Chapter-2

2 Methodology and Instrumentation

2.1 Materials Used for the Synthesis and Other Experiments of CQDs

Fresh and healthy Banana leaves (*Musa acuminate*) and Pride of India plant (*Lagerstroemia speciosa*) leaves were obtained from the campus area of IIT (BHU) Varanasi. All metal salts and chemicals used in our experiment were of analytical grade and pure form without any further purification. The metal salts $NaAsO_2$ (SDFCL Mumbai, India), $Hg_2(NO_3)_2.2H_2O$ (SRL Chemicals Pvt. Ltd.), $CaCl_2$ (Avantor Performance Materials India Limited), $3CdSO_4.8H_2O$ (SRL Chemicals Pvt. Ltd.), $ZnCl_2$ (Merck Specialities Pvt. Ltd.), $Ag(NO_3)$ (Spectro Chem Private Limited Mumbai, India.), $Pb(NO_3)_2$ (Merck Specialities Pvt. Ltd.), $SnCl_2.2H_2O$ (Merck Specialities Pvt. Ltd.), NaCl (Merck Specialities Pvt. Ltd.), ethanol (SRL Chemicals Pvt. Ltd.) were purchased by local chemical distributors. HEK-293 and SiHa cells were obtained from the Cell repository at the National Centre for Cell Science (NCCS), Pune, India. Sigma provided FBS (St. Louis, USA). Trypsin-EDTA and Pen Strep (Penicillin and Streptomycin) were supplied by Gibco (New York, U.S.A). DMEM, acridine orange, propidium iodide, and DAPI were provided by Himedia (Mumbai, India). MTT, DMSO, and ethanol were offered by Sisco Research Laboratory (SRL, India). Plastic products, including T-25 flasks, 24 and 96 well plates supplied by Genetix Biotech Pvt. Ltd., DPPH extrapure supplied by Srichem Pvt. Ltd., while methanol is supplied by Molychem Pvt. Ltd. Indium tin oxide (ITO) glass substrates with a sheet resistance (20 Ω per square) were purchased from Vinkarola USA. Chemicals and solvents, such as acetone, dichlorobenzene (DCB) and propane-2-ol without additional purification. Poly3,4ethylenedioxythiophene: polystyrenesulfonate (PEDOT: PSS)

were purchased from sigma Aldrich, polyspirobifluorene (PSBF) and Polyfluorene (PFO) were purchased from Merck and Sigma Aldrich respectively, octadecylamine functionalized SWNT were purchased from sigma Aldrich, DI water using a Millipore Milli-Q ultrapure water purifier with a resistivity of 11-18 MΩ cm (Millipore, Billerica, MA).

2.2 Preparation of CQDs

2.2.1 Banana-derived CQDs for OLED Application

Synthesis of CQDs has been done by one-step hydrothermal method. Fresh and healthy banana leaves were obtained from Department of Physics, IIT (BHU), Varanasi. Deionized ultrapure Type II water was used with a resistivity of 10-15 MΩ.cm for all the experiments. 180 grams of fresh banana leaves were obtained and thoroughly washed with DI water. They were cleaned with pure Ethanol to remove impurities. The leaves were folded and boiled in a beaker after adding 100 ml of DI water. The extract was filtered using Whatmann filter paper. The filtrate was subjected to hydrothermal treatment in a 200 ml capacity Teflon-lined cylinder in a stainless steel hydrothermal autoclave at 125°C for 7 hours. After cooling it down to room temperature, the dark brown solution was centrifuged at 11000 rpm for 10 minutes. The resulting supernatant was used for subsequent experiments. All chemicals used were of analytical grade used without modifications.

2.2.2 Banana-derived CQDs for Heavy Metal Detection

Banana leaves were used as an organic precursor for the synthesis of CQDs by hydrothermal method. Banana stems and leaves have high fiber content (stem>58% DM, leaf >72% DM), nitrogen-free extract content (>50% DM), and water-soluble carbohydrate contents (WSC>5% DM), with certain nutritive value. Tannins of banana stem and leaf were 0.11% DM and 0.24%

DM. There were lactic acid bacteria (>105cfu/g FM) in the banana stems and leaves[100]. The hydrothermal reaction was conducted in the Teflon-lined autoclave, and the reaction was carried out at a maximum of 230°C. Banana leaves obtained from the campus area of IIT (BHU), were washed several times with ultrapure distilled water. Cleaned leaves were dried at room temperature overnight. Then, they were finely cut into very small pieces and soaked in ethanol. For making extract, the suspension was heated on a hot plate at 100 ^{0}C for 2 hours [101]. The resulting extract solution is rich in chlorophyll and is green in color. Other plant pigments like carotenoids and anthocyanins soluble in ethanol may also be present in this solution. The prepared extract was then transferred to a 200 mL Teflon-lined stainless steel autoclave for 8 hours at the desired synthesis temperature. The obtained solution was cooled down to room temperature (RT) naturally. The synthesis temperature was set at 120°C, 160°C, 200°C, and 230°C for various batches. After the synthesis, the resulting black-brown carbonaceous material was centrifuged at 11000 rpm for 15 minutes to remove the larger particles. The obtained solution was purified using a 1.5 micron PTFE filter. The purified CQDs were put in a Vacuum oven at 100 ^{0}C for 4 hours. The obtained dry CQD was dissolved in DI water and adjusted concentration to 1 mg/mL after sonication and centrifugation.

2.2.3 Banana-derived Chl-CQDs for Cervical Cancer Application

Banana leaves obtained from the IIT (BHU) campus were typically washed three times with ultrapure distilled water. At room temperature, cleaned leaves were dried. To prepare the extract, small pieces of banana leaves are boiled in ethanol at a temperature of 100°C for 4 hours on a hot plate. The extract was separated from banana leaves and filtered using Whatman filter paper to remove big particles. After transferring the Extract to a 200 mL Teflon-lined stainless steel laboratory autoclave, it was heated to 150°C for 8 hours and then cooled to room

temperature. The brownish solution was centrifuged for 15 minutes at 12000 rpm to remove big or agglomerated particles, and the supernatant containing Chl-CQDs was further purified using a 0.2 μm membrane. To make powder, purified Chl-CQDs were heated in a vacuum oven set to 100 °C for four hours. Dissolve the dry powder in DI water and sonicating for 1 hour, the suspended Chl-CQDs were centrifuged for 15 minutes at 10,000 rpm. The resultant Chl-CQDs were dissolved in DI water and adjusted to a concentration of 2 mg/ml.

2.2.4 Pride of India plant-derived CQDs for Environment Application

CQDs was synthesized through hydrothermal process by using Pride of India plant (Lagerstroemia speciosa) leaves obtained from the campus area of IIT (BHU) as precursor material. Lagerstroemia speciosa leaves were hydrothermally treated in DI water for 15 hours at a constant temperature of 180°C to convert organic compounds present in the plant to carbon dots. Afterwards, a dark brown solution was obtained which was cooled down to room temperature. The solution was centrifuged at 12000 rpm for 15 minutes. The resulting supernatant was used for subsequent experiments after 0.2 μm syringe filtration. Figure *2.1* shows synthesis process of CQDs using hydrothermal process.

Figure 2.1 Schematic illustration of synthesis process of CQDs by using hydrothermal method

2.3 Apparatus Used in the Experiments

All characterization of synthesized CQD and heavy metal-doped CQD were performed using a variety of spectroscopic techniques. UV-visible absorbance spectra were measured using Eppendorf kinetic BioSpectrophotometer; Eppendorf AG, Germany. Fluorescence emission spectra were obtained using a Fluorolog FL3C-21, Horiba spectrofluorometer with excitation slit width set at 3nm bandpass and emission at 3nm bandpass in 1 cmx1 cm quartz cell. The

size and shape were analyzed using an FEI Tecnai G2 High-resolution transmission electron microscope (HRTEM) operating at 200 kV with a liquid nitrogen-cooled sample holder. X-ray diffraction (XRD) spectra of CQDs were obtained using a bench-top X-ray diffractometer Rigaku Miniflex 600 Desktop X-Ray Diffraction System, RIGAKU Corporation. CQDs' zeta potential was measured using the Malvern zeta sizer pro. Functional groups were identified using Fourier transform infrared spectroscopy and were obtained by Jasco FR/IR-4600 spectrometer in KBr medium. Chemical compositions were characterized via X-ray photoelectron spectroscopy using by Thermoscientific K-Alpha spectrometer. X-ray photoelectron spectroscopy was recorded using an Axis Ultra system equipped with a monochromatic Al Kα X-ray source (1486.6 eV). The energy resolution was set at 0.1 eV. The XPS peaks were analyzed using CASA XPS software [102]. The XPS peaks were fitted with Gaussian Lorenztzian peaks after subtracting the Shirley-type background. Raman spectra was recorded with 532 nm laser in the back-scattering geometry in a CRM spectrometer equipped with a confocal microscope and 100x objective (model alpha 300 of WiTec Germany). The biological assays were performed by incubating the sample in a humidified CO_2 chamber (Termo Hepa class 100). Absorbance was recorded at 570 nm using an ELISA plate reader (Biorad iMarkTM). Phase contrast microscope, fluorescence microscope and AO/PI (100μg/mL) photographs were captured under the fluorescence microscope (EVOS cell imaging system, Invitrogen). The colorimetric assay measured the released LDH activity as per the manufacturer's instructions (Promega).

The thickness of LiF and Al layers was measured using the DTM-101 quartz crystal thickness monitor. The current voltage was characterized using Keithley 2400 programmed source meter. The output and transfer characteristics of VOLET were evaluated by a semiconductor

characterization system (Keithley 4200). Luminance characteristics was measured using LMT L1009 with Keithley 2400 programmable source meter. The spectrum of electroluminescence was measured using HR-2000CG-UV-NIR spectrophotometer an Ocean optics. All measurements were performed at ambient conditions and room temperature.

2.4 Experiments Related to SWNTs doped Optoelectronic Devices

2.4.1 Device Fabrication

Laser patterning was used to pattern the substrates made of indium tin oxide (ITO)-coated glass. The substrates were then washed with detergent, followed by cleaning with tap water. Sonicate the patterned ITO-coated substrates three times for 15 minutes in DI water. After 15 minutes of sonication with acetone and isopropanol, place the substrate in a vacuum oven set to 120^0 C for 45 minutes. The substrates were treated with ultraviolet–ozone (UV–ozone) light for 30 minutes before use to completely eliminate organic solvent residues. The PEDOT: PSS hole transporting layer was spin coated on the ITO-coated glass in air for 60 s at 2000 rpm. The film was then dried in a vacuum oven at 120 °C for 45 minutes. Afterwards 1 mg of SWNT was dispersed in 1 mL DCB and sonicate for 1 hour and 10 mg of PSBF was dissolved in 1 mL of dichlorobenzene, as emissive layer (PSBF and PSBF doped with SWNT) was spin coated for 60 seconds on the PEDOT: PSS substrate at 1500 rpm. Finally, to fabricate the OLED, lithium fluoride (LiF) 1 nm layer and a 100 nm aluminum layer were deposited on the emissive layer using thermal evaporation at a pressure of 10^{-6} Torr (Figure *2.2* (a)).

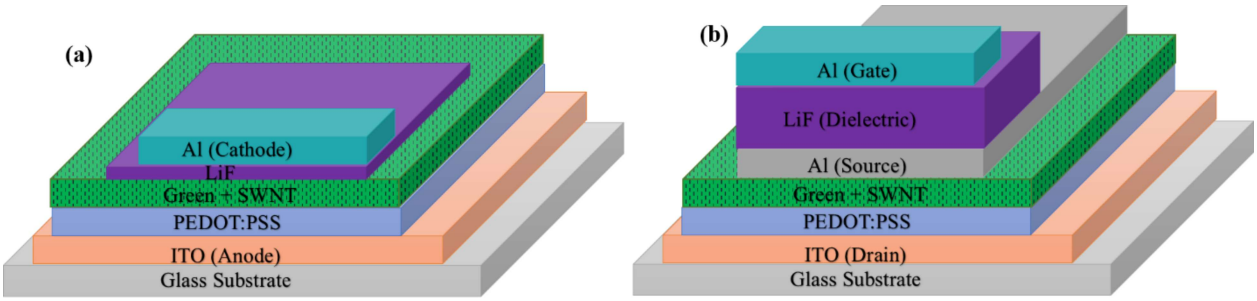

Figure 2.2 Multilayer device structure of (a) OLED and (b) VOLET

Additionally, for the fabrication of VOLETs, a thin layer of Aluminum 17 nm is deposited on an emissive layer referred to as the source electrode, followed by a layer of LiF 100 nm (Dielectric) deposited on the source electrode and then another 100 nm Aluminum layer as the gate electrode via thermal evaporation at a pressure of 10^{-6} Torr (Figure *2.2* (b)).

2.5 Experiments Related to CQDs as Electron Transport Layer in OLED

2.5.1 Device fabrication of OLED

Bottom emission OLED devices were constructed using poly (9, 9-di-n-octylfluorenyl-2,7-diyl) (PFO) as the emitting layer, PEDOT:PSS as a hole transport layer and CQDs as an electron transport layer. Polyfluorene (PFO) is a highly fluorescent conjugated polymer with emission in the bluish- green region. It has hole mobility (10^{-4}–10^{-5} $cm^2V^{-1}s^{-1}$) with high photoluminescence quantum efficiency (0.45-0.55), good thermal and chemical stability[103]. Devices with multilayer structure ITO/PEDOT: PSS/PFO/CQD/LiF/Al are fabricated on indium tin oxide (ITO) coated glass substrates having a sheet resistance of 20 Ω/cm and a thickness of 120 nm. ITO was patterned by laser patterning system onto a glass slide which was then cleaned using deionized water, acetone, and isopropyl alcohol sequentially for 20 min each using an ultrasonic bath and dried in vacuum oven at 120 ºC. HTL consisting of

(PEDOT:PSS) was spin coated on ITO at 2000 rpm for 1 minute and then dried in vacuum oven at 120°C for 45 minutes (thickness 50 nm). 10 mg/ml PFO was dissolved in toluene and then spin-casted as blue emissive layer at 1500 rpm for 1 minute. It was then baked at 120 ºC for 45 minutes. For the next layer, 10mg/ml CQD solution was spin-coated with a speed of 3000 rpm for 1 min as ETL followed by vacuum annealing at 120 °C for 45 minutes. Finally LiF (1nm thick) was deposited at a deposition rate of 0.1Å/sec and the fabrication process was completed. 100 nm-thick Al cathode was deposited through a metal mask by thermal evaporation in a vacuum chamber under the base pressure of 1.2×10^{-5} bar. The thickness of LiF and Al layers was controlled by quartz crystal thickness monitor attached with the thermal coating unit (HINDHIVAC). Two control devices with multilayer structure ITO/PEDOT:PSS/CQD/LiF/Al (Device A) and ITO/PEDOT:PSS/PFO/LiF/Al (Device B) were also prepared (Figure *2.3*).

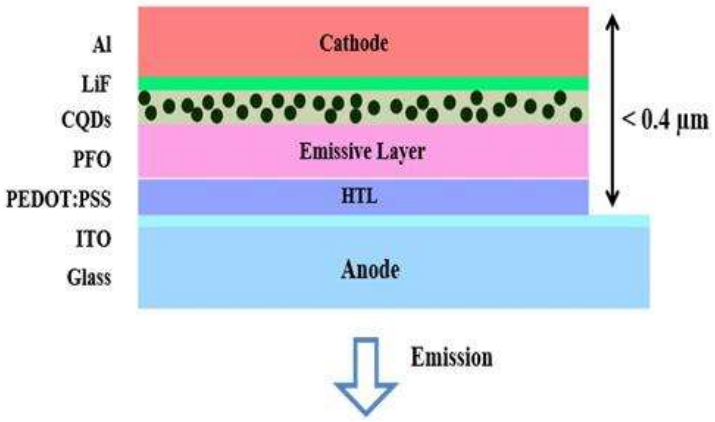

Figure 2.3 Multilayer device structure of OLED

2.6 Experiments Related to CQDs as dual probe sensor for Mercury and Arsenic

2.6.1 Computational Details

The role of CQD surfaces at different temperatures was assessed by modeling two different types of structures as shown in Figure *2.4* ; **ChlCQD** structure was used to model experimental surfaces at low temperatures and the structure consists of a modified chlorophyll moiety attached to a CQD. The methyl groups on the porphyrin rings of the pristine chlorophyll moiety were replaced with hydrogens and the long alkyl tail was replaced by a methyl group to generate a chlorophyll model. Similarly, **CQD** structure was used to model the experimental surface at high temperatures.

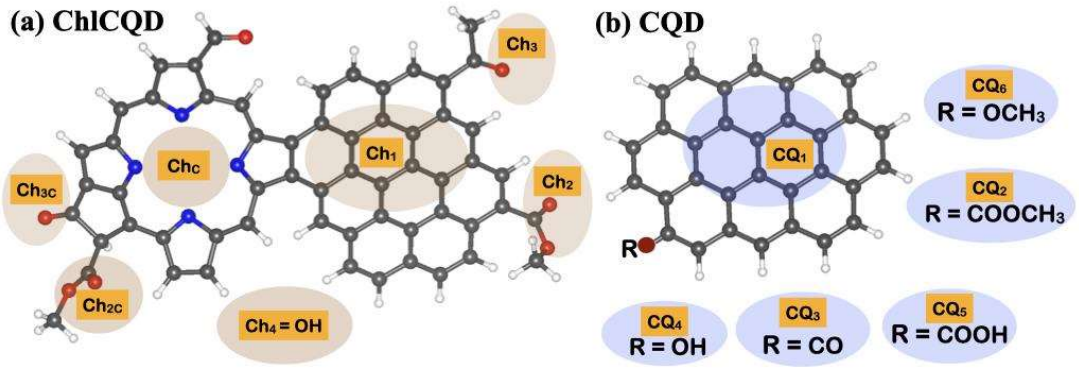

Figure 2.4 Optimized geometries of model surfaces of (a) ChlCQD (Chn) and (b) CQD (CQn) surfaces. The different interaction sites on the surfaces are highlighted where n represents ; surface (n=1), ester (n=2), keto (n=3) hydroxyl (n=4) carboxylic acids (n=5) an and ether (n=6) groups; centre of porphyrin ring (n=C) and ester group (n=2C) on chlorophyll moiety. The grey, white, red, and blue spheres represent C, H, O, and N atoms

The different functional groups were placed individually on the terminal sites of the modeled surfaces to assess the role of each functional group in interaction with As^{3+} and Hg^{+}. The

different sites (*n*) that were used consisted of surface (*n*=1), ester (*n*=2), keto (*n*=3), hydroxyl (*n*=4), carboxylic acid (*n*=5) and ether (*n*=6) groups as reported in Figure *2.4* (a-b) for **ChlCQD**(Ch_n) and **CQD**(CQ_n) model surfaces. The cavity in the porphyrin ring (*n*=C) and ester group on chlorophyll moiety (*n*=2C) were chosen as interaction sites on the chlorophyll moiety of **ChlCQD**. The hydroxyl group on **ChlCQD** was modeled by placing the hydroxyl group on one of the terminal sites of the extended CQD surface as shown in Figure *2.5* (a). This extended CQD surface for the hydroxyl group was chosen as for hydroxyl groups in the original **ChlCQD** model, As^{3+} was binding only to the surface in the vicinity of the hydroxyl group as shown in Figure *2.5* (b).

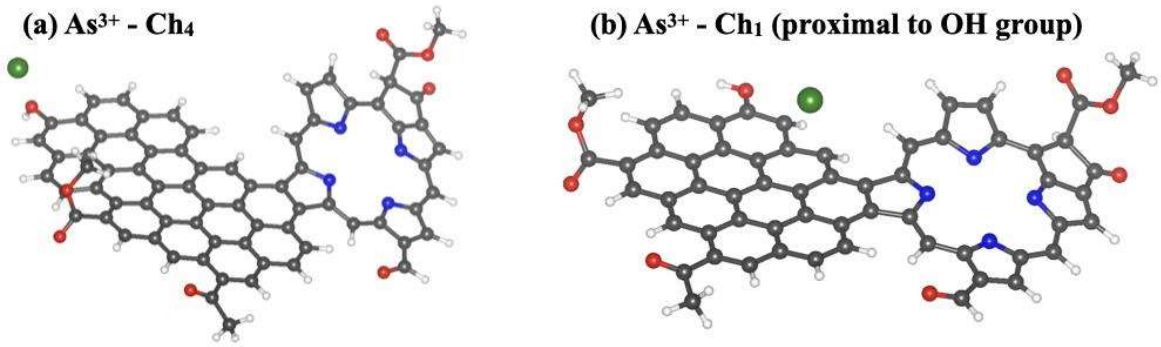

Figure 2.5 Optimized geometries of interaction of As^{3+} with (a) Ch4 and (b) Ch1 in vicinity of hydroxyl group placed near the chlorophyll moiety on ChlCQD surface. The grey, white, red, green and blue spheres represent C, H, O, as and N atoms

The +2 oxidation state of mercury is more stable than the +1 state and there is a probability of oxidation of Hg^{+1} to Hg^{+2} so we also performed calculations of Hg^{+2} with sites on **ChlCQD**. All the Density Functional Theory (DFT) calculations were performed using PBE1PBE functional in Gaussian 09 suite [104]. All the atoms were modeled using a 6-31g** basis set and Hg^{+} was modeled using the LANL2DZ basis set [105]. The frequency calculations were

performed on all the optimized geometries and showed the absence of imaginary frequencies. The TD-DFT calculations were performed using the PCM model with water as the solvent.

2.7 Experiments Related to CQDs as drug for Cervical Cancer

2.7.1 Cell lines and cell culture

HEK-293 and SiHa cells were obtained from the Cell repository at the National Centre for Cell Science (NCCS), Pune, India. The cells were grown in 25 cm^2 flasks with DMEM supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml) and incubated at 37°C in a humidified 5% CO2 chamber (Termo Hepa class 100).

2.7.2 Assay for cell proliferation

The cell proliferation assay was performed to determine the cytotoxicity of Chl-CQDs in normal kidney and cervical cancer cells [106]**.** Briefly, both HEK-293 and SiHa cells were seeded at a density of 10^4 cells per well in a 96-well plate overnight followed by treatment with various concentrations of CQD for 24 and 48 hours. After completion of treatment time, 10 μL of MTT (5 mg/mL stock) was exposed to each well and incubated for 2-3 hours at 37°C in a CO_2 chamber. Next, 100 μL DMSO was poured in each well and the absorbance was recorded at 570 nm using an ELISA plate reader (Biorad iMarkTM).

2.7.3 Assay for cell morphology

Cell morphology assay was executed to observe the morphological changes associated with drug action [107]**.** Both, HEK-293 and SiHa cells were exposed to various concentrations of Chl-CQD after overnight adherence at a density of 10^4 cells per well in a 96-well plate. After the treatment time the cells were rinsed with PBS and observed under a phase contrast microscope (EVOS cell imaging system, Invitrogen).

2.7.4 Assay for nuclear fragmentation

Nuclear fragmentation assay was used to visualize the nuclear changes after treatment with the drug [108]**.** Both HEK-293 and SiHa cells were planted overnight and subjected to desired concentrations of Chl-CQDs for 24 hours. Subsequently, the cells were allowed to fix in paraformaldehyde and then permeabilized with methanol (100 µL/well) for half an hour at -20 °C. Finally, cells were stained with DAPI (2 µg/mL) in the dark and observed under a fluorescence microscope (EVOS cell imaging system, Invitrogen).

2.7.5 Assay for apoptosis

Apoptosis assay was performed to assess the qualitative assessment of apoptotic process with the effect of drug [109]**.** Both the cells were exposed to various concentrations of Chl-CQDs after seeding in a 24 well plate overnight. Thereafter, the cells were washed, stained with equal ratio of AO/PI (100µg/mL) in the dark and photographs were captured under the fluorescence microscope (EVOS cell imaging system, Invitrogen).

2.7.6 Assay for plasma membrane integrity

Plasma membrane integrity assay was executed to examine the integrity of plasma membrane after effect of the drug. Both the cells were cultured overnight and subjected to various concentrations of Chl-CQDs for 24 hours. Next, the supernatant was collected and evaluation of the released LDH activity was measured by colorimetric assay as per manufacturer's instructions (Promega).

2.8 Experiments Related to CQDs as Ammonia and Lead detection

2.8.1 Fabrication of gas sensor

For device fabrication, the substrate indium tin oxide (ITO) having active area of 5x5 cm^2 dimension was etched through a simple one-step process. ITO coated glass substrate was taken, protected by plastic tape, and dipped into the acid etch. Etch mixture comprised 30 mL deionized water (DI), 10 mL hydrochloric acid (HCl), and 2-gram zinc dust. The substrate was kept for 5 minutes in etch solution and later rinsed under DI water. The plating tape was peeled off to measure the resistance of the ITO strip using a multimeter. The separation between the ITO electrodes was approximately 1 mm. Next, the patterned ITO glass was cleaned by standard degreasing procedure to ensure repeatability of low resistance ITO/organic interfaces[110]. The substrates were first soaked in a water-based neutral detergent (MERCK) while undergoing sonication for 15 minutes and then rinsed using DI water. They were again sonicated three times each for 15 minutes in DI water followed by 15 minutes sonication in acetone, another 15 minutes in isopropanol and were finally washed using isopropanol steam. The washed substrates was placed in an oven and dried at 120^oC for 45 minutes.

The film deposition of CQDs was carried out at room temperature in ambient air using the drop-casting method. Typically, 6 µL CQDs was dropped onto the substrates and baked at 120^oC for 120 minutes to get the final CQDs-coated sensor.

2.8.2 Detection of Vapour Phase

The fabricated device was placed inside a chamber having a volume of approximately 600 mL for conducting the vapour sensing experiments. By using a syringe, the analyte of fixed volume was injected into the test chamber. The vapour concentration can be calculated using the equation (1) [111]

$$\text{Conc} = \frac{\text{v}\times\text{D}}{\text{M}\times\text{V}} \times 2.24 \times 10^7 \qquad (1)$$

where 'Conc' is the required concentration of vapour in ppm, 'D' the liquid density in g mL^{-1}, 'v' the volume of liquid analyte in µL, 'V' the test chamber volume in mL and 'M' the molecular weight in g mol^{-1} of the liquid analyte. First syringe was kept inside the chamber adjacent to the device. Ammonia vapour was placed in the chamber by using the syringe to perform sensing experiments under ambient conditions.