CHAPTER – 4

EXPERIMENTATION

The complete experimental set-up along with the fabrication of sensor device is presented in Chapter-3. The detailed block diagram of experimentation and experimental procedure are presented in this chapter.

4.1 Flow Diagram of Experimentation.

The process of experimentation completes in three phases. Phase -1 covers the fixing of the fabricated Biosensor cantilever device to experimental set-up and exposing it to N₂ and CO gas mixtures for CO adsorption in the lab. This causes deflection of the cantilever which is recorded as voltage (Output). This deflection may also be caused due to the pressure of the gas flow. In order to ascertain that the deflection is purely due to adsorption of CO with porphyrin, a conformation test is conducted. First the biosensor with porphyrin coating and without porphyrin coating are exposed to the CO and N₂ gases and the results are analysed. Further, the conformation test is conducted on SU-8 coated silicon wafer functionalized with porphyrin, which is flat surface. This test is conducted in phase-2.

In phase-2, the wafer coated with porphyrin is exposed to CO for its adsorption. If CO is adsorbed on the surface of the wafer, it is implied that the deflection of the cantilever in phase-1 experiments are due to Fe-CO binding and not by the pressure of the gas flow. Phase-2 deals with characterizations tests for conforming the binding (adsorption) of CO with porphyrin. These tests supports the experiments conducted in phase-1.

The above experiments conform the CO adsorption with the biosensor. This biosensor is tested further for the following basic sensitivity characteristics.
In addition to the above, the biosensor is further tested for various porphyrins for CO adsorption. These results are presented and discussed in Chapter-5.

Subsequently, in Phase-3 experiments, Biosensor developed at IIT, Bombay, and the commercial CO sensor (TGS 5042) are tested on Twin cylinder water cooled stationary diesel engine at the Department of Mechanical engineering, Srikalahasteeswara Institute of Technology, Srikalahasthi college laboratory and the results are analysed. The complete process of experimentation is shown in flow diagram Fig (4.1).
The three phases of experimentation are explained above in detail and the experimental procedure is described below.

4.1.1 Experimental Procedure (Phase-1)

The experimental procedure is as follows: The fabricated biosensor device is fixed in its place as shown in Fig(3.1) in the experimental set-up i.e in one of the arm of the Wheatstone Bridge and the output of the Wheatstone Bridge is connected to TI Board. The four arms of the Wheatstone Bridge are balanced such that the output voltage of the bridge is zero by varying the variable resistor. But most of the times, even after balancing the bridge, the output voltage was found to have certain minimum value, which the present investigation has taken as base value.

The N\textsubscript{2} and CO cylinders are connected to gas mixing chamber. The N\textsubscript{2} is allowed first to pass to gas mixing chamber at constant flow rate. Then the flow controllers are adjusted for the required CO concentration, normally it is varied from 7 SCCM (Standard Cubic Centi Meter) to 70 SCCM. The flow cell lid is in forced fit to the base on the sensor device. Now the pump is switched on to draw the CO and N\textsubscript{2} mixture of required concentration from the mixing chamber through the sensor device. As the mixture of the gas passes over the cantilever, the CO gets in contact with the porphyrin. It is due to the affinity of CO with porphyrin the CO gets deposited on the cantilever and creates surface stress over the cantilever, so the cantilever deflects causing change in resistance. The change in resistance in terms of Voltage is measured by read-out method and is recorded using a Personal Computer (PC). The same procedure is repeated at various CO flow rates. The results recorded are plotted graphically using Origin Lab Software. The changes in resistance (Voltage) Vs Time are analysed. The response curve for CO adsorption on cantilever
coated with porphyrin and without porphyrin (Bare) cantilever are recorded and presented below.

**CO Response Curve of Cantilever Coated with Porphyrin (Sample test)**

Fig(4.2) shows the response curve of the cantilever coated with porphyrin for exposure of 20 SCCM of carbon monoxide. From the Fig (4.2) it can be observed that the base output voltage of the Wheatstone Bridge is around 0.75mV for the exposure of nitrogen in the first 500s of the commencement of experiment. It is pertinent to note at this point from the Fig (4.2) that the output voltage suddenly jumps to a value of 2.5mV and remains at that value for the next 500s during which the sensor is exposed to carbon monoxide. It is interesting to note further that the output voltage drops back to the base voltage after 1000s from the commencement of experiment due to the termination of the supply of carbon monoxide. The response and recovery time are observed to be 1s and 3s. This indicates clearly that the sensor is responding to carbon monoxide exposure.

**CO Response Curve of Bare Cantilever (uncoated) (Sample Test)**

The response of uncoated (Bare) SU-8/CB cantilever for 20 SCCM flow of CO gas is shown in Fig (4.3). It can be clearly observed that the cantilevers didn’t respond to either of the gasses, since the change in voltage of the cantilever for N₂ and CO is in between 0.00 to 0.0015mV. This clearly demonstrates that only porphyrin functionalized microcantilevers respond to CO gas, but bare cantilevers do not respond. Further by comparing Fig(4.2) and Fig(4.3), it can be noted that there is a significant increase in the value of output voltage for porphyrin coated cantilever when exposed to CO compared to bare cantilever exposed to CO of same volume flow rate.
From this it can be concluded that the deflection of porphyrin coated cantilever is due to adsorption of CO but not due to pressure of the CO Gas.

![Graph](image)

**Fig (4.2)** Response curve of CO and N\(_2\) gas with porphyrin coated cantilever sensor device

![Graph](image)

**Fig (4.3)** Response curve of CO and N\(_2\) gas with bare (uncoated) cantilever (sensor device)
Comparison of Present Investigation with the Earlier Work

The experimental results (response curves) shown in Fig (4.2), are compared with a similar work of Santhosh Paul et al [141]. Their results are presented in Fig (4.4). It is observed from their results that there is an increase in resistance due to CO adsorption in the region of response and decreases in resistance during recovery in the absence of CO for different concentrations. A similar trend is observed in the response curve of research work shown in Fig (4.2) of the cantilevers functionalized with Fe(III)porphyrin. It is further noticed that the response and recovery time of the present work is 1 sec and 3 sec, whereas it is 12 sec and 169 sec in the works of Santhosh Paul et al., [141]. This feature has added advantage of biosensor in detection of carbon monoxide. Hence, biosensors, functionalized with porphyrin are appropriately suited for automotive applications where quick response is required.

![Graph](image-url)

Fig 6. Response curve of the PPy-FeTPPCl sensor to 330 ppm and 100 ppm CO at room temperature (25° C) under dry conditions (< 10% humidity).

Fig (4.4) Results of researchers taken as model.
The adsorption of CO with porphyrin is further confirmed by means of conformation tests which are presented below.

4.1.2 Conformation Tests of CO binding to Porphyrin (Phase-2)

The characterisation i.e. conformation of CO binding to porphyrin on the cantilever’s surface has been done by using Fourier transform infrared (FTIR) spectroscopy supplied by Perkin Elmer and UV-VIS spectroscopy supplied by Perkin Elmer. Atomic Force Microscopy, (AFM) (Pico Scan 2100) is used for finding surface morphology. These processes for characterization are quite difficult on actual cantilevers because of their miniature size. Hence, a separate surface is prepared with similar properties to emulate the surface of the cantilever which is described below.

A thin film of SU-8 2002 is spin coated on a RCA (Radio Corporation of America) cleaned silicon wafer of p-type <1,0,0> orientation. This wafer was then given a blank exposure of UV as part of the Lithography process. Once the surface was ready, a drop of porphyrin is put on the surface which spreads to form a thin film. This surface is then utilised for FTIR, UV-VIS and AFM studies as samples. The salient features of FTIR, UV-Vis and AFM are presented.

**Fourier transform infrared (FTIR) spectroscopy**

Fourier transform infrared (FTIR) spectroscopy Fig(4.5) is a measurement technique that allows one to record infrared spectra as shown in Fig (4.6). Infrared light is guided through an interferometer and then through the sample (or vice versa). A moving mirror inside the apparatus alters the distribution of infrared light that passes through the interferometer. The signal directly recorded, called an "interferogram", represents light output as a function of mirror position. A data-processing technique called Fourier transform turns this raw data into the desired
result (the sample's spectrum): Light output as a function of infrared wavelength (or equivalently, wavenumber).

The samples prepared by the present investigation as explained above is exposed to infrared light and the results are recorded. Examination of the transmitted light reveals how much energy is absorbed at each wavelength. This can be done with a monochromatic beam, which changes in wavelength over time, or by using a Fourier transform instrument to measure all wavelengths at once. From this, a transmittance or absorbance spectrum can be produced, showing the IR wavelengths at which the sample absorbs. Analysis of these adsorption characteristics reveals details about the molecular structure of the sample. When the frequency of the IR is the same as the vibrational frequency of a bond then adsorption occurs.

The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far- infrared, named for their relation to the visible spectrum. The higher energy near-IR, approximately 14000–4000 cm\(^{-1}\) (0.8–2.5 μm wavelength) can excite over-tone or harmonic vibrations. The mid-infrared, approximately 4000–400 cm\(^{-1}\) (2.5–25 μm) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The far-infrared, approximately 400–10 cm\(^{-1}\) (25–1000 μm), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy. The names and classifications of these subregions are conventions, and are only loosely based on the relative molecular or electromagnetic properties. The mid-infrared, approximately 4000–400 cm\(^{-1}\) (2.5–25 μm wavelength) is used in this experiment.
In the present investigation, the investigator is interested in the spectrum of metal carbonyl group formation as iron porphyrin and carbon monoxide are interacting. The metal carbonyl group formation is further discussed at length for a clear insight on binding of CO with Metal (Fe).

**Metal Carbonyl group** are the compounds that contain carbon monoxide as a coordinated ligand. Carbon monoxide is a common ligand in transition metal chemistry, in part due to the synergistic nature of its bonding to transition metals. The bonding of CO to a metal consists of two components. The first component is a two electron donation of the lone pair on carbon (coordination exclusively through the oxygen is extremely rare) into a vacant metal d-orbital. This electron donation makes the metal more electron rich, and in order to compensate for this increased electron density, a **filled** metal d-orbital may interact with the **empty** pi* orbital on the carbonyl ligand to relieve itself of the added electron density. This second component is called **pi-backbonding** or pi-backdonation. This is shown in Fig (4.7) diagramatically as well as through a simple MO picture below. The MO is color coded and it can be identified by each component of the bonding interaction.
IR peaks of Metal carbonyl group are as follows [18]

Uncoordinated or "free" CO: 2143 cm\(^{-1}\)

Terminal M-CO: 2125 to 1850 cm\(^{-1}\)

Doubly bridging (mu-2): 1850 to 1750 cm\(^{-1}\)

Triply bridging (mu-3): 1675 to 1600 cm\(^{-1}\)

Fig (4.7) Bonding of CO to metal

C. Bernard et al.,[129] have explained the probable reaction that can takes place in porphyrin-CO binding which is shown in Fig (4.8).

Fig (4.8) Probable reaction of porphyrin bonding to CO.
**UV-VIS spectroscopy**

Ultraviolet-visible (UV-Vis) spectroscopy as shown in Fig(4.9) is used to obtain the absorbance spectra of a compound in solution or as a solid. UV-Vis spectroscopic data can give qualitative and quantitative information of a given compound or molecule. UV-Vis actually being observed spectroscopically is the absorbance of light energy or electromagnetic radiation, which excites electrons from the ground state to the first singlet excited state of the compound or material. The UV-Vis region of energy for the electromagnetic spectrum covers 1.5 - 6.2 eV which relates to a wavelength range of 800 - 200 nm. The Beer-Lambert Law, is the principle behind absorbance spectroscopy. For a single wavelength, $A$ is absorbance (unitless, usually seen as arb. units or arbitrary units), $\varepsilon$ is the molar absorptivity of the compound or molecule in solution (M$^{-1}$cm$^{-1}$), $b$ is the path length of the cuvette or sample holder (usually 1 cm), and $c$ is the concentration of the solution (M). But in the present investigation the wavelengths of 300 – 600 nm spectrum is covered for analysing the sample prepared and the results are presented in Chapter-5.

$$A = \varepsilon bc.$$  

(4.1)
Atomic Force Microscope (AFM)

With the rapid development of the MEMS/NEMS technology, the characteristic scale of the corresponding materials and structures are getting smaller and smaller. The traditional macroscopic test machine is limited by the scale under this situation, and new micro/nanoscale (zone) mechanical test systems and methods are both necessary. AFM system (Pico Scan 2100) as shown in Fig (4.11) is mainly used as a microscope to observe the surface topography of the sample in the present investigation. AFM system is a three-dimension moveable stage and used as a scanning platform to locate the observed point on the sample surface and the results are presented in Chapter-5.

![AFM System](image)

Fig (4.11) Instrument used for AFM

The conformation tests for CO adsorption are conducted and their results are discussed in Chapter-5, Results and Discussions. After the conformation tests, the real time experiments are conducted on actual engine, Twin cylinder diesel engine.

4.1.3 Experimentation on Actual Engine. (Phase-3)

The experiments on actual engine are carried out in the Mechanical Engineering Department, Srikalahasteeswara Institute of Technology, [SKIT], Srikalahasti.
Biosensor Devices developed at IIT, Bombay, and the commercially available CO Sensor (TGS 5042) are used in these experiments.

4.1.3.1 Experimentation with Biosensor Devices. (Fabricated at IIT Bombay)

The experimental set-up is shown in the Fig (4.12). A 4-Stroke Twin cylinder water cooled diesel engine used in the experimental set-up is presented in Fig (4.13). The specifications of the engine are given in the Appendix-III. A short length of pipe is fitted to the silencer (Exhaust Pipe) of the engine. A small amount of exhaust gas from silencer is drawn into the test box through this pipe.

The test box is a plastic rectangular box. This is selected in view of its availability, ease of drilling, fixing and visibility. Two openings are provided in the plastic box, one for the inlet of the exhaust gas from the silencer of the automobile and the other for the outlet of the exhaust gas from the plastic box. The SU-8 cantilever sensor device functionalised with porphyrin is placed in the plastic container. The wires connecting the sensor and the Wheatstone Bridge passes through a small hole in the rectangle box. Fevikwik is used to create an airtight seal around the wires.

The exhaust gas from the engine is drawn over the sensor device by means of gas sampling pump. The sensor senses the amount of CO in exhaust gases. This output from the Wheatstone Bridge is connected to T.I board and then the T.I board in turn is connected to computer system for continuous recording of the results for various loads of the engine and plotted the graphs using Origin Lab Software. The data recorded for various loads is presented in Appendix-IV and the results are shown in Table (4.1).
Fig (4.12) Experimental set-up of Twin cylinder diesel engine

Fig(4.13) Twin cylinder diesel engine
4.1.3.2. Experimentation with Commercially available CO Sensor (TGS 5042)

The commercially available CO sensor (TGS 5042) is a battery operable electrochemical carbon monoxide sensor manufactured by Figaro. As per the claim of the company this sensor exclusively reacts to only carbon monoxide and is shown in Fig (4.15). The specifications are given in the Appendix - V. Its electrolyte is environmentally friendly, as it poses no risk of electrolyte leakage, can detect concentrations of 0-10,000ppm, operates in a range from -40° and +70°C, and it has
lower sensitivity to interfering gases. The TGS 5042 has good long term stability, and high accuracy and it is typically used in portable CO detector applications. The sensor generates a minute electric current which can be converted into a measurable voltage by an op-amp current-to-voltage converter circuit. The circuit diagram is shown in Fig (4.16) and its assembly view is shown in Fig (4.17). The test chamber and the procedure utilised for the commercially available sensor is the same that of the above, except replacing the sensors (TGS5042). The output (Voltage) of the sensor is directly fed to TI Board. The data recorded for various loads is presented in Appendix-IV and the results are shown in Table (4.2).

Fig (4.15) TGS 5042 Sensor
Fig (4.16) TGS 5042 circuit diagram
Table (4.2) Experimental results of commercial sensor (5042) exposed to engine exhaust gases.

The data obtained during the experimentation are analysed and presented in Chapter-5.