of the two media, refraction appears no more and instead total internal reflection occurs as illustrated in Figure 2.11.

![Figure 2.11. Diagram illustrating total internal reflection angle (\(\theta_c\)) above critical (\(\theta_c\)). Symbols, \(\eta\) and \(u\) denote refractive indices and velocities respectively.](image)

The behaviour of light at the interface of the two media with varying refractive indices as described and illustrated diagrammatically above is applied in sensing technology during the designing and manufacture of optical-fibres (Figure 2.12).
Figure 2.12. Total internal reflection confining maximum radiant energy within the optical fibre.

An optical-fibre is therefore designed using two materials of different refractive indices. The inner material is known as the core and the outer material known as the cladding. The cladding material has low refractive index ($\eta_{\text{clad}}$) while the core material has a high refractive index ($\eta_{\text{core}}$). Since $\eta_{\text{clad}} < \eta_{\text{core}}$ total internal reflection occurs inside the core material and radiant energy is therefore guided along the length of the optical fibre via the core material. Surrounding the core and cladding materials is a plastic sheath to protect and stabilise the optical fibre.

Electromagnetic energy from the source is therefore not transmitted to the surrounding through the cladding material due to the low refractive index of this material. When this light is reflected totally within the optical fibre core material, it is assumed that the energy is not lost by transmission to the surrounding. This ensures that appropriate energy of a particular wavelength of interest reaches the sensing area and the evanescent wave phenomenon applied to analyse a very small surface area of the analyte of interest which will be a representation of the bulk homogeneous system of the analyte under study. However, attenuation does occur due to some defects in the optical fibre inherited during the manufacture.
2.15 Guiding radiant energy onto sensor.

When drilling the hole for coupling of the optical-fibre onto the sensor platform, the divergence angle of the radiant energy is also put into consideration. This ensures that appropriate drill angle is selected to yield an appropriate incident ray angle ($\theta_{c+}$) for the coupling (Figure 2.13). If the above procedure is followed, a sensor platform with efficient light coupling characteristics can be produced (Figure 2.13). Such a sensor platform facilitates guided light wave to be imparted evenly upon the sensing area on the platform and therefore uniformly interacts with the molecules in the sensing area.

![Figure 2.13](image)

$\theta_{c+} = \text{Normal incident energy}$

$\theta_{c+} = \text{Optical Fibre}$

$\theta_{c+} = \text{Sensor platform}$

$\theta_{c+} = \text{Emergent radiant energy to transducer & Detector}$

$\theta_{c+} = \text{Light wave Divergent angle}$

$\theta_{c+} = \text{Matrix & sensor; (inset matrix and sensor region is a small area of reagent analysed basing on evanescent wave)}$

$\theta_{c+} = \text{Sensor platform}$

$\theta_{c+} = \text{Light wave}$

$\theta_{c+} = \text{Divergent angle}$

$\theta_{c+} = \text{Matrix & sensor; (inset matrix and sensor region is a small area of reagent analysed basing on evanescent wave)}$

Figure 2.13. Illustration of coupling of optical fibre to sensor platform

Note: For Illustration only otherwise sensing area should be light tight therefore optical fibre usually couples in sensor platform.
2.16 Types of optical fibre chemical sensor configurations.

There are two types of optical fibre chemical sensor configurations leading to two types of optical fibre chemical sensing techniques namely intrinsic and extrinsic optical fibre chemical sensing. The first is a wave-guide-binding configuration (Figures 2.14 and 2.15) which facilitates recognition at the surface of the fibre core through the evanescent field. This type of sensing is achieved by exposing the core by removal of the cladding and jacket material and allowing the analyte material to bind to the core surface.

Figure 2.14. Schematic illustrating intrinsic optical fibre sensing.

Figure 2.15. Diagram illustrating an intrinsic optical-fibre chemical sensor configuration.
Figures 2.14 and 2.15 type of configurations show what is called intrinsic optical fibre chemical sensor.

In extrinsic optical fibre chemical sensor configuration, the sensor is held against the illuminated area at the end of a single fibre optic or a bundle of optical fibres (Figure 2.16). The fibre in this case is only acting as light guide to the analyte and this type of sensor is called extrinsic optical fibre chemical sensor. However each configuration will require an excitation light source such as xenon or tungsten lamps with monochromator and will also require detector(s). These peripherals to the optical fibre are achieved through interfacing of the optical fibre with the optical paths of appropriate spectroscopic instrument(s). As mentioned above, Figure 2.16 illustrates extrinsic optical fibre sensing technique.

In this research we adopt investigations basing on extrinsic optical chemical sensing configuration technique (Figure 2.16), due to the available analytical instrument; LS 55 spectrofluorometer. Also in this technique, a more robust optical fibre can be utilised rather than the cheap optical fibres that can be discarded because they cannot be reused after having immobilised the sensor film on them.
In the long run, using a more expensive, robust optical fibre becomes cheaper because it can always be re-used for future similar studies.

### 2.17 Optical sensing mechanism.

Optical sensing is brought about by interaction of materials with radiant energy of a specific wavelength from the electromagnetic spectrum where chemical or physical changes are monitored by instrumental optical system. A general sensor platform can be represented as in **Figure 2.17**.

![Figure 2.17. Schematic of sensing system platform](image)

Radiant energy may cause physical (Scheme 2.11) or chemical (Scheme 2.12) interactions with analytes in question.
Scheme 2.11. Physical way of interaction of sensor and analyte; \( n \) is the number of analytes.

Scheme 2.12. Chemical way of interaction of sensor and analyte.

An analyte under investigation is fed onto an immobilised reagent on a sensor platform. When this reagent and the analyte interact, physical or chemical changes take place that affect the structure and functioning of the reagent molecule. Signal(s) produced through transduction and amplification is/are recorded as electromagnetic energy of a particular wavelength resulting from the kind of interaction that initially took place. Thereby making the reagent molecule used to qualify as a sensor for the analytes that caused such changes after interaction.

On the whole, optical chemical sensing mechanism is achieved through four types of interfaces namely:

- Chemo-optical interface; thus interface between the sensing reagent and the optical fibre.
Electro-optical interface: interface between the electrical system of the analytical instrument and optical fibre.

Opto-electronic interface which is the interfacing of the optics and electronics systems in the analytical instrument with the optical fibre.

Photo-detector electronic interface which is connection between the photo detection devices such as photodiodes or photomultipliers with the electronic system of the analytical instrument and the optical fibre.

These interfaces maybe illustrated schematically as in **chapter (3) sub-section 3.8.4.**

The physical and chemical properties of a reagent reported or conveyed by the optical-fibre system are recorded through the aid of instrumental software. Fibre optical sensing techniques are more sensitive as compared to other bulk optical sensing techniques since radiant energy in the optical-fibre system is guided and therefore no need for extra coupling electrically to the sensor platform that may cause losses and interferences. In this regard therefore, the technique can be suitable for use in hazardous environments where flammable analytes are encountered. Optical fibre sensing technique is easier to use since there is less need for optical adjustments in order to achieve good results. This technique also allows for miniaturisation of sensing and detection systems or devices which is a more convenient modern way of sensing. The reason behind suitability and convenience for miniaturisation is because they can be held in specifically fabricated sensing compartments, easily be interfaced with appropriate optical fibres and can be positioned in some impossible areas if bigger sensing system were to be the only ones in use. Fibre optical sensing as a technique is suitable for organic vapour sensing because signal loses are minimal which is a prerequisite because of the low concentrations of reagents and analytes involved. This kind of system can easily be interfaced with appropriate instrumental systems of analysis such as absorption spectrophotometer, fluorometers and many other analytical Instruments. Above all the advantages, the sensor platform can be outside the instrument and therefore remote sensing is very possible which can also facilitate online monitoring of the organic pollutants.
Multiplexing can also be achieved in this kind of arrangement for the purposes of multi-sensing; (cf. nerve system), manipulation by harmless bending of the optical fibre to fit the occasion can be perfectly achieved due to smallness of the sensing platform and finally incorporation of both interrogating and response signals in the same optical fibre system can be achieved.

Previously, to ascertain the extend of pollution in the environment; scientist sampled affected areas for analysis. Highlighted in section 2.18 is sampling mechanisms for organic vapour pollutants (OPs).

**2.18 Sampling mechanisms for OPs.**

As highlighted in section 2.17, optical chemical sensing technique specifically online chemical sensing surpass manual sampling and manual analytical techniques because, environmental sampling for particular analytes can be tricky. This is due to the expansiveness of distribution of matter in the environment with mixtures of organic and inorganic pollutants and dusts.

Samples for the organic pollutants selected for this study can be present in the following media:

- **Air**
- **Water;** (Rivers, oceans, lakes, seas and pools)
- **Soil**
- **Effluents and wastes.**
- **Surfaces including:** (Rooftops, walls, windows, pavements, leaves etc)

Various environmental organisations have developed methods for sampling, extraction and analysis of organic and inorganic pollutants. Such organisations as Occupational Safety and Health Administration (OSHA), which is a branch of the department of labour in the USA have developed methods for environmental samplings in air, soil, water, wastes and surfaces [NIOSH Manual for Analytical Methods (NMAM)]. NIOSH is abbreviation for National Institute for Occupational Safety and Health. NMAM manual is a collection of methods for sampling and analysis of contaminants in workplace air, water, surfaces and in the blood and
urine of workers who are occupationally exposed. OSHA has their methods coded for easy reference. The coding follows examples as 0010/8310, a method for sampling PAHs from water, extraction and subsequent analysis. Where 0010 describes sampling, transportation, sample treatment and storage prior to analysis and 8310 describes analytical techniques for PAHs using HPLC in that case [OSHA-58 NMAM (Issue July 1986)]. Sampling of semi-volatile organic compounds (SVOCs) from stationery surfaces is described by OSHA method 0010/8270C. Air sampling method for PAHs is described by OSHA with appropriate coding for reference.

Prior to establishment of these sampling and analytical methods, careful development was done. Including contaminating media with these pollutants and sampling from contaminated media areas or surfaces. Evaluation of surface removal efficiency (SR\(_E\)), Extraction efficiency (E\(_E\)) and Reliable quantification limits (RQL) were carried out.

These parameters were evaluated as follows:

\[
E_E = \frac{100M_r}{M_s} \tag{2.4}
\]

\(E_E\) = Extraction efficiency.

\(M_r\) = Mass of analyte removed.

\(M_s\) = Mass of analyte placed on the media.

Evaluation of surface removal efficiency (SR\(_E\)) can be calculated by use of formulae 2.5 and 2.6.

\[
M_s = \frac{M - M_B}{E_E} \tag{2.5}
\]

\(M_s\) = mass recovered from sampled surface (placed on media) in \(\mu g\)

\(M\) = \(\mu g\) per sample
M_B = Mass found on blank

If the area which was sampled was 100 cm², then the surface removal efficiency can be corrected using **formula 2.6**.

\[
C_s = 100 \frac{M_s}{S}
\]  

2.6

Cs = μg of analyte per 100 cm²  
M_S = Mass of sampled surface  
S = surface area sampled (cm²)

The reliable quantification limit can calculated using **formula 2.7**.

\[
RQL = \frac{10(S_{std})}{A}
\]  

2.7

RQL = Reliable quantification limit, recorded as mass of sample, μg.  
S_{std} = Standard error estimate of regression.  
A = Analytical sensitivity (slope).

Other parameters used to establish these methods as reference sampling and analytical methods include Analytical Method Recovery (AMR) where digestion, solubility and matrix effects are considered in comparison with the standards for the samples in question. These methods or sampling are appropriate, but as they are manual, large systematic errors might be a problem. The accuracy of the method will very much depend on the person sampling, transportation, treatment and preparation of representative samples prior to analysis.

Online detection of pollutants from their source and subsequent control to minimise to less harmful amounts or conversion into less harmful components can be a more modern method of minimising environmental pollution. Such methods for online monitoring of noxious gases and allied products have been developed in some big industries. Such industries like oil refineries [Kenya
Petroleum oil refinery, Changamwe, Mombasa – Kenya] where if the amounts set as acceptable limits go beyond these limits, a siren is heard and a light flickers indicating the locality of the problem. The siren alerts Chemists and Engineers monitoring these levels and therefore the problem rectified before spilling hazardous amounts to the environment. Methods that can sense and hence monitor organic pollutants at their sources can be more appropriate in dealing with environmental pollution.

Fibre optical chemical sensing technology is envisaged as a method that can be developed for online monitoring of organic pollutants. Fibre optical sensor platforms can be integrated in or interfaced to the analytical instruments that are interfaced with computers with appropriate software developed for particular pollutants. Properties to be monitored e.g. change of chemical or physical properties of the sensor when brought in contact with the pollutants and subsequent changes at a specific wavelength can be observed. Signals produced can act as indicator and quantities for these pollutants monitored thus allowing appropriate measures to be taken to check the levels that can be acceptable and therefore less harmful environmentally.

Following the above sections, highlighting important aspects of optical chemical sensing techniques, we now look at a brief overview of some literature on sensors for ANTH, BaP, PRN and Py.

2.19 Literature review on sensors for organic pollutants of this study

Scientists globally, have done numerous researches in the quest to develop optical chemical sensors for organic, inorganic and gaseous pollutants. They have utilised immobilised solid thin films of materials such as: Porphyrins, metalloporphyrins, phthalocyanines, metallophthalocyanines, polymers, resins and metal oxides.
In this section, a summary of some published works including authors, sensing reagents, limit of detection, where reported and analytical methods employed for ANTH, BaP PRN and Py are summarised in Table 2.1

Table 2.1. Some sensors reported in literature for ANTH, BaP, PRN and Py

<table>
<thead>
<tr>
<th>Author</th>
<th>Chemical used as sensor</th>
<th>Matrix</th>
<th>Analytical techniques</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elosua et al 2008</td>
<td>Vapochromic cobalt complex; β-Co(py)Cl₂</td>
<td>PVC (Poly Vinyl Chloride), TBP (Tributylphosphate), tetrahydrofuran (THF), forming a plasticised matrix</td>
<td>Absorbance and reflectance</td>
<td>1.0 mg/L Py</td>
</tr>
<tr>
<td>Giuliani et al 1983</td>
<td>Oxazine perchlorate films</td>
<td></td>
<td>Optical waveguide; Transmittance</td>
<td>Py</td>
</tr>
<tr>
<td>Schmidt et al 2004</td>
<td>Silver metallic nanostructures</td>
<td>Mehtyltriethoxysilane (MTEOS) and Ethyltriethoxysilane (ETEOS)</td>
<td>SERS</td>
<td>ANTH, PRN</td>
</tr>
<tr>
<td>Chen et al 2004</td>
<td>MIP; with ANTH binding sites</td>
<td>Polyurethane</td>
<td>Fluorescence</td>
<td>ANTH 15</td>
</tr>
<tr>
<td>Fernandez-Sanchez et al 2003</td>
<td>Ionic amberlite;XAD-2, XAD-4 and XAD-7</td>
<td></td>
<td>Fluorescence</td>
<td>ANTH, BaP, PRN 3.0 ng/L</td>
</tr>
<tr>
<td>Kasili et al 2004</td>
<td>Anti-BaP</td>
<td>Silane-Imidazole</td>
<td>Fluorescence</td>
<td>BaP, 0.36 ng</td>
</tr>
<tr>
<td>Pierre et al 1996</td>
<td>Anti-BPT*</td>
<td>Optical fibre</td>
<td>Fluorescence</td>
<td>BaP; 0.001 ng</td>
</tr>
<tr>
<td>Cañizares et al 1996</td>
<td>MIP with ANTH binding sites</td>
<td>Polyurethane</td>
<td>Fluorescence</td>
<td>ANTH</td>
</tr>
</tbody>
</table>

* Antibody-BPT; - Benzo(a)pyrene tetrol (BPT), PVC (Poly vinyl chloride), TBT (Tributylphosphate), THF(Tetrahydrofuran).

Table 2.1 highlights some of the sensors and analytical techniques reported in literature for the organic pollutants selected for this research. In most of these sensors, other types of molecules and polymeric membranes rather than metalloporphyrins and FLXN have been employed. However, though not yet applied for the pollutant molecules selected for this research, porphyrins and metalloporphyrins have been used extensively in research as suitable films for sensing gases, metal ions and other π-conjugated systems. Such molecules can interact with these macromolecules and effect changes that can be monitored using various analytical techniques. For example, thin films for porphyrins and metallo-porphyrins have been used as pH sensors [Yuan et al 1993], [Cheng-Gang et al 2005], thiocyanate ions (SCN⁻) sensors [Shamsipur et al 1999], mercury ions (Hg⁺) sensors [Guo et al 2006]. In their research, Paolesse et al...
1999, using quartz crystal microbalance analytical technique demonstrated that, thin films of nickel porphyrin, copper porphyrin, zinc porphyrin and cobalt porphyrin were able to detect vapours for acetic acid, triethylamine, ethanol and toluene. Apart from porphyrins being used as sensors for metal ions, the complexes formed as metalloporphyrins are used as co-ordination sensors for various ligands, e.g. pyridine pollutant of this research, Cammidge et al 2000 and Wajaczyński et al 2002. These porphyrins and metalloporphyrins are also used as stationary phases in chromatographic analytical technique. They interact with many compounds due to their highly π-conjugated systems and therefore are suitable as stationary phases. They have been used in HPLC for separation of fullerenes $C_{60}$, $C_{70}$, $C_{94}$ [Xiao et al 1995], nucleosides and nucleotides [Biesaga et al 2001] and anions (Cl-, NO$_3^-$, ClO$_4^-$, I- and SCN$^-$), [Biesaga et al 2002] FLXN on the other hand has been used before for analysis of Ca$^{2+}$ in solution [Diehl et al 1956].

Instrumental analytical techniques employed in this research are highlighted in chapter 3.
Chapter 3: Analytical techniques

3.1 Chapter introduction.

Instrumental analytical techniques employed in this research include absorption and luminescence; luminescence here is basically fluorescence spectroscopy. Fluorescence spectroscopic analytical technique was preferred for thin films study.

Both thin film and solutions study required sample holders. Thin films required a fabrication of a sensor platform (see section 5.10 of chapter 5). Solutions have been studied using quartz cuvettes as sample holders, in which case, the cuvettes (Figures 3.1 and 3.2) are placed in appropriate sensor platforms inside the instruments. On the other hand, solid thin films study required fabrication of a flow cell as a sensor platform (see section 2.13), which is placed outside the instrument but interfaced to the instrument in use through the optical fibre as a means of guiding radiant energy onto the sensor film.

![Diagram](image.png)

Figure 3.1. Absorption measurement solution samples cuvettes in sample compartment.
Mostly in use are quartz cuvettes because they are cheaper than the superior fused silica. They are useful as sample holders in the UV-VIS spectrophotometric and fluorometric measurements for solution study. The reason why these materials are useful in this region is that they are transparent to the radiant energy ranging from 200-800 nm. Thus they do not absorb in this region of the electromagnetic spectrum. Sample cuvettes vary in design depending on the analytical technique. Sample holders for absorption measurements are designed in such a way that will allow incident radiant energy, \( I_0 \), to pass through the measurand so that a particular wavelength for the measurand gets absorbed and the residue radiant energy that pass through the solution emerge as energy transmitted \( I_t \). Sample cuvettes for solution absorption measurements are transparent on two opposite sides to allow light path through and the other two opposite edged sides are opaque (Figure 3.1). On the other hand, solution sample cuvettes for fluorescence measurement are transparent on all the four sides because the incident and emergent radiant energy form an angle of 90\(^0\) to each other at the sample surface (Figure 3.2).
3.2 Absorption spectroscopy.

The technique is based on the fact that particular molecular chromophores absorb radiant energy of particular wavelength(s) isolated from the incident radiation from the source by the monochromator. The source usually Hydrogen or Deuterium lamps and combined with tungsten lamps cover the electromagnetic spectrum (EMS) range from 190 nm to 1000nm. The emission spectra for these lamps are as illustrated in Figure 3.3.

![Figure 3.3. Emission profile for deuterium, tungsten and xenon lamps (from Fundamentals of optoelectronics by Artur Dybko).](image)

These lamps cover the ranges as illustrated in Figure 3.3; which shows that xenon arc can be used alone during analysis when scanning through the wavelength ranges from 200 to 800 nm.

The layout of a dual beam absorption spectrophotometer similar to the Shimadzu UV-VIS spectrophotometer PC 2401 used in this research is as illustrated in Figure 3.4.
In a dual beam absorption spectrophotometer, there are two beams as shown in the diagram. One beam is for sample irradiation and the other for blank irradiation. The blank is usually the matrix which would contain the sample but minus the sample (analyte). The blank irradiation is subtracted automatically by the instrument software to correct for the background noise in order to establish a baseline. The instrument software also takes a ratio of transmitted energy $I$ and incident radiant energy $I_0$ (see equation 3.3) in order to give the value of absorbed energy which is then recorded as absorbance of the analyte vs the wavelength. A simplified diagram of Figure 3.4 is as presented in Figure 3.5.
3.3 Absorbance and excitation of electrons.

For a molecule to be able to absorb UV or Visible light, it must have a chromophore, which is a group or moiety in the molecule that is capable of absorbing in this range due to unsaturated conjugated systems with π-electrons or some heteroatoms that have non-bonding electrons. This phenomenon is governed by equation 3.1.

\[ E_{ex} - E_0 = h\nu \]  

Where; \( E_{ex} \) = excitation energy; or energy absorbed, \( E_0 \) = energy of the molecule at ground state, \( h \) = Plank’s constant which has a value of \( 6.63 \times 10^{-34} \) Js, = \( 4.14 \times 10^{-15} \) eV and \( \nu \) = the frequency of the radiation.

Molecules or atoms absorb energy of a particular wavelength from the excitation source of the electromagnetic spectrum that merges the energy difference between their ground state, which is their most stable state, to their higher vibrational level also known as the excited state which is more unstable (equation 3.1). When these species absorb energy, it is their valence electrons that gain the energy and get promoted to these higher vibrational levels.

Since these higher energy levels are unstable states this prompts the molecule to lose that excess energy through charge transfer to neighbouring species, bond formation or emission. The profile of this energy is then converted into electrical energy by the photomultiplier detector (Figure 3.6) and recorded on the display unit such as a computer monitor as electronic absorption spectrum that is characteristic of the molecules involved.
Figure 3.6. The diagram shows the schematic of a photomultiplier. Light photons entering the glass entrance window impinge on the photocathode. The result is emission of one electron for approximately every five light photons. The electron produced is accelerated towards a dynode chain. The accelerated electron has sufficient kinetic energy to liberate approximately five electrons when it strikes each dynode. The effective electron gain at the collecting anode is $10^6 - 10^8$. The output of a photomultiplier is a signal with a characteristic shape and the amplitude of the signal is proportional to the number of photons entering the photomultiplier tube (HV= High voltage; Pre-Amp = Pre-amplification).

Diagram from RadioGraphics 1999 by Ranger T. N.

The energy profile of the photomultipliers is as presented in Figure 3.7.

Figure 3.7. Response curves for photomultipliers [LS 55 Perkin Elmer fluorescence spectrometer manual].
Measurements done using the absorption spectroscopic technique strictly need concentrations that will give linear absorption ranges for any meaningful true values. The range of concentrations that will give linear absorbances are dictated by the molar absorptivity or optical density of the analyte and limited by the Beer-Lambert’s law. The amount of radiant energy absorbed by a chromophore in the molecule if the concentration is in moles $\text{dm}^{-3}$ is defined by the molar absorptivity, $\varepsilon$, which depends on the probability of a species absorbing that photon from a particular wavelength that pass through it. Hence the probability of the type of electronic transition taking place at this concentration. The molar absorptivity is related to absorbance, $A$, and concentration, $c$, when radiation pass through the solution of path length of $(b \text{ cm})$ as illustrated in equation 3.2.

$$A = \varepsilon b c$$  \hspace{1cm} 3.2

The Beer-Lambert’s law is a derivation from the ratio of the transmitted radiation $(I)$ to that of incident radiation $(I_0)$ as illustrated in equations 3.3, 3.4 and 3.5.

$$T = \frac{I}{I_0}$$  \hspace{1cm} 3.3

$$A = -\log_{10} T = \log \frac{I_0}{I}$$  \hspace{1cm} 3.4

$$A = \log \frac{I_0}{I} = \varepsilon bc$$  \hspace{1cm} 3.5

$I_0 =$ the radiant energy before passing through the solution,
$I =$ radiation after passing through the solution,
$T =$ the transmittance; fraction of $I_0$ transmitted through the solution.

The Beer-Lambert’s law can also be applied when analysing solutions that contain more than one absorbing species. This is applicable if there is no interaction among the species concerned. The total absorbance of the multi-
component system is the sum of all the individual absorbencies and can be represented by the equation 3.6.

\[ A_{\text{total}} = A_1 + A_2 + \ldots + A_n = \varepsilon_1 b c_1 + \varepsilon_2 b c_2 + \ldots + \varepsilon_n b c_n \]  

Subscripts refer to individual absorbing species.

Multi-component absorbance system will not be applied in this thesis but has been highlighted above for information only.

However, the Beer-Lambert’s law is a limiting law. Deviations from linear relationship of absorbance vs concentration can occur at high concentrations above maximum detectable limit (MDL) and very low concentrations below the limit of detection (LOD) respectively of the absorbing chromophore (see figure in section 2.9.1 of chapter 2). These deviations from linearity may give errors in measurements. When the absorbing species undergo dissociation or association or react to form products that absorb different absorption bands aside those of the analyte, then the linearity is also affected and the deviation caused in this manner is referred to as chemical deviation.

Absorption spectrophotometry gives a hint on the type of electronic transitions involved, so that when the spectrum is correctly interpreted, transitions such as \( \pi-\pi^* \), \( n-\pi^* \) or even \( \sigma-\pi^* \) can be identified. Usually the \( \pi-\pi^* \) are higher in energy therefore their absorption bands are more towards the blue end of the electromagnetic spectrum basically in the near UV-VIS region of the electromagnetic spectrum (EMS), followed by \( n-\pi^* \) (although this can be earlier than \( \pi-\pi^* \) depending on molecular environment), and \( n-\sigma^* \), which have the next highest energy transitions and lastly the \( \pi-\pi^* \) the lowest energy transitions bands, also referred to as Laporte forbidden bands. Larpote forbidden bands have lower energy and hence low absorbancies most cases equal to or below 0.1 depending on the limit of detection (LOD) and MDL of concentration for the chromophore of the molecule in question which is governed by its absorptivity.
3.4 Luminescence spectroscopy.

3.4.1 Introduction.

After irradiation and subsequent absorption of a photon, many chemical compounds will emit light in the visible region of the electromagnetic spectrum. This emission is called luminescence and it is of two types.

These two types depend on the multiplicity of electron spins. If the emission occurs from an excited state that has the same singlet spin multiplicity as the ground state, then the emission is called fluorescence. On the other hand, phosphorescence occurs when the excited state spin multiplicity flips to the Laporte forbidden triplet state which is different from that of the singlet ground state. Fluorescence and phosphorescence do not occur at the same wavelength of excitation but at longer wavelength and therefore of lower energy than that of the excitation wavelength. When the molecule is promoted to the excited electronic state, it immediately loses some of the energy through vibrating. This kind of energy lose is called vibrational relaxation. This explains why the luminescence energy is always less than the excitation energy. This energy difference is called Stoke’s Shift; named after a scientist who first observed this phenomenon. Jablonski diagram (Figure 3.8) summarises the principles of luminescence spectroscopy.
Figure 3.8. Jablonski diagram showing the general energy vibrational levels of molecules excited to higher levels and their subsequent dissipation of energy absorbed.

In the Jablonski diagram, $h\nu_{\text{ex}}$ is excitation energy, $h\nu_{\text{fluo}}$ fluorescence energy, $h\nu_{\text{phos}}$ phosphorescence energy, S is singlet state and T is triplet state.

Fluorescence measurements follow the basic principles as depicted in schematic instrumental layout as represented in Figure 3.9.
The components of the layout of LS 55 Fluorometer of Figure 3.9 can be described as follows:

**The radiation source:** Provides radiant energy from the EMS. In fluorometric techniques, radiation sources are usually deuterium, D₂, combined with tungsten, W, lamps to cover the EMS range from 190 – 900 nm.

**Excitation monochromator:** Usually a prism for versatility, which spreads out the normal incident light in various radiation bands. This is achieved by taking the advantage of the variance of refractive index with wavelength. The light is spread out into individual frequencies of wavelengths and therefore, the desired excitation wavelength (λₑₓ) as selected and keyed in through the instrumental software by the analyst can then be isolated from other wavelengths directed and focused by appropriate mirrors onto the sensing area of the instrument. Thus the analyst must have a rough idea of the absorption wavelength(s), λₘₐₜ, of the analyte under study, which is usually obtained from measurements of the analyte’s electronic absorption spectra. The quartz glass prism monochromator bends short wavelength more than it does to longer wavelengths because the refractive index is greater for shorter wavelengths than it is for the longer
wavelengths. The refractive index is greatest as the prism material approaches the wavelength at which it becomes opaque to radiant energy.

The greater the change in the refractive index, $\eta$, with wavelength, the greater the dispersive power of the prism.

**Excitation polariser:** The work of the excitation polariser is to resolve the light components by eliminating vibration of light components of electrical and magnetic fields in other directions and directing them in the desired plane **Figure 3.10.** This is then called plane polarised light.

![End view of plane of magnetic fields](image)

**Figure 3.10.** The selective removal of components from light: (a) ordinary light showing various magnetic fields that comprise it. (b) Selected radiation a, b and c. (c) selected radiation resolved into vertical and horizontal components; and (d) light after the vertical component is removed. The remaining light is now polarised in the horizontal direction. [Undergraduate instrumental analysis Fifth edition, revised and expanded; James W. Robinson (1994)]

After the excitation wavelength passes through the analyte of interest, the overall absorbed radiant power loses some energy through, IC, ISC, etc (Jablonski diagram, in this section above as cited in literature in many journal papers and books with topics on luminescence). The resultant energy wavelength is then passed on by the emission polariser to the emission monochromator.

The emission monochromator auto selects the characteristic emission wavelength, $\lambda_{em}$, of the analyte under study. The emission radiant energy is
converted by the detector, usually photomultiplier detector (section 3.3) into electrical energy and the signal generated is displayed graphically on display unit which may be a computer monitor or screen. In the presence of plane polarised light, fluorescence and or phosphorescence properties of the analyte can then be monitored using the same principle so long as the instrument is switched on to the mode desired, whether fluorescence or phosphorescence mode (in reference to the LS 55 Fluorometer).

3.5 Quenching of the excited state of a complex.

In the presence of another chemical, the excited state intensity of a molecule may decrease or be eliminated completely. This phenomenon is called quenching of the excited molecule. Quenching is bimolecular process which involves energy transfer between the excited state of one molecule called the donor (D) and the ground state of another molecule called the quencher (Q). This quenching process may lead to a chemical reaction between the donor and the quencher or simply energy transfer between the two species without any new products formed. Such interactions are significant in sensing technology.

The fundamental luminescence for a monochromatic excitation light is as presented in equation 3.7.

\[ I_{\text{obs}} = K I_0 \Phi F \]  \hspace{1cm} 3.7

Where \( I_{\text{obs}} \) is the observed luminescence intensity of the sample being irradiated at a particular wavelength ‘\( \lambda \)’ with an incident or excitation intensity (quanta/sec) \( I_{\text{exc}} \), \( I_0 \) is incident radiation; \( K \) is proportionality constant which is usually constant as long as the detector wavelength and geometry do not change, while \( \Phi \) is the probability that once a molecule has absorbed a photon, it will emit a photon and \( F \) is the fraction of incident light absorbed by the sample.
The fraction of radiation absorbed \( F \) is given by Beer-Lambert’s law as illustrated in equation 3.8.

\[
F = 1 - e^{-2.303\varepsilon\ell C}
\]

3.8

Where \( \varepsilon \) is the molar absorptivity at a particular wavelength, \( C \) is the concentration in moles/litre and \( \ell \) is the optical path length. If \( \varepsilon\ell < 1 \), the exponential part of the equation can be expanded giving equation 3.9.

\[
F = 2.303\varepsilon\ell C
\]

3.9

Equation 3.9 can be substituted in equation 3.7 to yield equation 3.10.

\[
I_{obs} = 2.303KI_0\Phi \varepsilon\ell C
\]

3.10

### 3.6 Quenching and the Stern-Volmer equation.

Quenching involves removal of energy from the excited state of a molecule by a different molecule. When a quencher (Q) is added to a solution of a fluorophore (M), various processes can occur as listed below:

\[
M \rightarrow M^* \quad I \quad excitation
\]

3.11

\[
M^* \rightarrow M + h\nu \quad k_e \quad emission
\]

3.12

\[
M^* \rightarrow M + heat \quad k_r \quad relaxation
\]

3.13

\[
M^* + Q \rightarrow M + Q^* \quad k_q \quad quenching
\]

3.14
As illustrated above, the molecule M gets excited by absorbing radiant energy of excitation energy $h\nu$ and of intensity $I$. The excited state of this molecule $M^*$ can be deactivated through processes illustrated in equations (3.11 – 3.14). In these processes, $k_e$ is the emission rate constant, $k_vr$ is the vibrational relaxations rate constant and $k_2$ is the bimolecular quenching constant. If the steady state approximation is made for the excited state of M, then equation 3.15 can be obtained.

$$\frac{I_0}{I} = 1 + \frac{k_2 [Q]}{(k_e + k_vr)}$$

3.15

Where $I_0$ is the measured luminescence intensity of M and $I$ intensity without quencher (Q).

The ratio of the luminescence in the absence of quencher to that in the presence of quencher, gives an equation containing only fundamental rate constants and measurable quantities. Equation (3.16) is called the Stern – Volmer equation, and it is one of the fundamental equations of photophysics. Equation (3.16) is normally written as:

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

3.16

Thus $K_{SV}$, which is the Stern-Volmer quenching constant is as stated in equation 3.17.

$$K_{SV} = \frac{k_2}{(k_e + k_vr)}$$

3.17

The lifetime of an excited state ($\tau$) is defined as the reciprocal of the rate constant ($k$); $\tau = \frac{1}{k}$. If the quencher is not present in the matrix, the donor can lose energy only through luminescence and vibrational relaxation so that the total rate
constant becomes $k_e + k_{vr}$. Therefore the lifetime of the excited state in the absence of a quencher is as illustrated in equation 3.18.

$$\tau_0 = \frac{1}{(k_e + k_{vr})}$$  \hspace{1cm} 3.18

When equation 3.18 is substituted in the Stern-Volmer equation, equation 3.19 is obtained.

$$K_{SV} = k_2 \tau_0$$  \hspace{1cm} 3.19

The Stern-Volmer constant $K_{SV}$ can be obtained from the slope after plotting $\frac{I_0}{I}$ vs $[Q]$. If $\tau_0$ is known, the bimolecular quenching constant $k_2$ can be obtained using equation 3.19. The bimolecular quenching constant is the constant for the actual reaction between the donor and quencher.

### 3.7 Fluorescence quantum yield.

#### 3.7.1 Introduction.

Fluorescence quantum yield is defined as the ratio of the photons absorbed to that of the photons emitted. This then particularly infer to deactivation of energy of photons by emission. Quantum yield can also be defined as presented in equation 3.20. Like any other measurements, inaccurate quantum yields are a very common occurrence. To avoid inaccurate $\Phi_F$, comparative methods are employed [Williams et al 1983, and Jobin Yvon Horiba Ltd].

$$\Phi_F = \frac{\text{Measured fluorescence lifetime, } \tau_F}{\text{Time const. for emission in the absence of competing process, } \tau_R}$$  \hspace{1cm} 3.20
Accurate comparative recording of quantum yields involves use of standards and test samples. The standard samples have known $\Phi_F$. The standards chosen for a particular test sample are those with same excitation wavelength as the test sample and also emit in the same region of the electromagnetic spectrum. When the same excitation wavelength is used, it is assumed that the same numbers of photons are absorbed by individual fluorophores within the samples under comparison. It is also very important that the measurements for test and STD samples are recorded under identical conditions including solvents in which they are dissolved. However, where the choice of solvents is different due to absorption properties of the samples, then their refractive indices, $\eta$, are utilised when evaluating the true values. Thus respective slopes (corrected fluorescence intensity vs absorbance) for the test sample and the STD sample are multiplied by squared refractive indices, $\eta^2$, of the solvents used in each case. Carefully optimised concentration ranges and calibrated instruments and standards are vital.

Concentrations that record absorbancies between 0.02 – 0.1 in a 1 cm cuvette are acceptable since they give linear response in measurements and linearity is a good indication of reliable results [Dhami et al 1995]. Lower concentrations within linear concentration range eliminate inner filter/re-absorption effects. Inner filter effects tend to give inaccurate quantum yields. Cuvettes used for holding solutions under test in this research were of 1 cm light path length. Other cuvette path lengths of 2.0 cm, 5.0 cm are available in the market and these longer path lengths cuvettes eliminate error to a great extent. In case these longer path length cuvettes are used then the maximum absorbance is recorded at 0.2 (for a 2.0 cm cuvette) and 0.5 (for a 5.0 cm cuvette).

In this regard, quantum yields, ($\Phi_F$) and life-times, tau ($\tau$), are important to note during evaluation of suitability of molecules as sensors. Interaction of pollutant molecules with the sensing molecules may result in changes in the quantum yield and life-times of the sensor molecule. Measured fluorescence life-times, $\tau_F$, is the excited state life-time which has been affected by such process as internal conversion (IC), intersystem crossing (ISC) to triplet state and interactions of the sensing molecule through charge transfer (CT) and spin-orbit couplings. Intrinsic
life-time, $\tau_R$ represent decay time without competing processes as mentioned above. Known quantum yields and measured fluorescence life-times can help evaluate intrinsic life-time using equation 3.20. Therefore the effect of competing processes to the sensor alone and the sensor in the presence of pollutants can be evaluated. Fluorophores exhibit reduction in quantum yields when they form complexes. Quantum yields are sensitive to the molecular environment including, pH, temperature and also changes to molecular structure which could be due to complex formation. Higher quantum yields are desirable in sensing and typical quantum yields for the commonly used fluorophores range from 0.05 – 1.0.

In order to evaluate the quantum yield of an analyte, the raw luminescence data for both the analyte and the STD are corrected (Equation 3.21). Using comparison method, [Jobin Yvon Horiba method, William et al 1983], a curve for corrected fluorescence intensity vs absorbance is then plotted. The ratio for the slope of test sample and the standard is obtained.

The ratio obtained, when multiplied by ratio of squared refractive indices for the solvents used for test analyte and standard gives the quantum yield for the test sample. Absolute quantum yield for the test sample is then obtained after multiplication with the known standard quantum yield.

$$F_{\text{corr}} \approx F_{\text{obs}} \text{anti} \log \left( \frac{OD_{\text{ex}} \times OD_{\text{em}}}{2} \right)$$

3.21

Where, $F_{\text{corr}}$ is corrected fluorescence, $F_{\text{obs}}$ is observed fluorescence and OD stands for optical density (absorbance), with subscripts ex and em standing for excitation and emission respectively. It is assumed that there is no $OD_{em}$.

In order to get the value for the $OD_{ex}$, Beer Lambert’s law expression (Equation 3.22) is employed.

$$\log_{10} \frac{I_0}{I} = OD$$

3.22

$OD = $ Optical density = Absorbance.
\( I_0 = \) incident radiant energy
\( I = \) radiant energy after absorbance

Quantum yield can be evaluated using **equation 3.23**.

\[
\phi_{F_{\text{test}}} = \phi_{F_{\text{std}}} \frac{\text{Gradient}_{\text{test}} \left(\eta_{\text{test solvent}}^2\right)}{\text{Gradient}_{\text{std}} \left(\eta_{\text{std solvent}}^2\right)}
\]

3.23

Where, the subscripts ‘test’ and ‘std’ refer to test and standard samples respectively while ‘\( \eta \)’ refer to refractive index of solvents.

Fluorescence quantum yield measurements for this research were only evaluated for CoP(ph-OMe)\textsubscript{4} and therefore the results are presented in (appendix 3).

### 3.8 Fluorescence lifetime spectroscopy.

#### 3.8.1 Introduction.

The technique employed during fluorescence lifetime measurements in this study is based on time correlated single photon counting (TCSPC). This is a statistical method of measuring lifetime and therefore when using TCSPC technique, highly repetitive light source is essential in order to facilitate accumulation of sufficient photons which is a prerequisite for statistical data exactitude. The principle underlying lifetime measurement based on TCSPC entail detection of single photons and measurement of their arrival time concomitant to a reference signal which is supplied by the excitation light from a pertinent light source. It is illustrated in the operation manual that the electronics of this process can be compared to a fast stopwatch with two inputs where the clock is started by a start signal pulse and stopped by a stop signal pulse (**Figure 3.11**), such that the time measured for one start-stop sequence is represented by the addition of one more count to a histogram as in **Figure 3.11** in which the channels on the x-axis represent the time and y-axis photon counts.
Figure 3.11. Diagram illustrating the principle of measurement for life times based on TCSPC (Reproduced from the operation instructions manual for Spectrofluorometer OB920 Edinburgh instruments)

The start or stop pulse is generated by one single emission photon. Photomultipliers are used as detectors in this kind of measurement since they have an intrinsic high gain. It is vital for statistical reasons that no more than one single photon event per light flash is detected since multi-photon events affect the histogram statistics which lead to erroneous measurement in the results. Thus a pulse pile up problem results from multi-photon events per light flash. To curb the problem of pulse pile up, the repetition rate of the pulse is put into consideration and consequently the photon rate kept low at 5% or less the pulse rate. The electronics governing the operation for TCSPC technique are programmed in such a way that at the input, incoming pulses are evaluated based on a threshold pulse height and only those higher than a set threshold are accepted for further signal processing and the smaller amplitude noise pulses are readily eliminated.

The signal processing in TCSPC technique is based on a list of main components namely: constant fraction discriminators (CFD), electrical delays (Del), Time-to-amplitude converter (TAC), Amplifier (Amp), Analogue-to-digital converter (ADC) and Digital memory (Mem). These main components are as illustrated in Figure 3.12.
Figure 3.12. Main components for signal processing in TCSPC.
(Reproduced from the operation instructions manual for Spectrofluorometer OB920 Edinburgh instruments)

The constant fraction discriminators on both start and stop input, analyses the pulse shapes of individual pulses. The portion of steepest slope of the initial edge on the incoming pulses, this portion of which is of the negative pulses is taken as criterion for temporal position and the specific position of the slope is depended on the fraction, CFD (which is also known as the shaping delay) and the zero crossing level. The threshold, fraction (CFD) and zero crossing depends on the type of detector used and therefore matching to an appropriate detector is vital.

At the output of CFD, the pulses are re-shaped to standard height and shape, delayed by an electronic shifting delay, Del, whose implementation will either result in a right or left shift of the entire lifetime measurement on the time axis. At the other ends of Dels is the TAC, which is a fast clock, started by the start pulse and stopped by the stop pulse. The amplifier, Amp, then amplifies the TAC output and effectively stretching the time axis. Thus minimum and maximum TAC amplitudes determine the time range. Then the ADC resolution determines how many discrete time values are possible. A collective of possible TAC pulse measured amplitudes are then allocated in different time bins and the width of the time bin is the ration of full time range to a resolution of the ADC in channels and will give the time resolution measured in either, picoseconds (ps) or nanoseconds (ns) channels. A detailed explanation of mechanism of the electronics for TCSPC technique is beyond the scope of this thesis.
3.8.2 Radiant energy sources: Flash lamps.

Lamps used in fluorescence lifetime measurements vary depending on the gas inside. The type of gas determines the spectral distribution output. It is cited in literature (Edinburgh instruments user manual for OB920 Spectrometer and Lakowikz et al 1994) that hydrogen lamps are the best for use in lifetime measurements due to lack of tailing which would otherwise interfere with the output signal.

There are two modes of operation for these lamps, namely:

- Free running mode
- Gated mode

Gated mode is the most popular in lifetime measurements and its mechanism is presented in section 3.5.2.1. Free running mode mechanism is not discussed here.

3.8.2.1 Gated lamps.

In gated lamps, the discharge is controlled by a thyatron tube. This allows the gate to operate during the laser pulse only therefore events outside laser pulse are suppressed. In OB920 fluorescence lifetime spectrometer used in this study, the thyatron facilitates high intensity, reproducibility and repeatability of each pulse at the rate of 50 kHz. This ensures a uniform intensity profile for the entire spectral distribution output for the lamp. This lamp can be operated with many gases or mixtures of gases. Common in use are pure hydrogen (H₂) and nitrogen (N₂). In this study, hydrogen gas was used.

In this type of set up, the frequency of the lamp is independent of the capacitance, gas and pressure of gas. Therefore, the pressure and gas can be conveniently changed. The pulse rate and stability depends on the electrodes (Figure 3.13).
It is therefore vital to ensure that the electrodes are thoroughly cleaned using a recommended method by the manufacturer and properly aligned with the optical axis in the instrument. Proper cleaning and alignment prevents degradation of pulse shape. The discharge chamber of the lamp is under vacuum sealed with O-rings and it is insulated using pyrex. Vacuum couplings connect the discharge chamber to gas handling system and a fibre optic interfaces the discharge chamber to the synchronisation photomultiplier. This lamp is operated at a high voltage of 8 kV from its power supply.

### 3.8.3 Acquisition of lifetime data.

In this study, hydrogen gas was used to produce pulsed sparks from the electrodes aligned in the optical path. The pulses provide a continuum, which is spectrum of H$_2$ gas. The energy in this region of the EMS matching that of the excited states of the test sample is used to excite the test sample molecules to higher energy levels. Photons are then counted with time as the test sample decays to ground state.

Parameters that have to be set for the hydrogen gas include:

- The frequency of the lamp pulses; 40 MHz
The gap between a flat electrode and a pointed electrode; 1 mm which is set using a filler gauge.

Voltage for photomultiplier detector; 6.82 ETV.

Pressure of 0.40 bar.

The bulk of the system being measured for life times has several decays with different exponentials. A representative decay parameter for the sample within the system has to be isolated known as the sample decay model, \( R(t) \) using software supplied with the instrument for this purpose. The accuracy of the sample decay model depends on how a mathematical fit is performed. A tail fit with minimum chi-square tends closer to the true value of the decay time of the sample. The tail fit is performed using a software program which is nF900 for time-decay measurements, on OB920 fluorescence lifetime spectrometer. Another alternative way for evaluating the data is through reconvolution using the same software. Lifetimes can also be evaluated manually by a plot of the logarithm of the number of photon counts vs time taken for acquisition of raw data. In the latter, the slope of the curve gives \( R(t) = -\frac{1}{\tau} \) and when tau, \( \tau' \) is evaluated, it gives the lifetime of the test sample. The best value can be verified by comparison with standards and literature. In this study, zinc phthalocyanine (ZnPc) was used as a comparison standard for CoP(ph-OMe)₄.

Experimental work for this study is presented in chapters; 4 (solution study) and 5 (solid thin films study). For solid thin films study, interface of the sensor platform to the analytical instrument as highlighted in section 2.13 is as illustrated in section 3.8.4 and Figure 3.14.

3.8.4 Interface of sensor platform to spectrophotometer.

As mentioned in chapter 2, in optical fibre chemical sensing technique, to facilitate transmission of appropriate radiant energy to a remotely placed sensor platform, interface to the analytical instrument is vital. This interface is achieved by use of the optical fibre. The optical fibre transmits the radiant energy thereby connecting the remote sensor platform to the instrument, the interfaced computer and
analytical software. Thus the instrument, as in the case of LS 55 spectrofluorimeter used in this study, has designed slots for a quick-fit of the optical fibre. This kind of interface can be illustrated diagrammatically as in **Figure 3.14**.

![Figure 3.14. Signal processing in a fibre optical chemical sensor.](image)

The layout in **Figure 3.14** illustrates interface of the sensor platform through the optical fibre to the analytical instrument. These interfaces can facilitate remote sensing.
Chapter 4: Solution study.

4.1 Chapter introduction.

Instrumental spectroscopic methods of analysis were employed in this study. These solutions were held in cuvettes like those highlighted in section 3.1 of chapter 3. Organic pollutant molecules and CoP(ph-OMe)$_4$ are sparingly soluble in water and therefore dichloromethane was used as a solvent for them when evaluating the viability of CoP(ph-OMe)$_4$ as a sensor. On the other hand, FLXN is very soluble in water and therefore distilled water was used as its solvent. Conversely, when evaluating the suitability of FLXN as their sensor, these organic pollutants were also dissolved in distilled water and filtered. All the solution study experiments were carried out at ambient temperature and pressure conditions within the analytical laboratory.

Using hazardous chemicals at work place can put peoples’ health at risk, causing diseases including; dermatitis, cancer and asthma. Therefore before ordering any chemicals for this study, COSHH assessment was done. It is the evaluation of health risks the chemical can pose and hence precautionary measures taken when working with such chemicals. Thus COSHH stands for Control Of Substances Hazardous to Health.

4.2 Apparatus, chemicals and instruments for solution study.

Anthracene, benzo(a)pyrene, dichloromethane, pyrene and pyridine were purchased from Sigma-Aldrich while CoP(ph-OMe)$_4$, FLXN were sourced from Fisher-Scientific. These reagents were of analytical grade with purity ranging from 80 to 99 % and therefore used as purchased without further purification. Only in cases of reagents, compensation for 100 % purity was performed through calculations (equation 4.2).
The apparatus used were readily available in the laboratory or purchased as required and these included; volumetric flasks for preparation of stock solutions, micropipettes and tits for siphoning and delivery of liquids and solutions in micro volumes, weighing crucibles for holding reagents to be weighed and small glass vials for dilutions and or mixing reagents, weighing micro-balances for taking weights of reagents, cuvettes for holding solutions in the sample compartment of the instrument for measurement.

Instruments used in this study were Shimadzu UV-VIS PC spectrophotometer for absorbance and LS 55 Perkin-Elmer fluorometer for fluorescence measurements. Lifetime measurements were performed on lifetime spectrophotometer OB920 of Edinburgh instruments Ltd.

4.3 Method development and experiments.

Known concentrations of solutions for the sensing molecules and those of pollutant molecules were prepared as stocks in appropriate solvents and then dilutions made from them to µM or nM concentrations depending on their molar absorptivities; thus absorption capacity of a given molecule. Absorption measurements are carried out within a scale of signals ranging from 0.0 to 1.0, because absorbance is a logarithmic ratio of the incident radiant energy to that of transmitted energy. Mostly recommended for linear calibration curves are measurements done within the signals ranging from 0.005 to 0.5 so that deviations from the limiting Beer-Lambert’s law are contained. Solutions for this study were prepared and a series of measurements done however, to establish absorbance within 0.01 to 0.8 absorption ranges. This range gave a linear calibration plot. The aim was to establish the highest concentration of the sensing molecule that can be fixed as the concentration for the pollutants are varied in order to monitor any changes to the signal of the sensor molecule. Conversely, fluorescence measurements are carried out within the emission signal scale ranging from 0.0 to 1000.
4.3.1 Experiments.

Seven replica measurements were carried out on same concentrations for the sensor molecules alone and sensor molecules with the pollutants so as to establish repeatability of measurements. This approach gives representative data for evaluation and conclusions.

4.3.2 Absorption measurements.

In this study when evaluating CoP(ph-OMe)$_4$ as a sensor for the pollutants, dichloromethane (CH$_2$Cl$_2$) was used as a solvent for both this sensing molecule and the organic pollutants. While for measurements with FLXN and the pollutants, the solutions were prepared in distilled water.

4.3.3 Absorption measurements for the pollutants.

Also measurements for the absorption spectra for the organic pollutants of this research were performed. **Figures 4.1, 4.2, 4.3 and 4.4** are absorption spectra for ANTH, BaP, PRN and Py respectively.

![Figure 4.1. Absorption spectrum for anthracene in CH$_2$Cl$_2$.](image)
As illustrated in figure 4.1, ANTH absorbs strongly between 290 and 390 nm with 6 vibrational energy band levels. It has a high absorption coefficient of about $7 \times 10^6$ L mol$^{-1}$ cm$^{-1}$ and exists as greyish solid crystals.

![Absorption spectrum for 0.1 µM BaP in CH$_2$Cl$_2$.](image)

The appearance of BaP is yellow solid crystals. It absorbs strongly in the UV-VIS region of the electromagnetic with high molar absorptivities; $4.3 \times 10^5$ L mol$^{-1}$ cm$^{-1}$.

![Absorption spectrum for 0.2 µM pyrene in CH$_2$Cl$_2$.](image)
Pyrene strongly absorbs in the UV-VIS region of the electromagnetic spectrum as illustrated in Figure 4.3. It has a high absorption coefficient, of $7.5 \times 10^6 \text{ L mol}^{-1} \text{ cm}^{-1}$ and the crystals are greyish in colour.

![Graph showing absorbance vs wavelength](image)

Figure 4.4. Absorption spectrum for 0.24 µM pyridine in CH$_2$Cl$_2$.

Pyridine exists as colourless liquid and absorbs strongly with molar absorptivity $1.75 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$, of in the UV-region of the electromagnetic spectrum (Figure 4.4).

### 4.3.4 Absorption measurements for CoP(ph-OMe)$_4$.

Initially measurements for the sensor molecule alone were performed to ascertain its reproducible signal at concentrations ranging from 0.01 µM to 4 µM and also as a standard to reveal any changes after interaction with the pollutant molecules. Stock solution of CoP(ph-OMe)$_4$ was prepared in a 50 ml volumetric flask and from this stock solution, appropriate dilutions of known concentrations were made.

Before preparing the stock solutions, the purity for CoP(ph-OMe)$_4$ was compensated for by adjusting the weight of the calculated amount, $W_{\text{calc}}$ for the
desired concentration of the stock solution to that actual weight, $W_{\text{actual}}$ which would reflect 100 % purity for CoP(ph-OMe)$_4$ (equation 4.1). Instead this sensing molecule is available at 80 % purity.

$$W_{\text{actual}} = W_{\text{calc}} \left( \frac{100\%}{80\%} \right)$$  \hspace{1cm} 4.1

When equation 4.1 is simplified, equation 4.2 as shown below can be derived:

$$W_{\text{actual}} = 1.25(W_{\text{calc}})$$  \hspace{1cm} 4.2

From the above formulation, instead of weighing the originally calculated amount ($W_{\text{calc}}$), the actual compensated 100 % purity weight ($W_{\text{actual}}$) which is 1.25 times the originally calculated weight for the desired concentration was weighed. The ($W_{\text{actual}}$) was then dissolved in a volumetric flask as stated above to give the desired concentration of the stock solution. From the stock solution, volumes for dilutions as desired were evaluated using equation 4.3 and prepared accordingly to give a range of µM dilute solutions required.

$$C_1V_1 = C_2V_2$$  \hspace{1cm} 4.3

Where $C$ and $V$ stands for concentration and volume respectively.

Using equation 4.2, a stock solution containing 11.63 mg (0.01163 g) instead of 9.3 mg (0.0093 g) was prepared and this solution was an equivalent of $1.47 \times 10^{-5}$ moles for CoP(ph-OMe)$_4$ which has a formula weight of 791.77 g/mole.

A set of dilutions were made by pipetting aliquots of known µ-volume from the stock solution into a spotlessly clean cuvette containing 2.5 ml of the solvent used in dissolving the reagents. Same amount in aliquots were added in succession as measurements were being performed. This ensured that the concentration
was increasing in a certain given magnitude. To curb any solvent evaporation, the cuvette has a tight fitting stopper (lid). Every time the amount in volume of 5 µL from the stock solution were added to the cuvette, thorough mixing was performed by shaking the stoppered cuvette, then cleaning its external surface of any specs of dirt before reintroducing into the sample compartment of the instrument for measurement. From such dilutions, 0.06, 0.08, 0.1, 0.2 and 0.4 µM for CoP(ph-OMe)$_4$ initially were achieved. **Figure 4.5** represents the absorption spectra for CoP(ph-OMe)$_4$ alone from these solutions. As observed in **Figure 4.5**, this sensing molecule has a main absorption band at 414 nm called the soret-band which is a kind of absorption band that appears typically between 400 nm to 500 nm. The Q-band which typically appears between 500 nm to 800 nm is at 530 nm for CoP(ph-OMe)$_4$.

![Absorption Spectra](image)

**Figure 4.5.** Absorption spectra for CoP(ph-OMe)$_4$ dissolved in CH$_2$Cl$_2$.

From the spectra in **Figure 4.5**, a calibration plot for CoP(ph-OMe)$_4$ as illustrated in **Figure 4.2** was obtained.
The calibration plot in Figure 4.6 has shown linearity as the concentration for CoP(ph-OMe)$_4$ increases in the solution. A straight calibration plot may indicate that there was no possibility of aggregation or decomposition of this sensing molecule due to interaction with the radiant energy. This shows that there is repeatability; a quality that is required for sensing molecules.

Subsequent measurements of this sensing molecule with the organic pollutants entailed dissolutions in dichloromethane (CH$_2$Cl$_2$) as highlighted earlier. All the organic pollutants selected for this study are completely soluble in CH$_2$Cl$_2$. In Figures 4.7, 4.8, 4.9 and 4.10, are representations of the absorption spectra for CoP(ph-OMe)$_4$ solution in CH$_2$Cl$_2$ when this sensing molecule interacted with anthracene, benzo(a)pyrene, pyrene and pyridine respectively.

![Figure 4.6. Calibration plots for absorption vs [CoP(ph-OMe)$_4$] μM solution in CH$_2$Cl$_2$.](image)
In Figure 4.7, it can be observed that the major absorption peak at 414 nm for the sensing molecule CoP(ph-OMe)₄, decrease in absorption intensity with increasing concentration for anthracene. There is also a shift of 12 nm (414 nm to 426 nm) towards the red end of the electromagnetic spectrum. A shoulder peak forms at 465 nm which increases in absorption intensity as the concentration for anthracene increases in solution recorded with fixed concentration of this sensing molecule. Isosbestic points are observed at 446 nm and 496 nm. Also observed is formation of a broad absorption band between 580 nm and 745 nm with absorption $\lambda_{\text{max}}$ at 654 nm. This broad band increases in absorbance as the anthracene concentration is increased at fixed concentration of CoP(ph-OMe)₄.

Figures 4.8 and 4.9 represent absorption spectra for CoP(ph-OMe)₄ after interaction with BaP and PRN respectively.
As illustrated in **Figure 4.8**, BaP interacts with CoP(ph-OMe)$_4$, and reduces intensity of its major absorption peak at 414 nm, but there is no observed shift in the peak position. Conversely, after PRN interaction with this same sensing molecule shifts the major absorption peak at 414 nm with a magnitude of 6 nm towards the red end of the electromagnetic spectrum (414 to 420 nm).
Presented below in Figures 4.10 and 4.11, are spectra showing the way pyridine interacts with CoP(ph-OMe)₄.

Figure 4.10. Absorption spectra for fixed 0.02 µM [CoP(ph-OMe)₄] with increasing µM [pyridine] in CH₂Cl₂ (0 - 1.2 µM).

Figure 4.11. Absorption spectrum for fixed 0.02 µM CoP(ph-OMe)₄ with increasing pyridine concentration illustrating new peaks at 438, 553 and 594 nm (spectrum for 1.2 µM Py extracted from Figure 4.10 above).

In Figure 4.10, the reduction in absorbance at 414 nm and formation of new peak at 438 nm are elaborative while new absorption bands formed towards the red end of the electromagnetic spectrum which were eliminated in this spectrum for clarity are illustrated in Figure 4.11. These bands at 553 nm and 594 nm were very low in intensity if represented in Figure 4.10 and therefore isolated and presented in Figure 4.11 for clarity.
Following those absorption spectra illustrated above are their calibration plots. **Figure 4.12** represents a calibration plot for decreasing absorption intensity at the major absorption peak of the sensing molecule as the concentration for anthracene increases.

![Figure 4.12](image1.jpg)

**Figure 4.12.** Calibration plot at 414 nm for interaction of fixed 0.006 µM of the sensing molecule CoP(ph-OMe)$_4$ with increasing concentration for anthracene in µM.

The calibration plot relating to the results for interactions of BaP with CoP(ph-OMe)$_4$ are represented **Figure 4.13**. In this plot, it is also shown that a new peak is formed at 405 nm and increases in its absorption intensity as the concentration for BaP increases in a fixed 0.324 µM CoP(ph-OMe)$_4$ solution.

![Figure 4.13](image2.jpg)

**Figure 4.13.** Absorption calibration plots for increasing concentration for benzo(a)pyrene with fixed concentration 0.324 µM for CoP(ph-OMe)$_4$. 
**Figure 4.14** represents plot for the results after interaction of PRN with CoP(ph-OMe)$_4$.

![Absorption calibration plot for increasing concentration for pyrene with fixed concentration 0.05 µM CoP(ph-OMe)$_4$.](image)

Pyrene akin to other two PAHs (ANTH and BaP) of this study also reduces the absorbance of the sensing molecule as they interact in solution. The effect is as represented in absorption spectrum and its calibration plot. After interaction with this sensing molecule, PRN also forms a low intensity broad band between 580 nm to 750 nm with absorption maximum at 632 nm. It is also observed after interaction of PRN with CoP(ph-OMe)$_4$, that the major absorption band for the sensing molecule shifts from 414 nm to 420 nm which is a difference of 6 nm (Energy = $3.32 \times 10^{-17}$ J). Conversely, when Py interact with CoP(ph-OMe)$_4$ as represented in **Figure 4.15**, reduction at the major absorption wavelength of 414 nm for this sensing molecule and subsequent formation of a new absorption wavelength at 438 nm are observed. This occurrence gives a shift of 24 nm (Energy = $8.29 \times 10^{-18}$ J) in wavelength towards the red end of the electromagnetic spectrum. Other additional absorption bands after interaction of Py with CoP(ph-OMe)$_4$, formed at 553 nm and 594 nm were presented earlier in this section. The plot in **Figure 4.15** represents the calibration for interaction of CoP(ph-OMe)$_4$ with Py. In this figure, only the major absorption band for sensing molecule alongside the major absorption peak formed after interaction of this sensor with Py are illustrated but not the minor peaks as explained earlier.
Interaction of Py with CoP(ph-OMe)$_4$ is envisioned to be through adduct formation and axial coordination to the central metal ion, Co$^{2+}$. Axial coordination is made possible between Py and CoP(ph-OMe)$_4$ due to availability of electron-acceptor d$\pi$-orbitals of the central metal ion Co$^{2+}$, in the latter and n-electrons pair donated by the former. Axial ligation of Py to CoP(ph-OMe)$_4$ is envisioned as presented in chapter 2 section 2.5. Adduct formation is a possibility due to the presence of conjugated bonds in both the sensing molecules and the pollutant molecules that readily make available dienes and dienophiles (chapter 2 section 2.5). On the other hand, the interaction of these PAHs with CoP(ph-OMe)$_4$ is envisaged to be through adduct formation or through exciton or phonon couplings. In this case, the envisioned product of adduct formation is as presented in section 2.5 of chapter 2 exemplifying interaction with one of the enlisted PAHs known as ANTH.

The phenomenon observed when PAHs and Py reduce or enhance the absorption intensity of the sensing molecule and subsequent formation of new absorption peaks may be indicative of complex formation [D’Souza et al 2005] and [Rodriquez et al 2002]. Splitting of peaks or formation of new soret or B-band peak at 438 nm from 414 nm and formation of two broad, weak Q-band peaks at 553 nm, 594 nm towards the red end of the electromagnetic spectrum.
after Py interacts with the sensing molecule is due to co-ordination to the central metal ion Co$^{2+}$ [Mamothibe et al 2001]. Co-ordination of Py to the central metal ion Co$^{2+}$ in porphyrin reduces HOMO energy gap and hence a low energy shift for B- and Q-bands towards the red end of the electromagnetic spectrum is observed. Changes in intensities of the electromagnetic spectrum so observed and shifting of spectral bands are due to perturbation of (Lowly Unoccupied Molecular Orbital (LUMO) and Highly Occupied Molecular Orbital (HOMO); [Vinodu et al 2001]. Examples of such perturbations of which may be:

- Structural deformation after interaction with another molecule.
- A tilt of the substituent groups at the periphery of porphyrin.

Structural deformation normally decrease $e_g(\pi^*)\rightarrow d\pi$ orbital interaction which may result in decrease in the LUMO energy of the metalloporphyrin molecule. Conversely, a tilt in the substituent groups at the periphery of porphyrinato macro-molecule may cause delocalisation of electronic charges towards the porphyrin ring. More electrons towards the ring system of porphyrins or metalloporphyrin results in an decrease in the energy for HOMO of the porphyrin. On the whole, these effects reduce HOMO→LUMO energy gap and therefore a shift towards the red end of the electromagnetic spectrum for B and Q absorption bands is observed.

4.3.5 Absorption measurements for FLXN.

The same procedure as applied for CoP(ph-OMe)$_4$ was used for establishing absorption pattern for FLXN. This included; establishment of linear concentration range for FLXN, preparation of stock solutions by first compensating for percent purity and subsequent dilutions to low µM concentrations, using same equations as applied in preparation for those of CoP(ph-OMe)$_4$ in section 4.3.3 above. The only difference is that, these solutions were prepared and diluted using distilled water because FLXN dissolves in water and not the CH$_2$Cl$_2$ used for experiments of CoP(ph-OMe)$_4$. 
Employing similar procedure as in section 4.3.4, concentrations ranging from 8 to 40 µM for FLXN were prepared and measured. The absorption spectra for FLXN alone are as presented in Figure 4.16. The calibration plot for this concentration range is as presented in Figure 4.17.

Figure 4.16. Absorption spectra for FLXN alone dissolved in water.

As it can be observed in Figure 4.16, FLXN has a main absorption band at 494 nm and minor ones at 284 and 232 nm. The calibration plot for the peak at 494 nm is as presented in Figure 4.17.
The molar absorptivity for FLXN as calculated from measurements of this research, range from $10^3 – 10^4$ L M$^{-1}$ cm$^{-1}$.

Similarly, in this experiment of FLXN measurements with the organic pollutants followed the same method as highlighted in section 4.3.3 above for CoP(ph-OMe)$_4$. Fixed concentration of 0.026 µM for FLXN was made in a cuvette containing 2.5 ml of distilled water and absorption measurements carried out for FLXN alone and subsequently with added and increasing aliquot volumes from stock solutions of organic pollutants initially prepared in mg/100 mL of distilled water before filtering them using watman filter paper. These amounts initially pipetted µl and later converted in ml are as presented in Table 4.1.

Molecular weights for the pollutants are; ANTH 178.21 g/mol, BaP 252.31 g/mol, PRN 202.25 g/mol and py 79.1 g/mol.
Table 4.1: Amount of pollutants in stock solutions and the volumes pipetted from them for dilutions to be measured.

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Initial amount</th>
<th>Amount of volume converted from µl into ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTH</td>
<td>0.61 (mg)</td>
<td>0.02, 0.04, 0.06, 0.08, 0.2, 0.3, 0.4, 0.6, 0.8 and 1.0</td>
</tr>
<tr>
<td>BaP</td>
<td>0.439 (mg)</td>
<td>0.01, 0.02, 0.07, 0.12, 0.17, 0.22, 0.27, 0.37, 0.47, 0.67, 0.87, 1.07 and 1.27</td>
</tr>
<tr>
<td>PRN</td>
<td>0.459 (mg)</td>
<td>0.01, 0.03, 0.05, 0.07, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0</td>
</tr>
<tr>
<td>Py</td>
<td>0.5 (ml)</td>
<td>0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1</td>
</tr>
</tbody>
</table>

Figures 4.18, 4.19, 4.20 and 4.21 represent absorption spectra for a fixed concentration for FLXN with varying concentrations for ANTH, BaP, PRN and Py respectively.

Figure 4.18. Absorption spectra for fixed 0.026 µM FLXN with increasing amount of ANTH in ml at 494 nm.
Figure 4.19. Absorption spectra for fixed 0.026 µM FLXN with increasing amount of BaP in ml.

Figure 4.20. Absorption spectra for fixed 0.026 µM FLXN with increasing amount for PRN in ml.
Figure 4.21. Absorption spectra for fixed 0.026 µM FLXN with increasing amount for Py in ml.

Enlargements of spectra in Figure 4.21 which represents results after interaction of Py with FLXN between 220 nm to 270 nm and that between 420 nm and 520 nm are illustrated in Figures 4.22 and 4.23 respectively.

Figure 4.22. An enlargement for spectrum in Figure 4.21 of the absorption bands between 220 -270 nm for increasing [Py] in ml with fixed 0.0264 µM FLXN.

The spectrum for Py alone appears as represented in, section 4.3.3 of this chapter.
It is worth noting that, when dienes and dienophiles form adducts, the original shapes of their absorption spectra prior to the association are similar to the resultant spectra after interaction. This is because, the molecules involved have their structures and the electronic configurations preserved. This phenomenon may be applicable to the resultant spectra after interaction of organic pollutants and sensing molecules in this research. Figures 4.24, 4.25, 4.26 and 4.27, that follow here below are calibration plots after interaction of FLXN with the organic pollutants.

The plot in Figure 4.24 illustrates reduction at the major absorption peak for a fixed 0.026 µM FLXN with increasing amount of ANTH in ml. Prior to addition of ANTH aliquots, the signal for the sensing reagent increased in intensity as its
concentration increased in solution. As ANTH is added and the concentration increased in solution, the absorbance signal for FLXN decreased in intensity. The next Figure 4.25 illustrates interaction of FLXN with benzo(a)pyrene.

Figure 4.25. Absorption calibration plot for fixed 0.026 µM [FLXN] in solution with increasing [BaP] in ml at 494 and 234 nm peaks.

Figure 4.25 illustrates how BaP after interaction with FLXN reduces the absorption intensity at the major absorption peak 494 nm. It is also shown that the peak at 234 nm reduces in absorption intensity as the concentration for BaP increase in solution. The next illustration bellow is a calibration plot for interaction of PRN with sensing molecule FLXN.

Figure 4.26. Absorption calibration plot for fixed 0.026 µM [FLXN] with increasing [PRN] in ml at 494 nm absorption peak.

Pyrene, as illustrated in Figure 4.26 reduces the main absorption peak for FLXN at 494 nm. It is also observed that the peak at 237 nm reduces in absorption
intensity on interaction of pyrene with the sensing molecule FLXN. **Figure 4.27** below illustrates calibration plot for interaction of sensing molecule FLXN with Py.

![Figure 4.27](image.png)

**Figure 4.27.** Absorption calibration plot for fixed 0.0264 µM [FLXN] in solution with increasing [Py] in ml at 494 nm absorption peak.

The calibration plot in **Figure 4.27** exemplify increasing absorption intensity at the major absorption peak at 494 nm for fixed 0.026 µM [FLXN] with increasing amount for Py in ml. It is also illustrated that the absorption intensity between 220 nm to 270 nm in increasing with increase in Py concentration. This may point at the possibility of adduct formation and hydrogen bonding which is in line with explanation in the previous paragraph about preservation of original spectra for the associating molecules when adducts are formed. Polycyclic aromatic hydrocarbons (ANTH, BaP and PRN) caused decrease in absorbance for the sensing molecule FLXN at 237 nm peak, which lies between 220 nm – 270 nm, but in the presence of pyridine, this same peak increased in intensity. Interaction of organic pollutants with the sensing molecule FLXN is envisaged to be through adduct formation or through hydrogen bonding as represented in (chapter 2 section 2.8). This schematic in that section is exemplifying interaction of FLXN with Py through hydrogen bonding. Evidence of hydrogen bonding in pyridine has been published in literature [Nibu et al 2006]. Nibu and co-workers proved from their IR studies that pyridine’s N-atom can be protonated. The source of the proton during their investigations was hydrogen fluoride (HF).
4.3.6 Fluorescence solutions study.

4.3.6.1 Introduction.

Dilutions of these solutions used for fluorescence measurements were prepared as mentioned in earlier paragraphs. Fluorescence measurements were carried out on Perkin Elmer LS 55 fluorometer. The excitation and emission bandwidth were set at 15 nm and 20 nm respectively. Wider bandwidth minimises Rayleigh and Raman scattering.

4.3.6.2 CoP(ph-OMe)$_4$ with pollutants.

This sensing molecule (CoP(ph-OMe)$_4$, has a main emission peak at $\lambda_{em}$ 659 nm originating from $S_{e(1)}$$\rightarrow$$S_{g(0)}$ transition and a small one at $\lambda_{em}$ 723 nm originating from $S_{e(1)}$$\rightarrow$$S_{g(1)}$ transition. The excited states leading to those emissions resulted from the excitation $\lambda_{ex}$ 423 nm of $S_{g(0)}$$\rightarrow$$S_{e(2)}$ transitions (Scheme 4.1). The emissions originated from $S_{e(1)}$$\rightarrow$$S_{g(0)}$ & $S_{e(1)}$$\rightarrow$$S_{g(1)}$ because the energy from $S_{e(2)}$$\rightarrow$$S_{e(1)}$ dissipates rapidly through internal conversion (IC) since the molecule is quite unstable and only spends a very short time at $S_2$ vibrational energy level. This energy so dissipated rapidly is called vibrational energy. Scheme 4.1 below illustrates the above enlisted transitions.

![Scheme 4.1](image)

Scheme 4.1. Illustrating excitation and fluorescence spectra for CoP(ph-OMe)$_4$. 
Figures 4.28, 4.29, 4.30 and 4.31 are emission spectra for the sensing molecule CoP(ph-OMe)₄, after interaction with ANTH, BaP, PRN and Py and their calibration plots are represented in Figures 4.33, 4.34, 4.35 and 4.36 respectively. It is also noted that, apart from reducing the emission spectra for the sensing molecule, the PAHs also formed Sensor-PAH exciplex between 700 to 900 nm. These exciplex peaks get enhancement in fluorescence intensity as the concentration for PAHs increase in a fixed concentration of CoP(ph-OMe)₄. This phenomenon is presented in Figures 4.37, 4.38 and 4.39 after the pollutants; ANTH, BaP and PRN respectively interact with CoP(ph-OMe)₄. Such exciplexes formed after interactions of PAHs with polychlorobenzenes in cyclohexane have been published in literature [Grosso et al 1998]. Grosso and co-workers reported that PAHs were electron donors while polychlorobenzenes electron acceptors. Also reported in literature by [Sherifian et al 1992] are exciplexes formed between PAHs and substituted triphenylamines. The substituted triphenylamines acted as donors while the PAHs as acceptors. Sherifian and company also cited that PAHs are both electron donors and electron acceptors. The work of the two research groups, Grosso and co-workers and Sherifian and co-workers both reported fluorescence quenching of PAHs emission intensities.

Figure 4.28. Fluorescence spectra for fixed 0.02 μM CoP(ph-OMe)₄ with increasing concentration for ANTH (0.0 to 0.06 μM) in CH₂Cl₂.
Figure 4.29. Fluorescence for fixed 0.07 μM CoP(ph-OMe)_4 with increasing concentration of BaP (0.0 to 0.07 μM) in CH₂Cl₂.

Figure 4.30. Fluorescence for fixed 0.1 μM CoP(ph-OMe)_4 with increasing concentration of PRN (0.0 to 0.05 μM) in CH₂Cl₂.

Figure 4.31. Fluorescence spectra for fixed 0.052 μM CoP(ph-OMe)_4 with increasing concentration for Py (0 – 0.02 μM) in CH₂Cl₂. There is initial enhancement in fluorescence and subsequently diminish in fluorescence intensity.
Figure 4.31 represents fluorescence spectrum for increasing concentration of Py in the solution of fixed [CoP(ph-OMe)₄] while Figure 4.32 is the absorption spectrum measured of this resulting solution after fluorescence measurements for CoP(ph-OMe)₄ with 0.02 µM concentration for Py. This spectrum indicates formation of a new complex with absorption bands at 438, 553 and 594 nm, similar to the one formed during absorption measurements (section 4.3.3) due to the interaction of Py with this sensing molecule. The absorption intensity of this complex (0.25 au) in comparison with the intensity resulting from 0.052 µM concentration of the sensing molecule alone as per absorption spectra of section 4.3.3 without Py (0.5 au). This reveals a 50 % reduction in fluorescence intensity of the sensing molecule. When this 50 % reduction in absorption intensity is related to the fact that absorbance of a given analyte is directly proportional to its concentration (refer to Beer-Lambert’s law), then it may imply that a quantity yield of 50 % of a new complex is possibly achieved. It follows that this complex formed after the solution of CoP(ph-OMe)₄ with Py has been exposed to energy during fluorescence measurement has new peaks characteristic to the spectrum of a separate aliquot of the same solution which had only absorption measurements done earlier on. The new peaks are illustrated on the spectrum of Figure 4.32. Usually CoP(ph-OMe)₄ alone has absorption peaks at 335 nm, 414 nm and 530 nm. Therefore the absorption spectrum of the resultant solution formed after interaction of CoP(ph-OMe)₄ with Py after fluorescence measurements may indicate the presence of Cobalt metalloporphyrinato-pyridyl complex.
Figure 4.32. Absorption spectrum for the solution of CoP(ph-OMe)$_4$ with Py after fluorescence measurements
(The complex formed has similar spectrum to that formed during absorption measurements performed earlier.)

The emission peaks of the sensing molecule CoP(ph-OMe)$_4$ are still observed at 659 nm and 723 nm but only reduce or increase in intensity as aliquots of organic pollutants are added to the fixed concentration of this sensing molecule. Reduction and increase in fluorescence intensity after interaction of the sensing molecule with the pollutants is as illustrated in the plots in Figures 4.33, 4.34, 4.35 and 4.36 for ANTH, BaP, PRN and Py respectively.

Figure 4.33. Fluorescence calibration plot for interaction of fixed 0.02 µM for CoP(ph-OMe)$_4$ with increasing concentration for ANTH.

As illustrated in these plots, polycyclic aromatic hydrocarbons (PAHs) upon interaction with CoP(ph-OMe)$_4$ cause reduction or enhancement at 659 nm and 723 nm peaks of the sensing molecule. Interaction of the sensing molecule with
ANTH enhanced the emission peaks while with BaP and PRN reduced the emission intensities.

Figure 4.34. A calibration plot illustrating changing in fluorescence intensity for fixed 0.07 μM CoP(ph-OMe)$_4$ with increasing BaP concentration at 660 nm in CH$_2$Cl$_2$.

Figure 4.35. A calibration plot for fluorescence data of fixed 0.07 μM CoP(ph-OMe)$_4$ with increasing concentration for Py (0.0 to 0.05 μM) in CH$_2$Cl$_2$. 
Figure 4.36. A calibration plot illustrating fluorescence intensity on interaction of fixed 0.07 μM CoP(ph-OMe)_4 with increasing concentration for pyridine. Initially there is enhancement then subsequent diminishing intensity as the concentration for Py increases.

The calibration plot for Py illustrates initial increase and subsequently reduction in fluorescence intensity as the concentration of Py increases in the sensing matrix. Initial enhancement in fluorescence intensity as Py interact with the sensing molecule may be interpreted as first adsorption of pyridine within the cavity of the sensing molecule at the central metal ion Co^{2+} followed by co-ordination of the first pyridine molecule to the central metal ion of the porphyrinato complex and then quenching as the second pyridine molecule co-ordinates and the concentration for the new complex increases, which subsequently reduces the fluorescence intensity of the free metalloporphyrin. The possibility of complex formation between CoP(ph-OMe)_4 has been illustrated by the absorption spectrum of the resulting solution of CoP(ph-OMe)_4 with Py after fluorescence measurements (section 4.5.2). With increasing concentration for PAHs, there is also increase in the intensity of the major and minor exciplexes peaks at, 320, 816 and 865 nm for ANTH, BaP and PRN respectively after interaction with CoP(ph-OMe)_4. Exciplex formation is observed when λ_{ex} 418 nm wavelength is used instead of λ_{ex} 423 nm and it can also be observed that using 418 nm for excitation, all the three PAHs reduced the fluorescence intensities for this sensing molecule at 659 nm and 723 nm. These exciplax peaks are as presented in Figures 4.37, 4.38 and 4.39 for ANTH, BaP and PRN respectively.
Figure 4.37. Exciplex fluorescence spectra for fixed 0.02 µM CoP(ph-OMe)$_4$ with increasing concentration of ANTH (0.0 to 0.06 µM) in CH$_2$Cl$_2$.

Figure 4.38. Exciplex fluorescence spectra for fixed 0.07 µM CoP(ph-OMe)$_4$ with increasing concentration for BaP (0.0 to 0.07 µM) in CH$_2$Cl$_2$.

Figure 4.39. Exciplex fluorescence spectra for fixed 0.001 µM CoP(ph-OMe)$_4$ with increasing concentration for PRN (0.0 to 2.2 µM) in CH$_2$Cl$_2$. 
In Figure 4.39, there is reduction in fluorescence intensity at 659 nm but increase in fluorescence intensity at the exiplex peaks 808 and 865 nm for PRN. This reduction in fluorescence intensity at the main peak (659 nm) of the sensing molecule while increase in the fluorescence intensity of the exiplex peaks is also observed in Figures 4.37 and 4.38 for ANTH and BaP respectively, after interaction with CoP(ph-OMe)₄. The exiplex peaks as mentioned are at 774, 820 and 862 nm; and 816 and 863 nm with ANTH and BaP respectively.

### 4.3.6.3 Fluorescence solution study for FLXN.

Solutions were prepared as per the developed method of section 4.3.4. Measurements were performed on a series of dilute solutions to ascertain the fluorescence pattern for FLXN with the pollutant molecules. The stock solutions from which µM volumes, for pollutant molecules were pipetted, which were later converted to ml volumes for plots were the same ones used during absorption measurements.

Fluorescence spectrum for FLXN is usually at a λₑₘ 514 nm with the excitation at λₑₓ 485 nm. Figures 4.40, 4.41, 4.42 and 4.43 represent fluorescence spectra after interaction of FLXN with ANTH, BaP, PRN and Py respectively. Fluorescence calibration plots for enlisted figures are as presented in Figures 4.44, 4.45, 4.46 and 4.47 respectively.
When a fixed concentration of 0.026 µM for the sensing reagent FLXN interacted with ANTH, the fluorescence intensity for the sensing reagent at 514 nm increased, broadened and shifted 4 nm to 510 nm wavelength towards the blue end of the electromagnetic spectrum as illustrated in Figure 4.40. Other spectra for this measurement have been omitted for clarity.

Figure 4.41. Fluorescence spectra for fixed 0.019 µM [FLXN] with increasing [BaP].

**Figure 4.41**, illustrates increasing fluorescence intensity for the sensing molecule FLXN and subsequent shift from 514 nm to 504 nm (10 nm shift) towards the blue end of the electromagnetic spectrum as the [BaP] increase in solution.

Figure 4.42. Fluorescence spectrum for fixed 0.035 µM [FLXN] with increasing [PRN] at 514 nm.
Pyrene and pyridine reduced the fluorescence intensity for FLXN with their increasing concentration in solution as illustrated in Figures 4.42 and 4.43 respectively. There is a slight shift in wavelength of 2 nm towards the red end of the electromagnetic spectrum with increasing concentration for PRN in the matrix of the sensing molecule FLXN, but a shift is not observed with Py. Presented in Figures 4.44, 4.45, 4.46 and 4.47 are fluorescence calibration plots after interaction of FLXN with increasing concentrations for ANTH, BaP, PRN and Py respectively.
Figure 4.45. Fluorescence calibration plot increase in fluorescence intensity for fixed 0.019 µM [FLXN] with increasing [BaP]

Figure 4.46. Fluorescence calibration plot for fixed 0.035 µM [FLXN] with increasing [PRN]
Figure 4.47. Fluorescence calibration plot for fixed 0.035 µM [FLXN] with increasing [Py]

From absorption and fluorescence data, it can be observed that organic pollutants for this research change the way the sensing molecules CoP(ph-OMe)$_4$ and FLXN interact with the radiant energy of the electromagnetic spectrum. Having established the interaction pattern of the sensing molecules with the pollutants, the next study is therefore, for their solid, thin films.

The sensing molecules; CoP(ph-OMe)$_4$ and FLXN thin films were therefore subsequently immobilised in PDMS matrix for further investigations in evaluating their suitability as sensing molecules in their solid form, for the organic pollutants of this research. The results for the thin films study are presented in chapter 5.
Chapter 5: Solid thin films study.

5.1 Introduction.

Development of a Fluorescence Based Fibre Optical Chemical Sensing System (FBFOCS) for anthracene, benzo(a)pyrene, pyrene and pyridine has been considered and analyses leading towards achieving such a system are illustrated in this chapter. The mechanism for this sensing system is based on monitoring fluorescence properties of solid, thin films of sensing reagents selected for this research (these reagents are presented chapter 2 section 2.5 and 2.7) as they interact with the organic pollutants selected for this study (these pollutants are presented in chapter 2 section 2.10).

Thin films of the sensor molecules alone and with organic pollutants were investigated in this study using spectroscopic methods of analysis. Solid thin films of chemicals can be immobilised by a variety of techniques. Some of these techniques are more suitable for bulk polymer materials, such as vacuum evaporation which will not be described in this thesis. In this study, manual deposition on glass substrates of PDMS matrix containing sensing molecules was employed. A mixture of the sylgard 184 base monomer, linking monomer and known amount of the sensor molecules gave a texture that could easily be pipetted using micropipette and deposited onto the glass cover slip held onto ceramic white tiles.

5.2 Apparatus, chemicals and instruments for solid thin films study.

Poly-Dimethyl Siloxane (PDMS) curing monomer and base monomer, which are products of Dow-Corning were sourced from Ellsworth Adhesives Europe (Glasgow). Their trade names are sylgard 184 base elastomer (Figure 5.1) and sylgard 184 linker or curing monomer (Figure 5.2) respectively. Both are
colourless highly viscous fluids. Sources for sensor molecules and the organic pollutants are already highlighted in chapter 4 section 4.2 and their structures highlighted in section 2.3 to 2.10 of chapter 2.

![Figure 5.1](image1.png)

**Figure 5.1.** Structure of sylgard elastomer base; subscripts 'n' on the brackets represent the number if units of the back-bone of the polymer.

![Figure 5.2](image2.png)

**Figure 5.2.** Structure of sylgard monomer which is also the curing agent is a cross linker and binds to glass cover slips if used as substrates through hydrogen atom.

The way the base elastomer and curing monomer interact to form a polymer may be as outlined in **Scheme 5.1**.
Scheme 5.1. Illustration of the reaction between Sylgard 184 elastomer base and Sylgard 184 linking/curing monomer.

The apparatus included glass cover slips as substrates onto which thin films of sensor molecules were immobilised, ceramic white tiles for holding these glass cover slips when undergoing the curing process; glass vials for weighing in the sylgard base monomer, spatulas for scooping this base monomer, glass rods for stirring to mix e.g. the base monomer and linking monomer and other apparatus like volumetric flasks and micropipettes and tits, the use of which is as highlighted in chapter 4 section 4.2. The oven was also employed for final heat curing of the sensor molecules' films. The weighing balance too was essential. The instruments employed in this study include Shimadzu PC spectrophotometer 2401 for absorption measurements and Perkin-Elmer LS 55 fluorometer for
fluorescence measurements. A flow cell was also fabricated to hold the glass cover slips onto which sensor molecules films are immobilised, for fluorimetric measurements and a glass syringe was used as a reservoir for the organic pollutants and the hot plate for generating vapours from PAHs. To facilitate use of the Perkin-Elmer LS 55 fluorometer for measurements, an optical fibre was employed to interface the flow cell system to the fluorometer and a small vacuum pump for drawing organic vapours in and out of the flow cell. The complete assembly of the flow cell with tubing is referred to in this thesis as sensor platform. This flow cell made of Perspex for holding solid thin films of sensor molecules was fabricated in our university workshops after designing and appropriate tubing purchased (made from PVC material); the tubes were typically 1.44 mm and 3.4 internal and outside diameters respectively, which was cut in appropriate lengths as required. Other plastic or stainless steel micro-accessories for the tubes so that when coupled to sensor platform, they give a tight fitting were also purchased.

5.3 Method development and experiments.

A test experiment was performed on organic reagents pre-immobilised with the CoP(ph-OMe)$_4$. This as a start would give a hint on how actually these pollutant molecules affect the films of the sensing reagent and or PDMS matrix. Thus when immobilised with the sensing molecule, we are certain that indeed the pollutants are present in the sensing matrix. Prior to pre-immobilising with the organic pollutants, immobilisation of the PDMS matrix alone was done and absorption and fluorescence measurements performed on them for reference.

5.4 Pollutants pre-immobilised with CoP(ph-OMe)$_4$

Dow-Corning, the manufacturer of PDMS suggests that, to achieve a complete curing of the polymer, the mixture should contain 1:10 sylgard 184 base elastomer and linking monomer respectively. The immobilisation was such that
would suit extrinsic analytical technique (see section 2.16 chapter 2). Thus the films were immobilised on 22 mm² glass cover slips rather than the optical fibre. This way, the optical fibre could only be employed as an interface to the Perkin-Elmer LS 55 fluorometer and therefore only a transmitter of radiant energy to the film.

5.5 Pre-immobilisation procedure.

All the necessary apparatus were put in readiness before the procedure was started. Ten glass cover slips were already placed on clean white ceramic tiles on the bench, the linking monomer and micropipettes and tips, clean tissues and glass rods put in readiness for use. The stock solution of the sensing molecule had also been prepared already.

Amount of 1.00 g of sylgard base monomer was weighed in a dry clean glass vial using a microbalance. A stainless steel spatula was used to add the this base monomer into the vial. In this vial, 400 µL of CoP(ph-OMe)₄ pipetted from a stock solution of 0.261 µM were added and mixed thoroughly giving an orange mixture due to the presence of CoP(ph-OMe)₄; otherwise, PDMS alone is colourless. In this mixture, the concentration for CoP(ph-OMe)₄ was estimated to be 0.095 µM. Aliquots of the mixture were pipetted and deposited at the centres of glass cover slips.

Increments of aliquots of 5 µL for ANTH from a stock solution of 0.0071 mol l⁻¹ were added at the centre of a given glass cover slip containing the sensor in PDMS and then mixed thoroughly giving a homogenous look of the mixture. Thus, if 5 µL were added on the first glass cover slip, the next one will have 10 µL etc. A total of ten cover slips were prepared, four of them being of the sensor molecule alone in the PDMS matrix. Initially films of the PDMS alone had been prepared and spectra for both absorption and fluorescence measured for comparison with those of sensor with pollutants. While depositing the mixture on glass cover slips, it was discovered that, if agitated, the cover slip contents could easily flow off. Therefore, it was vital to leave them on the tiles on the bench undisturbed until they achieve initial curing. The initial curing was for 1 hr and then complete curing was through heat at 92 °C in the oven for a further 30 minutes, giving a rubbery
thin film adhered to the glass cover slip completely cured. These films were then stored in a desiccator pending analysis. A repeat of this process with other pollutants was carried out.

Curing of the mixture mentioned above simply mean that, the two components of PDMS react to form a solid elastomeric membrane on a substrate onto which it is deposited. It can either form a porous rubbery solid thin film or also form a porous rubbery, solid, thick mass depending on the amount and surface onto which the mixture is deposited. On a flat surface the fluid flows forming a thin film and in a container it is moulded in the shape of that container.

5.6 Absorption measurements for the sensor films pre-immobilised with the pollutants.

As mentioned earlier, absorbance for these thin films was performed on Shimadzu UV-VIS spectrophotometer PC 2401. The model available in the laboratory during measurements has no sample compartment for holding thin films. So improvisation was made such that the films were held against the solution samples compartment and in the optical path using laboratory clay at the tips of the four corners of the glass cover slips. After which the compartment sealed off from light by closing it. Initially the instrument had been set to absorbance vs wavelength measurements mode and therefore full scans from 200 – 900 nm were performed.

Figures 5.3, 5.4 and 5.5 represent absorption spectra of sensor molecules pre-immobilised with ANTH, BaP and PRN respectively.
Having pre-immobilised CoP(ph-OMe)$_4$ sensor molecule with ANTH, there is reduction in absorbance at the main absorption peak of this sensing molecule while the characteristic ANTH peaks can also be clearly observed and they and increase in absorbance with increasing [ANTH]. It can also be observed that, the main absorption peak for the sensing molecule appear to be at 406 nm as opposed to 414 nm in solution as shown in Figure 5.3. There is also that broad band between 480 and 580 nm.

Figure 5.4. Absorption spectra for thin films for 0.095 µM CoP(ph-OMe)$_4$ with and without BaP
In **Figure 5.4**, the main absorption peak for the CoP(ph-OMe)$_4$ is at 412 nm instead of the 414 nm of its solution. There is that broad band between 495 to 595 nm. The absorption bands for BaP are clearly!visible as well and they are increasing in absorption intensity as the amount of BaP increase.

In **Figure 5.5**, the main absorption peak for this sensing molecule which is usually at 414 nm in solution has shifted to 410 nm. Part of the absorption band for PRN can also be seen clearly and is increasing in intensity as the amount of PRN increase. There is that characteristic broad absorption band appearing at 490 and 580 nm.

It was expected that there could be less homogeneity when making the mixture of the sensor with the PDMS and hence uneven distribution of the sensor molecule inside the polymer. However, it was observed that the mixture appeared homogenous taking on the colour of the solutions of the sensing molecules. When the absorption measurement on another batch of immobilised films of same concentration was performed, the intensity of the films from this same batch had intensity magnitude of ±2 % as exemplified with absorbance for CoP(ph-OMe)$_4$ in PDMS matrix shown in **Figures 5.6 and 5.7**. This occurrence confirms that, there was an even distribution of the sensing molecules inside the matrix.
Thus, Figures 5.6 and 5.7 illustrate that the distribution of the sensing molecule in the PDMS matrix was relatively homogeneous to a reasonable error magnitude of $\pm 2\%$. 
5.7 Fluorescence measurements for the sensor films pre-immobilised with the pollutants.

The fluorescence for only BaP films was measured and these measurements were performed on Perkin-Eimer LS 55 fluorometer, which has the frontal surface attachment. Thus, this instrument has a provision for holding solid samples for analysis in the optical path and one film measured at a time. Excitation wavelength of 423 nm as for solutions of CoP(ph-OMe)₄ was used to irradiate and excite the molecules in the films and the emission spectra recorded as represented in Figure 5.8.

![Figure 5.8. Fluorescence and excitation spectra of solid thin films for 0.095 µM CoP(ph-OMe)₄ with and without BaP immobilised in PDMS matrix on glass cover slips.](image)

5.8 Testing the porosity of PDMS matrix.

Pyridine vapours were used to test for porosity of PDMS matrix and also as a preliminary test to observe the changes when Py vapours interact with the CoP(ph-OMe)₄. Simple laboratory apparatus including glass gas jar, micropipette, and laboratory sealing plastic film were used in this test. Two glass
cover slips of the encapsulated sensor were placed in a gas jar as shown in Figure 5.9 and 50 µL of a pure pyridine analyte introduced through a micropipette at the centre inside the jar but not touching the glass cover slips. The jar was then sealed tightly using laboratory film and left to stand at room temperature of 25.5 °C inside the fume cupboard for 5 minutes. It was observed that pyridine vapours had diffused onto the glass cover slips and the films on the cover slips changed colour from reddish-orange to yellowish-green. The glass cover slips were then removed from the jar and left to dry on clean ceramic white tile on the laboratory bench. After 10 minutes, they were dry enough for absorption and fluorescence measurements. The colour of initial films changed on exposure to pyridine as shown in Figure 5.10. The absorption spectra for CoP(ph-OMe)$_4$ films without and with pyridine measured on Shimadzu PC 2401 spectrophotometer are presented in Figures 5.11 and 5.12 respectively. The fluorescence spectra for the same films are presented in Figure 5.13. Assembled apparatus for this test are as presented in Figure 5.9 as mentioned above.

![Diagram showing apparatus initially used to facilitate the interaction of pyridine with CoP(ph-OMe)$_4$ at initial porosity test](image)

Figure 5.9. Diagram showing apparatus initially used to facilitate the interaction of pyridine with CoP(ph-OMe)$_4$ at initial porosity test.
There was colour change from reddish-orange to yellowish-orange of the film after interaction with pyridine as shown in Figure 5.10 which left no doubt that there was a kind of chemical reaction on exposure of these films to pyridine.

Figure 5.10. (1) Thin film for CoP(ph-OMe)$_4$ alone on glass cover slip encapsulated in PDMS and (2) and (3); CoP(ph-OMe)$_4$ films as in (1) above after exposure to pyridine vapour.

Absorption spectra for the films without and with Py are as presented in Figures 5.11 and 5.12 respectively.

Figure 5.11. Absorption spectrum of thin film for 6.2 $\mu$M CoP(ph-OMe)$_4$ in PDMS matrix.
The main absorption peak of the sensing molecule in the film is at 432 nm and a minor one at 536 nm as shown in (Figure 5.11). After interaction with Py as illustrated in (Figure 5.12), there are absorption peaks at 420, 440 and 552 nm. Thus, the absorption spectrum of solid thin film for CoP(ph-OMe)$_4$ show the soret band at 432 nm and Q-band at 536 nm. With pyridine, it can be observed that there are shifts in the soret and Q-bands, these shifts towards the red end of the electromagnetic spectrum also occur in spectra measured of solution samples after interaction of Py with CoP(ph-OMe)$_4$. The absorption spectrum of this sensing molecule in solution show the soret band at 414 nm and the Q-band at 530 nm and with pyridine in solution; new peaks at 438, 553 and 595 are formed. The shift at different absorption bands of these solid thin films for CoP(ph-OMe)$_4$ with Py towards the red end of the electromagnetic spectrum as compared to those in solution may be attributed partly to lack of solvent effects and due to polymeric matrix effect. Davis et al (2008) have published such differences between absorption spectra of solid thin films of molecules and those of their solutions. Davis and co-workers demonstrated that immobilised solid thin films of molecules exhibited red shifts as compared to these molecules in solutions and this also affected fluorescence intensity. Liu et al (2006) demonstrated that Langmuir-Blodgett thin films of molecules exhibited enhancement in fluorescence.
intensity and they attributed this phenomenon to molecular aggregates, planarity and rigidity of the films.

Figure 5.13 presents fluorescence spectrum before and after CoP(ph-OMe)$_4$ thin film interacted with pyridine.

![Fluorescence spectrum](image)

Figure 5.13. Fluorescence spectrum for thin film of 6.2 μM CoP(ph-OMe)$_4$ in PDMS matrix showing: 1.) Excitation and fluorescence of the film without Py and 2.) Excitation and fluorescence of the film with Py.

Figure 5.13 illustrates increase in fluorescence intensity of the film of the sensing molecule after interaction with pyridine. The amount of Py fed onto the sensing molecule film was not controlled therefore the film adsorbed that much that increased the intensity of 6.2 μM of this sensor molecule by 75 %. The second film too, had similar spectrum and intensity magnitude, showing that the amounts of Py adsorbed by both two films of the sensing molecule were equal. It is illustrated on results in the later sections of this chapter that initial pumping of pyridine vapours onto the sensing film increases the signal just the same way as illustrated in Figure 5.13 and subsequent quenching as more Py vapours are added and their concentration increase onto the sensing film.
5.9 Preliminary studies for FLXN.

Slurries for FLXN in PDMS matrix were prepared and smeared onto glass cover slips. Known amount of 18 mg of FLXN was made in minimum amount of water into slurry, which was then mixed with sylgard base elastomer and subsequently with sylgard linking monomer. The slurry was red in colour due to the presence of FLXN. Same curing procedure as for CoP(ph-OMe)₄ was adopted. After curing, absorption measurements were carried out on these films. In this method, a pre-weighed clean, dry, calibrated glass syringe was used to draw vapours from the reservoir. This was referred to as syringe vapour sucking (SVS). After collection of the vapours from the headspace of the container of the pollutant, the syringe was re-weighed and any increase in weight noted. The increase in weight was presumed to be that of the vapours in mg. This assumption was made because precautionary measures like using a clean tissue when handling the syringe to avoid any additional weight from greasy hands and cleaning of weighing balance pan were observed. The vapours were then injected into the cell containing the sensing film and measurements performed using a spectrophotometer. Represented in Tables 5.1, 5.2, 5.3 and 5.4 are data for weight taken in mg for ANTH, BaP, PRN and Py vapours respectively.

Table 5.1. Weight for ANTH vapour in mg

<table>
<thead>
<tr>
<th>Weight of syringe alone in mg</th>
<th>Syringe with ANTH vapour; mg</th>
<th>Weight of ANTH vapour in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>54232.8</td>
<td>54233.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>54235.6</td>
<td>2.8</td>
</tr>
<tr>
<td>54239.1</td>
<td>54242.6</td>
<td>3.6</td>
</tr>
<tr>
<td>54250.1</td>
<td>54252.6</td>
<td>17.3</td>
</tr>
<tr>
<td>54255.1</td>
<td>54257.6</td>
<td>19.8</td>
</tr>
<tr>
<td>54261.5</td>
<td>54318.9</td>
<td>22.3</td>
</tr>
<tr>
<td>54276.1</td>
<td>54287.6</td>
<td>24.8</td>
</tr>
<tr>
<td>54295.1</td>
<td>54300.1</td>
<td>28.7</td>
</tr>
<tr>
<td>54315.1</td>
<td>54318.9</td>
<td>31.1</td>
</tr>
<tr>
<td>54325.1</td>
<td>54328.9</td>
<td>33.1</td>
</tr>
</tbody>
</table>

Table 5.2. Weight for BaP vapour in mg.

<table>
<thead>
<tr>
<th>Weight of syringe alone in mg</th>
<th>Syringe with BaP vapour; mg</th>
<th>Weight of BaP vapour in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>66595.5</td>
<td>66596.1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>66596.7</td>
<td>1.6</td>
</tr>
<tr>
<td>66597.8</td>
<td>66599.7</td>
<td>2.3</td>
</tr>
<tr>
<td>66600.6</td>
<td>66602.6</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>66600.6</td>
<td>5.1</td>
</tr>
</tbody>
</table>
Table 5.3. Weight for PRN vapour in mg.

<table>
<thead>
<tr>
<th>Weight of syringe alone in mg</th>
<th>Syringe with PRN vapour; mg</th>
<th>Weight of PRN vapour in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>54185.2</td>
<td>54195.9</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>54201.7</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>54209.3</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>54221.3</td>
<td>36.1</td>
</tr>
<tr>
<td></td>
<td>54257.4</td>
<td>72.2</td>
</tr>
<tr>
<td></td>
<td>54308.0</td>
<td>122.8</td>
</tr>
<tr>
<td></td>
<td>54332.5</td>
<td>147.3</td>
</tr>
</tbody>
</table>

Table 5.4. Weight for Py vapour in mg.

<table>
<thead>
<tr>
<th>Weight of syringe alone in mg</th>
<th>Syringe with py vapour; mg</th>
<th>Weight of py vapour in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>54132.4</td>
<td>54133.9</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>54134.9</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>54136.6</td>
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<tr>
<td></td>
<td>54136.7</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>54137.4</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>54138.5</td>
<td>6.1</td>
</tr>
</tbody>
</table>

The vapours for the PAHs were generated by heating the sample by radiated heat energy from the hotplate. The sample container was held at a vantage position above the hot plate where the sample could only generate vapours but preserved from decomposition. The absorption spectra for solid thin films for FLXN with PAHs showed peaks for these organic pollutants which meant that the vapours were adsorbed within the sensing matrix as shown in Figures 5.14 and 5.15 for ANTH and PRN respectively.
Figure 5.14. Absorption spectra for increasing concentration of ANTH vapour with fixed concentration 18 mg thin film of FLXN in PDMS matrix.

Figure 5.15. Absorption spectra for increasing concentration of pyrene vapour with fixed concentration 18 mg thin film of FLXN in PDMS matrix.
Washing the sensor film in a suitable solvent reduces the concentration of loosely bound analyte and subsequently removes it all together from the sensor matrix. If this is the case, it means that there is a reversible interaction of the sensor and analyte. Therefore, an attempt to wash the film in order to establish reversibility of interaction showed as in Figure 5.16, that this method may not be a suitable one for analysis of PAHs with the thin films of the sensing reagents.

In Figure 5.16, it can be observed that the signal before washing the film was lower than after washing the film in CH$_2$Cl$_2$ solvent. This could represent a very large error magnitude in quantifying the amount of PAH vapour adsorbed onto the sensing film. Therefore absorption method of analysis for sensing of PAHs with solid sensor films may not be suitable in reference to this observation. In this regard, fluorescence spectroscopic method of analysis was considered suitable. Using fluorescence spectroscopic method, the flow cell can be held closer to vapour source and then interfaced to the instrument, which means that the vapours will not have re-solidified before interacting with the sensing film. This is because, as they are vaporised, they are directly pumped onto the film which is
very close to the reservoir, unlike sucking with the syringe before delivering onto the sensing film. A bifurcated optical fibre that facilitates connection of the sensing platform to the optical path of the fluorimeter can easily be coupled onto the flow cell. This bifurcated optical fibre connects the flow cell to the fluorimeter which is interfaced to the computer which has the software for the whole operation for fluorescence measurements.

5.10 Fluorescence Based Fibre Optical Chemical Sensing.

Immobilisations of subsequent films, for further investigations of these viable sensors were therefore adopted from the preliminary tests highlighted earlier in this chapter. Fluorescence Based Fibre Optical Chemical Sensing (FBFOCS) adopted as analytical technique for evaluating sensing capabilities for CoP(ph-OMe)$_4$ and FLXN. To facilitate interface of sensor films to Perkin-Elmer LS 55 fluorometer, a flow cell (Figure 5.17) was fabricated as highlighted in section 2.13 of chapter 2.

![Figure 5.17](image)

Figure 5.17. Flow cell for organic pollutants vapours with inlet and outlet tubes and a glass cover slip onto which sensor film is immobilised.

The individual components of Figure 5.17 of the flow cell are presented in Figure 5.18.
The flow cell in this study is simply a unit consisting of two square blocks of Perspex plastic material and a tight sealing gasket.

The dimensions of the units and features of the flow cell are as highlighted here below:

- The Base Block (BB), has measurements of (30 mm by 30 mm by 10 mm) length, width and height respectively and has a rut of (8 mm by 5 mm) diameter and depth respectively of volume 251.4 mm$^3$.
- Upper Block (UB), has measurements of (30 mm by 3 mm by 5 mm) length, width and depth respectively. Thus, it is thinner than the base block.
- Rubber Gasket (RG), has dimensions (30 mm by 30 mm by 1 mm), length, width and depth respectively.
- Screw holes at the four corners of the UB, BB and RG. Their purpose is to screw the units together using small screws.
- Circular, open grooves of 8 mm diameter are carved on; UP and RG, but dead-ended (though with opposite small holes) for the BB. The purpose of these grooves is to allow radiant energy to penetrate through to the UB, RG and BB; which will hold the sensing molecule film and the organic pollutants vapours.
Inlet and outlet holes at the base of the BB, which are each (4 mm) in diameter in which the delivery and outlet tubes with their seals fit snugly.

As highlighted above, that the lower base block is double the height of the upper block, it is because it consists of a cylindrical dead-end furrow of (8 mm by 5 mm) diameter and height (depth) respectively and of volume 251.4 mm³, the purpose of this rut is to hold and allow for interaction of pollutant vapours with the sensing film. However, at the base of BB, near the dead-end are inlet and outlet holes opposite each other. The purpose of these holes is to allow for connecting the inlet and outlet tubing linking the flow cell to the pollutants’ reservoir and the vacuum pumping unit respectively. This groove of BB is the size that will allow the glass cover slip of 22 mm² onto which the sensing molecule is immobilised to overlap it, giving room for tight fitting when a rubber gasket is placed over it before covering with the upper block (see Figure 5.17).

The unit is assembled such that the base block which has inlet and outlet tubes fitted with gas tight joints, has the side of the glass cover slip onto which the sensing molecule thin film is immobilised facing the groove. This facilitates exposure of the film to the organic vapours when they get pumped into the flow cell. The opposite side without the film faces upwards, and over the glass cover slip, the RG is placed; note that the gasket has a see-through circular opening as described in the previous paragraph and also as shown in Figures 5.17 and 5.18. This opening or runnel exposes the film area to the optical fibre guiding radiant energy to the sensing film. The UB is then placed over the BB unit carrying the film and gasket. The UB also has a circular opening as explained in the previous paragraph (also see Figures 5.17 and 5.18). The BB, the RG and the UB, all have holes of same size at their four corners. Through these holes, screws are used to hold the pieces together and so tightly such that no leakage of vapours can occur. The unit is then positioned near the fluorometer on a firm miniature stand so that the optical fibre can be coupled without strain. A syringe containing a known amount of the organic pollutant is held close to this sensor platform using a clamp stand so that it is easy to couple the inlet tube from the sensor platform tightly onto the syringe. The tube from the syringe containing organic pollutant
vapours is coupled onto the sensor platform. Nearby the sensor platform, a small vacuum pump is placed on a firm block of wood and the outlet tubing from sensor platform is coupled onto the suction end of this pump and another tube is connected onto the venting end which is led to a vessel holding a solvent that absorb the waste vapours.

The description in the last paragraph above is presented in **Scheme 5.2**.

A complete assembly of the sensing platform with the vapour source, pumping unit is then connected to the fluorimeter through the optical fibre and this whole set up is presented in **Figures 5.19 and 5.20**.
Bifurcated optical fibre feeds substantial information of the sample matrix to the fluorimeter. This information is then detected and converted into an instrumental graphical signal and recorded for further analysis and interpretation.

The tube from the vapour reservoir connects to the inlet tube of the flow cell therefore leads the vapours onto the sensor film. The outlet tube of the flow cell connects to suction tube of the vacuum pump. This arrangement creates suction pressure and this facilitates sucking vapours from the reservoir into the flow cell.
The venting tube of the vacuum pump connects to the waste trap and this facilitates draining excess vapours from the sensor into the trap that contain solvents to dissolve them for safe disposal.

**5.11 Experiments for CoP(ph-OMe)$_4$ with pollutants.**

After immobilisation and curing process as highlighted in section 5.5, measurements were performed on Perkin-Elmer LS 55 fluorimeter by exciting the sensing film with energy of 423 nm wavelength propagated through the optical fibre. Excitation slit width of 15 nm and emission slit width of 20 nm were appropriate for this measurement. After setting up the sensor platform, as explained in the previous paragraph with the film of the sensor molecule alone, a bifurcated optical fibre was used to interface the sensor platform with the fluorometer. The interfacing was achieved by plugging the optical fibre into the slots inside the instrument designed for that purpose and this connects the fibre with the radiant energy source. The optical fibre was subsequently coupled onto the sensor platform and the unit sealed from stray light using a black masking tape as illustrated in the Figures 5.19 and 5.20 of complete setup of sensor platform (section 5.10). The optical fibre is made of a material transparent in the range of 200 – 1000 nm. Fluorescence spectrum of the film for the sensor alone prior to addition of organic pollutant vapours was then measured so as to compare this signal with signals measured after the film interact with the organic pollutants.

Pyridine sample does not need heating to generate vapours and therefore its sample was drawn into a clean syringe. A vacuum pump is used which facilitates transfer of these vapours onto the sensing film. After the emission spectrum of the sensor film alone had been measured, this syringe containing pyridine sample was connected to the inlet tube of the sensor platform and clamped in position using a clamp stand as illustrated in (section 5.10). Pyridine vapours from the reservoir (syringe) are then pumped onto the sensing film through a tube which is 36.30 mm long and 1.44 mm diameter using a vacuum pump which is also connected to an outlet tube of 72 mm length and 1.44 mm diameter. This outlet
tube leads to a trap containing the solvent that will dissolve the waste vapours. Multiple emission spectra were recorded at successive intervals of 20 seconds as pumping of pyridine vapours onto the sensing film is continued. The draining tube is longer than the inlet tube so as to avoid any back flow in-case of fluctuations within the pumping unit.

After fluorescence measurements for CoP(ph-OMe)₄ with pyridine, the syringe is cleaned thoroughly, dried and subsequently known weights for ANTH, BaP and PRN are placed one after the other after cleaning the syringe and weighing. Fluorescence spectra for individual PAH after interaction with the film of CoP(ph-OMe)₄ were subsequently recorded. Vapours from PAHs’ crystals are generated using heat radiated from the hotplate onto the syringe containing them, thus a kind of thermal desorption method is used. The syringe is clamped and held at a position above the hot plate that can only get the heat (353 K) to generate vapours but not decompose the sample. The method for acquiring these spectra is as explained earlier for the sensor with pyridine in the previous paragraph of this section.

It is envisioned that these vapours will interact with the sensor film and affect the way this sensor interact with the radiant energy.

Plots for fluorescence Intensity vs time in seconds for the film of CoP(ph-OMe)₄ after interaction with ANTH, BaP, PRN and Py respectively are as presented in Figures 5.21, 5.22, 5.23 and 5.24.
Figure 5.21. Fluorescence Intensity vs time for fixed thin film 3.9 μM concentration of CoP(ph-OMe)$_4$ with increasing ANTH vapour.

Figure 5.22. Fluorescence Intensity vs time for fixed thin film 6.9 μM concentration of CoP(ph-OMe)$_4$ with increasing benzo(a)pyrene vapour.
Figure 5.23. Fluorescence Intensity vs time for fixed thin film 6.9 μM concentration of CoP(ph-OMe)$_4$ with increasing pyrene vapour.

Figure 5.24. Fluorescence Intensity vs time for fixed thin film 6.2 μM concentration of CoP(ph-OMe)$_4$ with increasing pyridine vapour.

These plots show reasonable repeatability, thus after every 20 seconds, which is settling time, the flow is relatively steady and still after repetition of runs for 20 minutes, the steadiness in flow is observed. Undershoots and overshoots are less observed in these plots with time. Measurement of the signal after feeding organic pollutant vapours to the sensing system is set at automatic repetitions of 50 runs.
at intervals of 20 seconds waiting time. This repeatability and reproducibility in the plots is attributed to the small diameter and short length features of the delivery tube. This facilitates quick response and minimise overshoots and undershoots to the sensing film, which may have been experienced in longer flexible tubes due to curves and bends. Curves and bends may trap the vapours and hinder smooth flow which can be observed in unpredictability in signals produced. That is why this mass flow system shows reasonable repeatability, stability and linearity as observed in signals generated after sensing of vapours interact with sensing film.

It can be observed from these calibration plots that there is diminishing in fluorescence intensity as the sensor molecule interacts with the increasing concentration of organic pollutants. This diminishing in fluorescence intensity is called quenching. There are two types of quenching; static and dynamic quenching. Static quenching is type resulting from a non-fluorescent complex formation. Dynamic quenching is due to collisions of pollutant molecules with sensing molecules and other interactions such as charge transfer between the sensor molecule and the pollutant molecules. Interaction of the sensor molecule with some pollutant molecules may result in both the two types of quenching. In this case, a Stern-Volmer calibration plot will have an upward or downward curvature.

The plots above were obtained from data of spectra as exemplified in Figures 5.25 and 5.26. These spectra examples represent the fluorescence spectra for the thin film of CoP(ph-OMe)₄ after interaction with increasing vapour concentration of benzo(a)pyrene and pyrene respectively.
5.12 Experiments for FLXN with pollutants.

Applying the same method and procedures as highlighted in section (5.5), using the sensor platform and syringe, fluorescence measurements for FLXN with the organic pollutants were performed. Since the same procedure as employed for CoP(ph-OMe)$_4$, was applied here, therefore presented in this section are plots for $I$ (observed fluorescence intensity) vs (time in minutes) of FLXN with the organic pollutants. The excitation wavelength for FLXN was 485 nm and its fluorescence wavelength at 514 nm. Figures 5.27, 5.28, 5.29 and 5.30 are plots for $I$ vs time in minutes for FLXN with ANTH, BaP, PRN and Py respectively.
Figure 5.27. Fluorescence Intensity vs time for fixed thin film 9.2 μM concentration of FLXN with increasing ANTH vapour.

As illustrated in Figure 5.27, ANTH reduces the fluorescence intensity for the sensing molecule FLXN.

Figure 5.28. Fluorescence Intensity vs time for fixed thin film 7.1 μM concentration of FLXN with increasing BaP vapour.

Benzo(a)pyrene increases the fluorescence intensity for FLXN after interaction as shown in Figure 5.28.
Fluorescence intensity for FLXN reduces with increasing concentration for PRN and illustrated in Figure 5.29. There is a sharp increase in the fluorescence intensity for FLXN with Py and then subsequent reduction in intensity as the concentration for Py increases onto the sensing film as presented in Figure 5.30.
5.13 Estimation of the concentration of pollutants using Clausius-Clapeyron equation.

The Clausius-Clapeyron equation can be used to estimate the vapour pressure of a chemical species at any temperature. This can be done when the vapour pressure $P_1$ of the same species at a given temperature, $T_1$ and their heat of vaporisation are known. For the organic pollutants of this study, such parameters are as shown in Table 5.5.

In this study, the calculated volume size of the delivery tube was 0.05912 cm$^3$.

### Table 5.5. Useful data for pollutants recorded in literature.

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Vapour pressure/atm</th>
<th>Temp/K</th>
<th>$H_{vap}$/J Mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTH</td>
<td>$1.32 \times 10^{-3}$</td>
<td>418</td>
<td>99700</td>
</tr>
<tr>
<td>BaP</td>
<td>$3.87 \times 10^{-6}$</td>
<td>298</td>
<td>70800</td>
</tr>
<tr>
<td>PRN</td>
<td>$7.74 \times 10^{-7}$</td>
<td>298</td>
<td>100200</td>
</tr>
<tr>
<td>Py</td>
<td>$1.32 \times 10^{-2}$</td>
<td>293</td>
<td>35090</td>
</tr>
</tbody>
</table>

Equation 5.1 is the Clausius-Clapeyron.

$$\ln \frac{P_2}{P_1} = -\frac{\Delta H_{vap}}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right)$$

5.1

Where, $\ln$ is natural logarithm, $P$ is vapour pressure, $T$ is temperature in degrees Kelvin, $R$ (8.3145 J mol$^{-1}$ K$^{-1}$) is the gas constant and $\Delta H_{vap}$ rate of change in heat of vaporisation.

From this study, the vapours for PAHs were generated at 353 K. Py vapours were at room temperature of 293 K. Applying Clausius-Clapeyron equation, the vapour pressure of PAHs at 353 K was calculated as below:

For example using ANTH data;
\[
\ln \frac{P_2}{0.00132} = \left( \frac{99700 \text{ J mol}^{-1}}{8.3145 \text{ J mol}^{-1} \text{ K}^{-1}} \right) \left( \frac{1}{353 \text{ K}} - \frac{1}{418 \text{ K}} \right) 
\]
(a)

\[
\ln \frac{P_2}{0.00132} = 5.16 
\]
(b)

\[
\frac{P_2}{0.00132} = 174.16 
\]
(c)

\[
P_2 = 0.23 \text{ atm} 
\]
(d)

The same calculations were applied to other pollutants and the data for their vapour pressures, \( P_2 \), were as presented in Table 5.6.

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Vapour pressure, ( P_2 )/ atm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTH</td>
<td>( 2.3 \times 10^{-1} )</td>
</tr>
<tr>
<td>BaP</td>
<td>( 2.70 \times 10^{-4} )</td>
</tr>
<tr>
<td>PRN</td>
<td>( 4.40 \times 10^{-4} )</td>
</tr>
<tr>
<td>Py</td>
<td>( 1.88 \times 10^{-2} )</td>
</tr>
</tbody>
</table>

**5.14 Calculating concentrations of pollutants from vapour pressures.**

Since vapour pressure expresses the concentration of the vaporised species in the gas/vapour stream, the concentrations of these pollutants can be calculated using the vapour pressures calculated at their \( T_2 \) as tabulated (see table in section 5.13). Vapour pressures of pollutants are useful in air pollution control because they are proportional to the concentration of the component species in their gas or vapour phase and therefore can be used to determine their concentration in ambient air.

Using the calculated vapour pressures, the concentrations of the organic pollutants were estimated using the ideal gas equation 5.2.
\[ PV = nRT \quad \text{(5.2)} \]

Rearranged;

\[ \frac{P}{RT} = \frac{n}{V} \quad \text{(5.3)} \]

\[ \frac{n}{V} = \text{Molarity} \quad \text{(5.4)} \]

Where \( P \) is the calculated vapour pressure, \( V \) is volume in relation to the species in question, \( R \) gas constant = 0.08206 L atm mol\(^{-1}\) K\(^{-1}\) = 8.3145 KJ mol\(^{-1}\) K\(^{-1}\), \( T \) is temperature at that calculated vapour pressure in degrees Kelvin.

Estimation of the volume of gas or vapour is done by taking the volume of the container multiplied by the pressure of the gas or vapour at a given temperature and pressure and then divided by the atmospheric pressure. This can be illustrated as in equation 5.5.

\[ V_{\text{cal}} = V_{\text{cont}} \left( \frac{P_{\text{act}}}{P_a} \right) \quad \text{(5.5)} \]

Where \( V_{\text{cal}} \) is the estimated volume from the calculation, \( V_{\text{cont}} \) is volume of the container, \( P_{\text{act}} \) is the actual pressure of that vapour or gas at that temperature and \( P_a \) is the atmospheric pressure.

The calculated concentrations of the pollutant vapours in molarities are as presented in Table 5.7.
Table 5.7. Calculated concentrations for the organic pollutants.

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Vapour pressure</th>
<th>Molarity; mol L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTH</td>
<td>2.30 x 10(^{-1})</td>
<td>7.55 x 10(^{-3})</td>
</tr>
<tr>
<td>BaP</td>
<td>2.70 x 10(^{-4})</td>
<td>1.58 x 10(^{-7})</td>
</tr>
<tr>
<td>PRN</td>
<td>4.40 x 10(^{-4})</td>
<td>3.17 x 10(^{-8})</td>
</tr>
<tr>
<td>Py</td>
<td>1.88 x 10(^{-2})</td>
<td>5.49 x 10(^{-4})</td>
</tr>
</tbody>
</table>

Densities in g/cm\(^3\) and molar weights [Py (0.978, 79.1); ANTH (1.009, 178.23); BaP(1.351, 252.3); PRN(1.271, 202.25)]

Assumption that the concentrations of the vapours reaching the sensor film is the same, at 353 K, at every stroke of the pump, then as we continue pumping in these vapours, the same concentration aliquot is added to the sensor film. The same also applies to the subsequent films used to detect other pollutants vapours. It is also assumed that since the inlet tube length is short (36.3 mm) and the fact that these vapours are being pumped very fast into the flow cell, the temperature of the vapours reaching the sensor film was still at 353 K.

Plots for increasing amount of pollutants in mol L\(^{-1}\) onto the thin film of CoP(ph-OMe)\(_4\) in PDMS matrix are as presented in Figures 5.31, 5.32, 5.33 and 5.34 with ANTH, BaP, PRN and Py respectively.

![Figure 5.31](image-url)
When ANTH vapours were pumped onto CoP(ph-OMe)$_4$ film, there was reduction in fluorescence intensity as the concentration for ANTH increased onto the sensing film.

Figure 5.32. Plot for increasing [BaP] in mol L$^{-1}$ onto 6.9 µM CoP(ph-OMe)$_4$ film in PDMS matrix.

Figure 5.33. Plot for increasing [PRN] in mol L$^{-1}$ onto 6.9 µM CoP(ph-OMe)$_4$ film in PDMS matrix.
Figure 5.34. Plot for increasing [Py] in ppm onto 6.2 µM CoP(ph-OMe)$_4$ film in PDMS matrix.

**Figures 5.35, 5.36, 5.37 and 5.38** represent plots for the organic pollutants in mol L$^{-1}$ with FLXN.

Figure 5.35. Plot for increasing [ANTH] in mol L$^{-1}$ onto 9.2 µM FLXN film in PDMS matrix.
As illustrated in Figure 5.35, ANTH reduces the fluorescence intensity of the sensing molecule FLXN. While BaP increases the fluorescence intensity for FLXN after interaction as shown in Figure 5.36.

Figure 5.36. Plot for increasing [BaP] in mol L\(^{-1}\) onto 7.1 µM FLXN film in PDMS matrix.

Figure 5.37. Plot for increasing [PRN] in mol L\(^{-1}\) onto 9.6 µM FLXN film in PDMS matrix.
Fluorescence intensity for FLXN films reduces with increasing concentration for PRN as illustrated in Figure 5.37. While there is a sharp increase in the fluorescence intensity for FLXN with Py and subsequent reduction in intensity as the concentration for Py increase onto the sensing film as presented in Figure 5.38.

![Figure 5.38. Plot for increasing [Py] in mol L\(^{-1}\) onto 3.2 μM FLXN film in PDMS matrix.](image)

**5.15 Application of the Stern-Volmer equation to the data of thin films study.**

The ratio of the emission in the absence of quencher to that in the presence of quencher, gives an equation containing only fundamental rate constants and measurable quantities. This equation is known as the Stern-Volmer equation;

\[
\frac{I_0}{I} = 1 + K_q[C_{\text{conc}}],
\]

Where, \( I \) is changing fluorescence intensity of sensor film with increasing concentration of organic pollutants, \( I_0 \) the initial fluorescence intensity of the sensor film alone and [Conc.] is the concentration of the organic pollutants.
Using the Stern-Volmer equation, plots for \( \frac{I_0}{I} \ vs \ [Conc] \) were obtained. **Figures 5.39, 5.40, 5.41 and 5.42** are Stern-Volmer plots for CoP(ph-OMe)\textsubscript{4} with the organic pollutants ANTH, BaP, PRN and Py respectively.

![Stern-Volmer plot for fixed thin film concentration 3.9 μM of CoP(ph-OMe)\textsubscript{4} with increasing ANTH vapour.](image)

Figure 5.39. Stern-Volmer plot for \( \frac{I_0}{I} \ vs \ [Conc] \) of fixed thin film concentration 3.9 μM of CoP(ph-OMe)\textsubscript{4} with increasing ANTH vapour.

In a Stern-Volmer plot, a straight line through ‘1’ indicates either static or dynamic quenching while a curved plot indicate both dynamic and static quenching.

![Stern-Volmer plot for fixed thin film concentration 6.9 μM of CoP(ph-OMe)\textsubscript{4} with increasing BaP vapour.](image)

Figure 5.40. Stern-Volmer plot for \( \frac{I_0}{I} \ vs \ [Conc] \) of fixed thin film 6.9 μM concentration of CoP(ph-OMe)\textsubscript{4} with increasing BaP vapour.
Downward and upward curvatures are observed of the plots in Figures 5.39 and 5.40 respectively, which means that there is both static and dynamic quenching mechanisms taking place after interaction of CoP(ph-OMe)$_4$ with ANTH and BaP respectively as explained earlier. Likewise, Figures 5.41 and 5.42 too have curvatures of the plots, which may still indicate both dynamic and static quenching taking place.

![Stern-Volmer plot for CoP(ph-OMe)$_4$ with increasing PRN vapour.](image1)

Figure 5.41. Stern-Volmer plot for $\frac{I_0}{I}$ vs $[\text{Conc}]$ of fixed thin film concentration 6.9 μM of CoP(ph-OMe)$_4$ with increasing PRN vapour.

![Stern-Volmer plot for CoP(ph-OMe)$_4$ with increasing Py vapour.](image2)

Figure 5.42. Stern-Volmer plot for $\frac{I_0}{I}$ vs $[\text{Conc}]$ of fixed thin film concentration 6.2 μM of CoP(ph-OMe)$_4$ with increasing Py vapour.
Figures 5.43, 5.44 and 5.45 represent Stern-Volmer plots for the organic pollutants; ANTH, PRN and Py with FLXN films respectively.

Figure 5.43. Stern-Volmer plot for increasing concentration for ANTH vapour in mol L\(^{-1}\) with fixed 9.2 µM FLXN.

Figure 5.44. Stern-Volmer plot for increasing concentration for PRN vapour in mol L\(^{-1}\) with fixed 9.6 µM FLXN.
The curvatures observed in Figures 5.43, 5.44 and 5.45 also show that when the pollutants highlighted interact with FLXN they cause static and dynamic quenching to the fluorescence intensity of this sensing molecule.

### 5.16 Limit of Detection, Method Detection Limit and Limit of Quantification.

Limit of Detection (LOD) is defined generally as the lowest amount of a substance that can be distinguished from the blank (absence of that substance in the matrix) within a 99 % confidence level. The International Union of Pure and Applied Chemistry (IUPAC) define LOD as the concentration or quantity derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure. Limit of detection can be affected by the accuracy of the model used to predict concentration from raw analytical signal. This may apply to this study where Clausius-Clapeyron equation is used to estimate the concentration of the pollutants. The estimation is based on the assumption that the heat (353 K) generated PAHs vapours from the source will actually reach the sensor film roughly at that temperature. This is due to the short distance travelled
by the vapours within the delivery tube and the fact that it is sealed from the effect of atmospheric air within the analytical laboratory. Therefore, the cooling of these vapours before they reach the sensing film may be insignificant. In effect, this will directly be applicable to the calculated vapour pressures at that temperature.

Limit of Quantification (LOQ) is defined as the lowest amount of analyte in a given matrix that can be quantitatively determined with suitable precision and accuracy. It follows therefore that, LOQ is precisely lower than LOD, because LOD just shows qualitatively, that the analyte is present even if it is unquantifiable.

Method Detection Limit (MDL) is defined as the concentration measured by an individual instrument and which is statistically different from that instrument’s background noise. This quantity usually requires 7 different measurements of the sample at a low concentration.

Many researchers have used the blank signal to evaluate the LOD, which is then converted to concentration. Whereby, they take a replicate measurements (usually 7) of the blank (matrix minus analyte), then from this data, evaluation of the Relative Standard Deviation (RSD) or Standard Deviation (STDEV) is carried out. Then, 3 x (RSD or STDEV) of the blank signal gives an initial value, which can then be estimated to LOD in terms of concentration. On the other hand, large environmental monitoring agencies like EPA have used spiked method. Whereby, the blank matrix signal is obtained, subsequently spiked with a series of known concentrations of the analyte in question. Relative Standard Deviation calculated and then the evaluation of LOD, MDL and LOQ carried out.

In this study, 7 replicate measurements of the sensing molecules’ films with pollutants estimated at initial vapour concentration; thus one spectrum at a time on different sensor films was measured with pumping in of pollutant vapours from the syringe. Relative Standard Deviations were then evaluated of these data and from these relative standard deviations, the Relative Limit of Detection (RLOD) calculated using equation 5.5.

$$RLOD = 3 \times RSD$$  \hspace{1cm} 5.5
These RLODs are then multiplied by the initial concentrations of pollutants pumped onto the sensor film as estimated using Clausius-Clapeyron and the ideal gas equations (see sections 5.13 and 5.14) and these results give LODs. The LOD multiplied by 3, gives Limit of Quantification (LOQ). These results are as presented in Table 5.8.

Table 5.8. Calculated RSD, RLOD, LOD and LOQ for the solid thin films of CoP(ph-OMe)$_4$ and FLXN with ANTH, BaP, PRN and Py vapours

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>RSD (%)</th>
<th>RLOD</th>
<th>LOD µM</th>
<th>LOQ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CoP(ph-OMe)$_4$</td>
<td>FLXN</td>
<td>CoP(ph-OMe)$_4$</td>
<td>FLXN</td>
</tr>
<tr>
<td>ANTH</td>
<td>0.014</td>
<td>0.008</td>
<td>0.042</td>
<td>0.024</td>
</tr>
<tr>
<td>BaP</td>
<td>0.045</td>
<td>0.011</td>
<td>0.135</td>
<td>0.033</td>
</tr>
<tr>
<td>PRN</td>
<td>0.039</td>
<td>0.047</td>
<td>0.117</td>
<td>0.141</td>
</tr>
<tr>
<td>Py</td>
<td>0.033</td>
<td>0.002</td>
<td>0.099</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Data for method detection limits for the CoP(ph-OMe)$_4$ and the pollutants is as presented in Table 5.9.

Table 5.9. Evaluation of Method Detection Limit for solid thin films of CoP(ph-OMe)$_4$ and FLXN with ANTH, BaP, PRN and Py vapours

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>t-test</th>
<th>RSD (%)</th>
<th>MDL µM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CoP(ph-OMe)$_4$</td>
<td>FLXN</td>
<td>CoP(ph-OMe)$_4$</td>
</tr>
<tr>
<td>ANTH</td>
<td>0.315</td>
<td>0.216</td>
<td>0.014</td>
</tr>
<tr>
<td>BaP</td>
<td>0.493</td>
<td>0.865</td>
<td>0.045</td>
</tr>
<tr>
<td>PRN</td>
<td>0.523</td>
<td>0.418</td>
<td>0.039</td>
</tr>
<tr>
<td>Py</td>
<td>0.345</td>
<td>0.357</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Table 5.10 illustrates data for LOD, LOQ and MDL in comparison with those from three laboratories namely; EPA (USA), DIONEX International (Salt Lake city Utah USA) and OSHA analytical laboratory (Salt Lake city, Utah, USA).
Table 5.10. Data for LOD, LOQ and MDL of this study and for some international agencies.

<table>
<thead>
<tr>
<th>ANALYST</th>
<th>Pollutant</th>
<th>LOD: µg/L</th>
<th>LOQ µg/L</th>
<th>MDL µg/L</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>ANTH</td>
<td>57.1 (32.16)</td>
<td>171.35 (96.24)</td>
<td>6.0 (2.31)</td>
<td>Fluorescence With PMT detector</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td>0.0054 (0.0013)</td>
<td>0.0162 (0.0039)</td>
<td>0.0008 (0.0004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRN</td>
<td>0.0007 (0.0002)</td>
<td>0.0002 (0.0006)</td>
<td>0.0001 (0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Py</td>
<td>4.35 (0.266)</td>
<td>13.04 (0.799)</td>
<td>0.499 (0.0317)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brackets; FLXN</td>
</tr>
<tr>
<td>DIONEX</td>
<td>ANTH</td>
<td></td>
<td></td>
<td></td>
<td>Fluorescence With PMT detector</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Py</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>ANTH</td>
<td></td>
<td></td>
<td></td>
<td>Fluorescence With PMT detector</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td>0.28</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRN</td>
<td>0.045</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Py</td>
<td>0.26</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brackets; FLXN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EHP With UV-Florescence detector</td>
</tr>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*** (GC-MS)</td>
</tr>
<tr>
<td>OSHA</td>
<td>ANTH</td>
<td></td>
<td></td>
<td></td>
<td>HPLC With UV-Florescence detector</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td>0.028</td>
<td>0.066</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRN</td>
<td>0.045</td>
<td>0.0207</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Py</td>
<td>0.26</td>
<td>1.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brackets; FLXN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HPLC With UV-Florescence detector</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*** (GC-MS)</td>
</tr>
<tr>
<td>NIOSH</td>
<td>ANTH</td>
<td></td>
<td></td>
<td></td>
<td>HPLC With UV-Florescence detector</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td>0.01 - 0.09</td>
<td>0.023 – 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRN</td>
<td>0.006 – 0.08</td>
<td>0.020 – 2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Py</td>
<td>0.001 – 0.03</td>
<td>0.0036 – 0.99</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brackets; FLXN</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HPLC With UV-Florescence detector</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*** (GC-MS)</td>
</tr>
</tbody>
</table>

Concentrations of PAHs, specifically of this study in some soil and water samples in various locations in Manhattan (USA) as per EPA survey of April 2002 are presented in Table 5.11.

Table 5.11. EPA* concentrations for ANTH, BaP and PRN in soil and ground water as per the survey of April 2002 in Manhattan (USA)

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>[ppm] in soil</th>
<th>[ppb] in ground water**</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTH</td>
<td>0.24 - 130</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>BaP</td>
<td>0.11 - 46</td>
<td>&lt;0.61</td>
</tr>
<tr>
<td>PRN</td>
<td>0.11 - 120</td>
<td>&lt;2.0</td>
</tr>
</tbody>
</table>

*Source of information - EPA website; **ground water concentrations are in ppb.
The data for LOQ values of this study are much lower as compared to the Permissible Exposure Limits (PEL) values of these pollutants (see table in section 1.2 of chapter 1). These PEL values are as recommended by the cited international agencies in that table. These LOQ amounts calculated in this study are also lower than the possible concentrations in contaminated soils (Table 5.10). This calls for the need to develop a sensor for monitoring these pollutants so that their amounts in the environment are reduced to PEL. Online sensors would be necessary so that the pollutants are controlled before they reach and contaminate the environment especially the soils, in higher amounts than the PEL recommended by environmental pollution monitoring agencies. It is very possible as per the data in Table 5.10 that the amounts in ground water are less as compared to those in the soil because PAHs are sparingly soluble in water.

The investigative data of this study show that CoP(ph-OMe)₄ and FLXN are viable sensors for the organic pollutants selected for this research.
Chapter 6. Discussion, conclusion and future work appraisal.

6.1 Discussion of thin films study results.

The aim of this research project was to investigate immobilised thin films of CoP(ph-OMe)$_4$ and FLXN as viable remote sensors for the vapours of organic pollutants namely; ANTH, BaP, PRN and Py.

This aim has been achieved as highlighted here below:

1) Immobilisations within PDMS matrix of the sensing molecules named above were very successful. Results of the spectral data obtained of these films showed that; these sensor molecules were entrapped within the PDMS matrix. This could be the case because absorption and fluorescence spectra showed characteristic absorption and fluorescence peaks of these sensor molecules. Yet the surface of the matrix showed the characteristic rubbery finish of cured PDMS polymer.

2) A model of remote sensing of the vapours of ANTH, BaP, PRN and Py by the films of these sensor molecules was established. This remote sensing was made possible by fabrication of a flow cell that held the sensing molecules’ films outside of the LS 55 fluorometer and subsequently the pollutant vapours fed onto the sensor film which had been immobilised on glass substrate. The whole unit was then subsequently interfaced to the fluorometer using the optical fibre that transmitted radiant energy onto the sensing films. This achievement established that, if these molecules are subsequently developed in future as sensors for the enlisted pollutants, they can be held in miniaturised compartments near the pollution sources and remotely interfaced to the analytical instrument via the optical fibre.

Selectivity of CoP(ph-Me)$_4$ and FLXN towards the selected pollutant molecules has not been tested in this research. However, due to interaction of the organic
pollutants selected with these sensing molecules and each giving characteristic absorption and fluorescence patterns, then these sensing molecules can be used as multi-sensors. Which follows that, they can interact with similar harmful compounds that can react with them, thereby changing the way these sensors interact with radiant energy and giving characteristic spectra.

Sensitivity of these molecules towards the pollutants selected and that of the analytical technique have been established by calculating LOD and MDL respectively, which are recorded in µg L⁻¹ units (see results in section 5.16 of chapter 5). These calculations gave results in µg L⁻¹ amounts, which are less than the Permissible Exposure Limits (PEL) listed in chapter 1 of this thesis and as highlighted in literature. This is true when the sensitivity is defined generally as, the minimum input of physical parameter that will create a detectable output change. Otherwise sensitivity is also recorded as the slope of the calibration curve.

The sensitivity of the analytical technique as illustrated by the quantities of MDL ranging from 0.0001 to 6.0 µg L⁻¹ is reasonably high. The method is more sensitive to BaP and PRN with both the sensing molecules CoP(ph-OMe)₄ and FLXN. The sensitivity of the sensing molecules towards the pollutants is also high; with the amounts ranging from 0.0002 to 57.1 µg L⁻¹ and also showing more sensitivity towards BaP and PRN with both the sensing molecules as shown by calculated LOD values (see Table 5.9 section 5.16 of chapter 5). Generally, FLXN is more sensitive to these pollutants as compared to CoP(ph-OMe)₄.

There could be some degree of durability, accuracy, precision and repeatability in the signal of these immobilised films of these sensing molecules because when the films were left safely stored in the laboratory for at least 30 days, the sensing molecules still gave the signals of equal magnitude as first obtained before storage.

It can be noted therefore that, if developed, such kind of sensing system is simple to operate, maintain and due to its miniature form, can easily be portable.

Despite the highlights on the achievement of the objectives and aims of this research, there were some expected limitations, namely:
Possibility of less homogeneity in the distribution of sensor molecule inside the polymeric matrix.

Delivery of pollutant vapours to the sensor film.

Need for use of heat in generating the PAHs vapours.

Limited literature on solid thin films study on organic vapours for PAHs.

Basing on the method used to deposit aliquots of the mixture of the sensor molecule with the PDMS, it was anticipated that, homogeneous distribution of the sensing molecule films in the matrix would be likely. However, on taking absorption measurements of a series of films, it was discovered that the signal magnitude showed only an error of ±2 %. This error magnitude is less than the acceptable error of ±5 %.

Delivery of pollutant vapours was through pumping, using a vacuum pump onto the sensing film. This method was expected to have limitations when it comes to establishment of uniformity in the flow of the vapours onto the sensing film. To minimise this limitation, a short flow tube (36.30 mm) in length and of a micro-diameter (1.44 mm) was employed and this seemed to work as shown by the steadiness in the signals of the calibration plots.

Another significant limitation was that, the PAHs have low vapour pressures and therefore, the need to generate them using heat. It is cited in literature by some researchers, the method of thermo-desorption used in delivering pollutants onto the receptacle inside the instrument for analysis. This follows that, when evaluating the quantities of the pollutants, a method which puts into consideration fluctuation in temperature is employed. This example is as given in this research where the Clausius-Clapeyron equation which puts into consideration the vapour pressures and heats of vaporisation of pollutants at specific temperatures is used. The known vapour pressures and heats of vaporisation at specific temperatures are inserted in the equation to calculate the vapour pressures at the experimental temperature (see Clausius-Clapeyron equation in section 5.13 of chapter 5).
Published literature on specifically delivering vapours onto the thin films is limited to mostly description of gases. In some cases as highlighted in literature, solutions of organic vapours are fed onto sensing films.

6.2 Conclusion and outline of future work

Cobalt tetra-(phenyl-methoxy)porphyrin (CoP(ph-OMe)_4) and Bis[N,N-bis(carboxy-methyl)amino-methyl]fluorescein (FLXN), through studies carried out for presentation in this thesis, have proved to be viable sensors for ANTH, BaP, PRN and Py. Future work will entail developing them as sensors for the enlisted pollutants. To be developed as sensors, the following detailed investigations may be carried out:

- Establishment of selectivity of these sensors over ANTH, BaP, PRN and Py over other similar pollutant compounds.
- Validate Limit of Detection (LOD), Practical Quantifiable Limits (PQL) and MDL of the pollutants selected for this study after interaction with these sensor molecules.
- Ascertain Linear Dynamic Response range (LDR) for their sensing.
- Verify the lifetime of the sensors over a period given the years.
- Subject them to pH changes to compare their response and changes with their neutral form after interaction with the pollutants and radiant energy. Protonation and deprotonation are influenced by pH. Moieties like (-COOH) for FLXN and (-OCH₃) for CoP(ph-OMe)₄ can be deprotonated with changes in pH.
- Authenticate the factors that may influence their sensing mechanism, including:
  - Temperature
  - Humidity
Pressure changes

Apart from being immobilised in PDMS matrices, other polymeric materials such as PVC may also be employed. Polyvinyl Chloride is a polymeric membrane for encapsulating the sensing molecules just like PDMS. Alternatively, instead of these sensing reagents being immobilised in polymers, they may also be made into polymers themselves and be immobilised on solid supports.
References


Metalloporphyrins are synthesised from the porphine which is a basic unit structure for certain proteins and vitamins in living organisms. In animals it is part of the haeme, which is an Iron complex that is responsible for transporting oxygen in body tissues. In plants it is a magnesium porphyrin complex called chlorophyll that is a centre responsible for photosynthesis and because of these complexes being part of the living systems, they are used also as delivery mechanisms for certain drugs into the systems of animals. This follows that they are good binding reagents for certain materials therefore can be used as sensing systems.

Elucidation of the structure of the free base porphine ring by Codding et al 1972, through X-ray crystallography reveals that it is a macrocyclic ring system consisting of four alternate pyrole rings which are joined by methine carbon atom bridges (Figure PH 1.1). The porphine is a geocentric macrocyclic ring with two sets of independent pyrole ring systems, Codding et al 1972. The structure indicates varying N-H, C-C bond lengths and angles for the pyrole rings and the bridging methine C-C bonds. The highlighted bold black bonds indicate dominant resonating structure (Figure PH 1.2) at the core of the porphine. Codding et al has also illustrated the differences (Δ) between corresponding bonds and angles as it is indicated in (Figure PH 1.2). The C-C bonds in the porphine vary between 1.492 Å to 1.526 Å which are less than the normal C-C length of 1.54 Å. He
concluded from his observations that the structure of the porphine macromolecule is planar. Both [Chen et al 1972] and [Codding et al 1972] agree that the STD deviations from planarity are \( \pm 0.002 \) this falls within the acceptable limits of \( \pm 0.02 \). According to Codding et al, by comparing the free base porphine structure and tetra phenyl substituted porphine, substituents at the bridging methine carbons locally change the \( \pi \)-electron density but the 'mother ligand' macrocyclic structure remains intact. It follows that its planarity is not affected much even in metalloporphyrins which are derivatives of the mother ligand; the porphine. Depending on the type of substituents, the \( \pi \)-electrons of the porphyrin get delocalised and extend to the system of the substituents at the periphery of the macrocyclic ring system of the porphyrin. A case in point to substantiate the latter will be for the electron withdrawing groups that may pull the electrons towards themselves and therefore causing a delocalisation of these electrons in the entire system. This delocalisation is supported by [Nalwa et al 1981] that these complexes show semi conducting properties. [Atermis et al, 1978] has shown that metalloporphyrins with \( M^{2+} \) have two planes of symmetry and that the variation of bond angles and rotation of substituted ligands with respect to the porphyrin plane do not greatly affect the energy levels of this macromolecule. Following their study, [Atermis et al 1978] have also described the absorption spectra for metalloporphyrins as classified into three categories: Normal, hypso and hyper absorption bands depending on the central metal ion. According to his classification, hypso porphyrins have three bands of \( \pi-\pi^* \) transition in their spectra. They have the Q-band (0,0) blue shifted at \(<570 \text{ nm} \). These hypso complexes, according to his study, exhibit phosphorescence and radiationless characteristics. It follows that cobalt complexes, like the one selected for this study, according to Atermis's classification fall in this category. The cobalt complex solution in \( \text{CH}_2\text{Cl}_2 \) selected for this research however does fluoresce at 659/660 nm and 722/723 nm with excitation wavelength of 423 nm and has a fluorescence quantum yield of 0.66 and life time of 4.78 ns which was measured and evaluated during this study using comparison methods with the standard ZnPc. According to [Atermis et al 1978], [OsCoTPP(py)_2] complex has an additional band at 597 nm and 595 nm. This complex is co-ordinated to two pyridine molecules. In their study, [Atermis et al 1978], observed broadening of
the Q(0,0) band and attributes this to be due to the overlap of CT bands. In this study, [CoP(ph-OMe)₄(py)]²⁺ complex is thought to be a product formed when pyridine molecules interact with CoP(ph-OMe)₄ and the complex has new absorption bands at 438 nm, 553 nm and 595 nm. Also observed is broadening of the absorption bands at 553 and 595 nm. These bands are absent in the spectrum of the CoP(ph-OMe)₄ alone without pyridine. Although according to [Atermis et al 1978], the absorption band at 595 nm is thought of as originating from an impurity in the sample, yet this peak is observed in the spectra for CoP(ph-OMe)₄ in this study when interacted with pyridine as well. From their study, [Chen et al 1972] and [Atermis et al 1978] have observed that the planarity of porphine macromolecule is least affected by the substituents to the ring system. It is therefore expected that the metalloporphyrins maintains the planarity as described in these works. It follows therefore that CoP(ph-OMe)₄ is near planar in structure.

![Figure PH 1.2. The Porphine ring with varying angles and bond lengths. Bold outlined structure illustrates dominant resonating structure of this macro cyclic mole](image-url)
APPENDIX 2

WG 1.1. Waveguides.

Waveguides are used to streamline and lead radiant energy or light waves to targeted areas. Guiding of radiant energy is done in modes called Mode Orders (MO). Different modes have different angles (Q) depending on the media at the interfaces (Figures WG 1.1 and WG 1.2). Wave guiding is applied in optical fibres and it is very necessary when the numerical aperture (NA) of the optical fibre is small. Figure WG 1.3 illustrate a guided wave in a given substrate, where Q stands for relevant angle in a given mode.

![Figure WG 1.1. Wave radiation in air.](image)
Transmission of the guided light along the length of a fibre is expected to be at a constant angle with respect to the optical fibre axis. Although this is assumed to be the case, scattering may cause variations in these angles. Scattering may be at the interface of the fibre and cladding due to irregularities in the core and cladding interface of the fibre.
FQ 1.1. Absorption and fluorescence spectroscopy data for evaluation of quantum yield of CoP(ph-OMe)$_4$.

FQ 1.1 Procedure

Stock solutions and subsequent dilute solutions were prepared as in previous studies of this research. From experimental trials, it was established that addition of aliquot volumes of the solutions of the analytes from their stock solutions of known concentration into the cuvette, followed by thorough mixing and then absorption and fluorescence measurements performed, minimised errors encountered due to evaporation of the solvent(s).

Zinc pthalocyanine was selected as the standard of known quantum yield to be used as a comparison to evaluating true quantum yield values for CoP(ph-OMe)$_4$. This is because the same excitation wavelength 423 nm for CoP(ph-OMe)$_4$ is also applicable to this standard. This will ensure that the same amount of energy is used for excitation of the molecules for both the standard and the test sample.

Zinc phthalocyanine standard solutions were prepared in 1% pyridine in toluene while CoP(ph-OMe)$_4$ solutions were prepared in CH$_2$Cl$_2$. Since the amount of pyridine was small in toluene, the refractive index for pyridine was insignificant. Evaluated experimental data for quantum yields are corrected using refractive indices of the solvents and the known ZnPc standard quantum yield. Prior to performing both absorption and fluorescence spectroscopic measurements on test solutions, blank solutions of purely the composition used as solvents without the analytes were measured in order to establish the baseline. Establishment of the baseline is in part sureness of reasonable elimination of errors from instrumental effects such as background absorption due to undesired radiation(s) and other contaminants from the solvent that may interfere with the true signal of the analyte(s) under study.

Absorption and fluorescence spectra for ZnPc are as presented in Figures FQ 1.1 and FQ 1.2. Absorption and fluorescence spectra for CoP(ph-OMe)$_4$ are not
shown in this section because they have been represented earlier in sections 4.3.4 of the main body thesis and 4.2.6.2 respectively. However, plots for data from those spectra are illustrated in this section since this particular data will be used alongside that of ZnPc for evaluation of quantum yield for CoP(ph-OMe)$_4$. Absorbance and fluorescence intensities of a range of concentrations for CoP(ph-OMe)$_4$ and ZnPc were recorded (Tables FQ 1.1 and FQ 1.2).

Corrected calibration graphs for evaluating, $\Phi_F$, are a plot of measurand sample fluorescence vs absorption intensities as presented in Figure FQ 1.3 and FQ 1.4.

Figures FQ 1.1. Absorption spectra for 0.08, 0.1, 0.15, 0.22 and 0.52 μM ZnPc STD in 1 % pyridine in toluene.

Figure FQ 1.2. Fluorescence spectra for 0.08, 0.1, 0.15, 0.22 and 0.52 μM Zinc phthalocyanine (ZnPc) STD in 1 % pyridine in toluene.
Table FQ 1.1. Absorption and fluorescence intensities for CoP(ph-OMe)$_4$

<table>
<thead>
<tr>
<th>[CoP(ph-OMe)$_4$] μM</th>
<th>Absorbance**</th>
<th>Fluorescence intensity**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>414 nm</td>
<td>530 nm</td>
</tr>
<tr>
<td></td>
<td>659 nm</td>
<td>723 nm</td>
</tr>
<tr>
<td>0.00</td>
<td>0.000*</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.06</td>
<td>0.097656</td>
<td>0.006302</td>
</tr>
<tr>
<td></td>
<td>20.53853</td>
<td>3.984578</td>
</tr>
<tr>
<td>0.08</td>
<td>0.137741</td>
<td>0.010254</td>
</tr>
<tr>
<td></td>
<td>46.70334</td>
<td>9.625138</td>
</tr>
<tr>
<td>0.10</td>
<td>0.176102</td>
<td>0.010910</td>
</tr>
<tr>
<td></td>
<td>65.69195</td>
<td>13.32851</td>
</tr>
<tr>
<td>0.20</td>
<td>0.356552</td>
<td>0.021637</td>
</tr>
<tr>
<td></td>
<td>160.2731</td>
<td>32.53695</td>
</tr>
</tbody>
</table>

*Absorbance and fluorescence intensities for the solvents.

** True values of analytes after subtracting solvent abs. and fluorescence intensity values.

In order to evaluate the quantum yield for CoP(ph-OMe)$_4$, the raw luminescence data for both CoP(ph-OMe)$_4$ and the std are corrected (Equation FQ 1.1).

Using comparison method, [Jobin Yvon Horiba method, William et al 1983], a curve for corrected fluorescence intensity Vs absorbance is then plotted. The

Table FQ 1.2. Absorption and fluorescence intensities for ZnPc

<table>
<thead>
<tr>
<th>[ZnPc] in μM</th>
<th>Absorbance**</th>
<th>Fluorescence intensity**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>423 nm</td>
<td>611 nm</td>
</tr>
<tr>
<td></td>
<td>679 nm</td>
<td>745 nm</td>
</tr>
<tr>
<td>0.00</td>
<td>0.000000*</td>
<td>0.000000</td>
</tr>
<tr>
<td></td>
<td>0.0*</td>
<td>0.0</td>
</tr>
<tr>
<td>0.08</td>
<td>0.000205</td>
<td>0.02900</td>
</tr>
<tr>
<td></td>
<td>79.0</td>
<td>15.0</td>
</tr>
<tr>
<td>0.10</td>
<td>0.000229</td>
<td>0.08279</td>
</tr>
<tr>
<td></td>
<td>213.0</td>
<td>40.0</td>
</tr>
<tr>
<td>0.15</td>
<td>0.001617</td>
<td>0.09879</td>
</tr>
<tr>
<td></td>
<td>254.0</td>
<td>50.0</td>
</tr>
<tr>
<td>0.22</td>
<td>0.004059</td>
<td>0.11707</td>
</tr>
<tr>
<td></td>
<td>301.0</td>
<td>58.0</td>
</tr>
<tr>
<td>0.52</td>
<td>0.008942</td>
<td>0.14880</td>
</tr>
<tr>
<td></td>
<td>392.0</td>
<td>74.0</td>
</tr>
</tbody>
</table>

*Absorbance and fluorescence intensities for the solvents.

** True values of analytes after subtracting solvent abs. and fluorescence intensity values.
ratio for the slope of test sample and the standard is obtained. The ratio obtained, when multiplied by ratio of squared refractive indices for the solvents used for test analyte and standard gives the quantum yield for the test sample. Absolute quantum yield for the test sample is then obtained after multiplication with the known standard quantum yield.

\[ F_{corr} \approx F_{obs} \times \text{antilog} \left( \frac{OD_{ex} \times OD_{em}}{2} \right) \]  

\( FQ1.1 \)

Assumption that there is no \( OD_{em} \), then \( OD_{ex} = 0.2322 \).

In order to get the value for the \( OD_{ex} \), Beer Lambert’s law expression (Equation FQ 1.2) is employed.

\[ \log_{10} \left( \frac{I_0}{I} \right) = OD \]  

\( FQ1.2 \)

\( OD = \) Optical density = Absorbance.

Excitation wavelength for CoP(ph-OMe)\textsubscript{4} is 423 nm and the emission wavelengths are at 659.5 ~ 660 nm and 722 ~ 723 nm. Energy in Joules for \( I_0 \) and \( I \) were obtained by using formula shown in equation FQ 1.3. The ratios for these energies were obtained and their logarithms evaluated for 660 nm and 723 nm. These logarithms were (0.1936) and (0.2322) respectively. Their antilogs at 660 nm and 723 nm were evaluated as (1.2482) and (1.3065) respectively. The values for the antilogs were multiplied by \( F_{obs} \) in order to obtain the value for \( F_{corr} \).

\[ E = \frac{hc}{\lambda} \]  

\( FQ1.3 \)

\( E = \) Energy in joules or KJ

\( h = \) Planck’s constant, 6.63 x 10\textsuperscript{-34} Js
c = Frequency of light, $3.0 \times 10^8$ ms$^{-1}$

$\lambda$ = Wavelength in nm.

Table FQ 1.3. Corrected fluorescence data for CoP(ph-OMe)$_4$ in CH$_2$Cl$_2$ required for plotting a curve for fluorescence vs Absorbance (the slope for the curve to be used for quantum yield, $\Phi_F$, evaluation).

<table>
<thead>
<tr>
<th>Absorbance (414 nm)</th>
<th>Corrected fluorescence intensity, $F_{corr}$ (660 nm)</th>
<th>(723 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.056</td>
<td>34.4</td>
<td>4.3</td>
</tr>
<tr>
<td>0.15</td>
<td>92.4</td>
<td>9.1</td>
</tr>
<tr>
<td>0.18</td>
<td>112.8</td>
<td>12</td>
</tr>
<tr>
<td>0.26</td>
<td>155.4</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Plots for emission (corrected) vs absorbance for CoP(ph-OMe)$_4$ and ZnPc std were obtained (Figures FQ 1.3 and FQ 1.4) and their gradients evaluated using trendlines from which the relative quantum yield is obtained using equation FQ 1.4. The absolute value is then evaluated by multiplying by the relative quantum yield by 0.3 which is the known quantum yield for ZnPc standard obtained from literature [Jobin Yvon Horiba method, William et al 1983].
Quantum yield for CoP(ph-OMe)$_4$ can be evaluated using equation FQ 1.4.

$$\phi F_{\text{test}} = \phi F_{\text{std}} \left[ \frac{\text{Gradient}_{\text{test}} (\eta_{\text{test}}^2 \text{solvent})}{\text{Gradient}_{\text{std}} (\eta_{\text{std}}^2 \text{solvent})} \right]$$

FQ1.4
Refractive indices for CH$_2$Cl$_2$ and toluene/pyridine mixture are 1.424 and 1.494 respectively (as per their label on respective containers). When values for the slopes of CoP(ph-OMe)$_4$ and ZnPc standard are substituted in equation 3.15, the relative quantum yield for CoP(ph-OMe)$_4$ obtained was 2.21. When corrected using the quantum yield 0.3 which is for ZnPc standard, it is found to be 0.66. In literature, [Adachi et al 2002], reported a fluorescence quantum yield for Cobalt(III) tetraphenyl porphyrin to be 0.56 while [Hoshino et al 1986] recorded a quantum yield for Cobalt(II)Tetraphenylporphyrin as 0.75. The value obtained from this study compares well with these two values as it lies between them. Quantum yield can also be described as in equation FQ 1.5.

\[ \Phi_F = \frac{\text{Measured fluorescence lifetime}, \tau_F}{\text{Time const. for emission in the absence of competing process}, \tau_R} \]  

FQ1.5

In this regard, quantum yields, (\(\Phi_F\)) and life-times, tau (\(\tau\)), are important to note during evaluation of suitability of molecules as sensors. Interaction of pollutant molecules with the sensing molecules may result in changes in the quantum yield and life-times of the sensor molecule. Measured fluorescence life-times, \(\tau_F\) is the excited state life-time which has been affected by such process as internal conversion (IC), intersystem crossing (ISC) to triplet state and interactions of the sensing molecule through charge transfer (CT) and spin-orbit couplings. Intrinsic life-time, \(\tau_R\) represent decay time without competing processes as mentioned above. Known quantum yields and measured fluorescence life-times can help evaluate intrinsic life-time using equation FQ 1.5. Therefore the effect of competing processes to the sensor alone and the sensor in the presence of pollutants can be evaluated. Fluorophores exhibit reduction in quantum yields when they form complexes. Quantum yields are sensitive to the molecular environment including, pH, temperature and also changes to molecular structure which could be due to complex formation. Higher quantum yields are desirable in sensing and typical quantum yields for the commonly used fluorophores range from 0.05 – 1.0.

The instrument used for this measurement was fluorometer FL920. It is computer interfaced and has a software nF900. Prior to life-time measurements, the instrument is switched on and the temperature of detector allowed to fall to -20 °C before the operation of the instrument begins. The electrodes provide sparks for the excitation of the gas molecules in order to produce a spectrum, the energy from which the test sample will absorb. These electrodes have to be cleaned using a fine grade sand paper and impurities on the surface from the sand paper removed using pure methanol solvent. After proper alignment of the electrodes in their compartment and reassembling of these components to the rest of the optical axis of the instrument, nF900 software is activated. The inlet line to the instrument for the gas to be used in generation of the spectrum is purged four times to remove any impurities. After which the gas pressure, pulse rate and PMT voltage are set accordingly depending on the type of gas used for generating the continuum. As mentioned earlier, H$_2$ gas was used in this study and the parameters for the gas are as outlined in section 3.8.2.1. After these parameters are set, the excitation and emission wavelength(s) for the test analyte are subsequently set. Known concentration of the solution of the analyte is placed in a 5 ml in the sample compartment, and the measurements are commenced. This experiment takes at least 8 hrs to achieve a representative spectrum for the raw data which is further processed to evaluate the life-time. After the sample spectrum is recorded, a spectrum of the scatter solution which scatters at least 80 % of the radiant energy used to excite the test sample is recorded. The scatter solution used in this study is 1 % colloidal silica in water. When a good tail fit or reconvolusion is carried out on the raw data using nF900 fluorescent software in the instrument in order to get the decay life-time of the sample, the scatter solution spectrum is subtracted from the test sample spectrum. A good tail fit or reconvolution gives the residuals of the scatter solution scattered about zero of the horizontal axis.
LT 1.2. Results of life-time measurements for CoP(ph-OMe)$_4$

Raw data for life-times was obtained and curve fitting done to get actual life-time.

![Image](image.png)

**Figure LT 1.1.** Raw data life-time decay of CoP(ph-OMe)$_4$ in CH$_2$Cl$_2$.

**Figure LT 1.1** shows the typical decay curve obtained for 0.02 µM CoP(ph-OMe)$_4$ without OP analyte(s). The spectrum shows raw data which requires tail fitting or reconvolution to evaluate the life-time, $\tau$, for the sensor molecule. The IR1 is the instrument response factor curve. This response factor curve is obtained through measurement using scatter solution (1% colloidal silica in water). The blue curve is the decay curve for test sample. When a tail fit or reconvolution is performed to the decay curve, IR is subtracted as background or instrument noise from test sample curve. Reconvolution of the raw data of the curve in **Figure LT 1.1** gave a value of $\tau = 4.78$ ns as decay time for the sensor molecule, CoP(ph-OMe)$_4$ alone. Reconvolution gives only shorter life-times. Evaluation of longer life-times is carried out through good tail fitting using software, nF900 in the fluorescence life-time instrument.
The **Figure LT 1.2** represents a reconvoluted curve for raw life-time data for CoP(ph-OMe)$_4$ without any OP. Life-time obtained was automatically recorded as 4.78 ns ($X^2 = 1.355$). The residuals (subtracted noise) are scattered around zero indicating a reconvolution of a reasonable accuracy. Accurate life-times are measured using comparative methods with the standards [William et al 1983]. A tail fit cannot give lower life-time estimates as that evaluated through reconvolution **Figure LT 1.2**. A tail fit was manually done in order to evaluate lower life-times and the results obtained clearly showed that this can give significant error **Figure LT 1.3**.
Figure LT 1.3. A demonstration of how tail fitting is not suitable for obtaining shorter life-time decay.

The tail fit for lower life-time values as shown in Figure LT 1.3 shows residuals that are not scattered at zero and the chi-square ($X^2$) is much higher than 1.0. This may lead to errors in the life-times. It is worth noting that wrong life-times are very easy to obtain and therefore comparative method for the life-times is recommended. For the sensor molecule CoP(ph-OMe)$_4$, in this study, Zinc phthalocyanine and Chlorophyll (A) are the standards normally used, because the excitation bands for these standards match closely to its excitation bands. The emission band between 600- 750 nm is also in the same range for both the Zinc phthalocyanine standard and CoP(ph-OMe)$_4$. Fluorescence decay life-time measurements of 0.1 µM of CoP(ph-OMe)$_4$ in the presence of 0.1 µM anthracene were carried out and a tail fit was performed to obtain a life-time of 8.37 ns with a chi-square value of 1.125 Figure LT 1.4.
As indicated, in Figure LT 1.4, the residuals (red curve) are well scattered about zero base-line. This shows a good tail fit that is likely to give values of life-times closer to the true value. A good tail fit is based on smaller value for chi-square ($X^2$), usually below 1.0 or slightly above. Reconvolution method did not automatically give any lower life-time figures for this data.

Figure LT 1.4. Good tail fit life-time decay curve of 0.1 μM CoP(ph-OMe)$_4$ in CH$_2$Cl$_2$ in the presence of 0.1 μM anthracene.

Figure LT 1.5. Good tail fit life-time decay curve of CoP(ph-OMe)$_4$ in the presence of benzo(a)pyrene
Figure LT 1.5 illustrates a good tail fit of fluorescence decay life-time for CoP(ph-OMe)$_4$ in the presence of benzo(a)pyrene. The life-time for this data is 8.70 ns with chi-square ($X^2$) value of 1.135.

Figure LT 1.6 presents tail fit curve for fluorescence life-time for CoP(ph-OMe)$_4$ in the presence of pyrene. The life-time for CoP(ph-OMe)$_4$ in the presence of pyrene is 8.00 ns with $X^2$ of 1.093.

![Figure LT 1.6](image)

Figure LT 1.6. Good tail fit life-time decay curve of CoP(ph-OMe)$_4$ in CH$_2$Cl$_2$ in the presence of pyrene.

In Figure LT 1.6 it is presented life-time decay curve for 0.2 µM CoP(ph-OMe)$_4$ in the presence of 0.5 µM pyridine whose results are; Tail fit data; $X^2 = 1.075$, $\tau = 10.45$ ns.

![Figure LT 1.7](image)

Figure LT 1.7. A good tail fit life-time decay curve of 0.2 µM CoP(ph-OMe)$_4$ in the presence of 0.5 µM pyridine.
Fluorescence life-time decay data for ZnPc standard is presented in Figures LT 1.8 and LT 1.9.

Figure LT 1.8. Raw data curve for the life-time decay of 0.357 µM ZnPc standard in 1% pyridine in toluene.

Figure LT 1.9. Good tail fit life-time decay curve of 0.357 µM ZnPc standard in 1% pyridine in toluene.

The tail fit gave the life-time decay for 0.357 µM ZnPc standard in 1% pyridine in toluene as 3.61 ns as shown in Figure LT 1.9. Sabata et al (2004) have obtained 3.9 ns life-time for ZnPc. The value obtained in this research is closer to this value.
from literature. Apart from using the nF900 software for reconvolution and tail fitting, the life-time values can also be obtained by plotting the logarithms of photon counts against time. Using this method, the plot in Figure LT 1.10 was obtained and a life-time for CoP(ph-OMe)$_4$ was estimated to be 4.22 ns, the calculation is illustrated after Figure 3.70. This value is close to the reconvoluted value of 4.78 ns obtained previously for this molecule.

![Fluorescence lifetime curve for 2 x 10^{-7} M CoP(ph-OMe)$_4$](image)

Figure LT 1.10. Estimation of the decay life-time for CoP(ph-OMe)$_4$ by getting the logs of photons counts and plotted vs time.

The exponential decay curve was re-plotted using log of photon counts per unit time. To get the slope numerical value, the trend line was added. The equation of the curve was then estimated from the trend line as a linear fit: The equation of the curve as estimated using the trend line:

$$y = -0.2372x + 2.8549$$

The slope of this curve is (-0.2372) and it is equal to, $\frac{1}{\tau}$.

$$-\frac{1}{\tau} = -0.2372$$

$$\tau = 4.22 \text{ ns}$$
From the experimental data, the life-times obtained are; 4.78, 8.37, 8.70, 8.00 and 10.45 ns for CoP(ph-OMe)₄ alone, and in the presence of ANTH, BaP, PRN and Py respectively. It is therefore observed that, in the presence of organic pollutants, the excited state life-time is altered. This evidence is also recorded in literature [Aizawa et al 2002]. Aizawa and co-workers recorded a life-time of 15 ns, after formation of anthracene-N,N-Dimethylanaline (ANTH – DMA)* exciplex. **Greiner 2000** recorded the life-time for anthracene alone in ethanol as 5.4 to 6.7 ns with increasing temperature. Life-times can reveal the type of quenching process. In static quenching, which is quenching due to formation of a sensor-quencher complex, the fluorescence intensity is reduced but the measured life-time remains unaltered. While in dynamic quenching, which is quenching due to IC, CT, ISC and spin-orbit couplings of the interacting molecules, there is reduction in fluorescence intensity and alteration in fluorescence life-time **[Davidson 1998 – 2009]**. Therefore life-times can be used to evaluate interaction modes of the sensing molecules and the pollutants. Quantum yields and fluorescence life-times for FLXN and in the presence of organic pollutants were not measured and therefore not presented in this thesis.

Factors that may affect fluorescence life-time include:

Ion intensity
Hydrophobic properties of the molecules
Oxygen concentration
Molecular binding,

Molecular interaction by energy transfer
It is recorded in literature [Benninger et al 2007], that increase in fluorescence life-time can be due to formation of a stable complex. This was established through their research on fluorescence life-time imaging of DNA-Dye fluorophore interactions. It is also cited in literature that hydrophobic binding of ligands to host molecules increase fluorescence life-times.