Chapter-3

Polyvinyl alcohol (PVA) based sensors for the detection of folic acid by fluorimetric technique; and sulphur dioxide vapours and formaldehyde detection by impedimetric technique.

Part A

CdTe/MSA QDs modified PVA-tryptophan hybrid (PTQ) sensor for in vitro detection of folic acid in blood serum of ovarian cancer and acute myeloid leukemia patients

(S. Chakravarty et.al, Sensors and Actuators B: Chemical, 2016, 232, 243-250)

Part B

Coumarin-6 modified PVA-formaldehyde composite as a cost effective sensor for impedimetric detection of sulphur dioxide vapours


Part C

Coumarin-6 modified PVA as a biodegradable impedimetric sensor for formaldehyde

(S. Chakravarty et.al, ACS Applied Materials and Interfaces, 2017, Submitted)
Part A: PVA based nano-biosensor for the detection of folic acid by fluorescence quenching.

3A.1 Introduction

Folic acid (FA) is the synthetic form of the naturally occurring folate, a member of Vitamin B family. It is a water soluble compound which is present in the substrates and coenzymes involved in the acquisition, transport and enzymatic processing of one carbon unit for amino acid and nucleic acid metabolism.[169] Also, some previous studies suggest that FA together with vitamin B\textsubscript{12} can participate in the synthesis of DNA and RNA.[170] In addition, it plays pivotal role in copying RNA from DNA. Thus, it is essential for accurate replication of DNA. [171] It is well known that FA concentration varies significantly in a number of health disorders like in cancer, cardiovascular disease, Alzheimer’s disease, depression, reduced cognition and neural tube defect (NTD).[172-175] Low or high level concentrations of FA in blood serum, plasma and red blood cells are exploited as an efficient biomarker for diagnosis of various disease conditions.

Since FA is essential for proper functioning of numerous biological functions in human, so it is pertinent to develop simple and sensitive methods to detect FA in biological systems. In this regard, researchers round the globe have developed different kinds of sensor arrays viz. electrochemical sensors, ELISA etc. ELISA kit, which is a widely used immunoassay technique with many advantages like high specificity and high sensitivity.[176] But, they have certain disadvantages like long reaction time, effect of temperature, unstable upon exposure to light etc.[176] However, in recent years, fluorescence based assays have emerged as an attractive alternative choice for such purposes. In this regard, fluorescence based assays have their own advantages and disadvantages. The main advantages are ability of signal multiplication and amplification, low cost and fast response time while disadvantages are less specificity, temperature effect, ionic concentration effect, light scattering etc. [177] Keeping these in mind, an effort has been made towards the design of efficient fluorescence based assay for FA sensing. There also exist few reports where fluorescence quenching based processes has been employed for FA sensing.[178-185] Hu et al. reported the fabrication of graphene oxide and Ag nanoparticles for the detection of FA, Yan et al.
reported the use of gold nanoclusters for FA and Zhang et al. reported the application of polyethylenimine-capped silver nanoclusters for FA. [182-184] However, most of the reported works suffer from many drawbacks like high fabrication cost, expensive precursors, devoid of long term stability, poor detection limit and poor selectivity. Taking these and the cost effectiveness of the sensor into consideration, we have selected a cheap and readily available precursor polyvinyl alcohol (PVA) as a template for the design of a hybrid sensor. PVA is a synthetic environmentally benign polymer having unique properties for such applications. It is highly hydrophilic with long term thermal and pH stability. [186] It also possesses excellent thin film forming capability. In addition, it has the potential to be blended with various materials like nanomaterials, peptides, aminoacidsetc. for use as a biosensor. In this regard, aminoacids having intrinsic fluorescence like tryptophan presents a very good choice that can be blended with PVA. The aminoacid tryptophan is widely used as a tool in the studies of protein structure, conformational changes and functions owing to its spectral properties. Its indolechromophore depicts high sensitivity to the interactions and changes of the environment. [121, 187-188] But, for long term applications of a hybrid biosensor, photostability emerges as a key concern. In order to address this issue, researchers have tried to incorporate quantum dots (QDs) into polymeric substrates. To date, only few reports are available which emphasizes interaction of QDs with FA. [189-191] In the present work, we have reported the synthesis of two hybrid hydrogels i.e. PVA-tryptophan (PT) and PVA-tryptophan-CdTe QDs (PTQ). A comparative study of the PTQ hybrid hydrogel with PT hybrid hydrogel for FA has been carried out.

In a nutshell, we have reported a novel, simple, selective and rapid sensor system based folic acid detection pro-kit, which will be helpful in fast and efficient quantitative estimation of folic acid in blood serum with a detection limit up to 42.29 ng/ml.

The schematic representation for the process is depicted below (Scheme 3A.1).
3A.2 Experimental Section

3A.2.1 Materials

Polyvinyl alcohol (PVA) [Sigma], NBOC-L-Tryptophan (SRL), Folic acid (SRL), [Hydrochloric acid, ethanol, Cadmium chloride, Sodium Borohydride] (Merck), [Mercapto succinic acid, P-Toluene sulfonic acid, Sodium Telluride] (LobaChemie), [Borax powder, Citric acid] (Fischer Scientific). All the chemicals and solvents used in the process were of analytical grade and used without further purification. For the analysis, MilliQ water was used as a solvent.

3A.2.2 Synthesis of fluorescent sensors

Synthesis of PT hydrogel

PT hydrogel was prepared using a simple protocol- PVA (1.742 g) was weighted into a beaker and dissolved in MilliQ water (50 ml) with constant stirring at room temperature for half an hour followed by heating the solution using a heating mantle to 80°C for another half an hour. Thereafter, half of the solution was disposed of and replaced with 25 ml tryptophan (0.0001M). The reaction mixture was then stirred for one hour with constant heating at 80°C. The mixture so obtained was cooled to 40°C with stirring and poured into a polyethylene plate containing a quartz slide. It was left for 3 days to allow
the film to form. The film was removed from the plate and cut to obtain the quartz slide with thin PT film on the surface. The above procedure was repeated for 0.0005M tryptophan solution.

*Synthesis of quantum dots (CdTe-MSA QDs) doped hybrid hydrogel: PVA-tryptophan quantum dots (PTQ)*

Herein, preformed CdTe QDs co-stabilized by mercaptosuccinic acid (MSA) [151] was incorporated into the PVA-tryptophan matrix. There are basically two modes of incorporating QDs into the hydrogel network—ex-situ and insitu synthesis. The insitu approach suffers from many drawbacks like polycrystallinity of QDs, broad size distribution, uncontrolled luminescence behavior etc.; so we have adopted an ex situ approach. In a nutshell, PVA (1.742 g) dissolved in Milli Q water (50 ml) is stirred at room temperature for half an hour followed by heating the solution to 80°C for another half an hour. Thereafter, half of the solution has been disposed of and replaced with 25 ml tryptophan (0.0001M). The solution of tryptophan with PVA is then stirred for another 10 minutes followed by addition of 1ml of QDs solution. The reaction mixture is again stirred for another one hour with heating at 80°C followed by cooling to 40°C with stirring. The subsequent solution has been used for spectrophotometric analysis with proper dilution.

**3A.2.3 Methods**

The sensing materials (PT and PTQ) were extensively characterized using Fourier Transform Infrared Spectrometer (FTIR) with an ATR (Attenuated Total Reflectance) attachment (Nicolet 6700, Thermo Fischer), Thermogravimetric Analysis (TGA) (Perkin Elmer, TGA 4000), Differential Scanning Calorimetry (DSC) (Perkin Elmer DSC 6000), Atomic Force Microscopy (AFM) (NT-MDT NTEGRA AFM, semi-contact mode), Gel Permeation Chromatogram (GPC) (WATERS 2414) and Field Emission Scanning Electron Microscopy (FESEM) (SIGMA – VP (ZEISS))

The formation of CdTe QDs was confirmed by Transmission Electron Microscopy (TEM). The sample preparation was done by dispersing few drops of QDs sample into a 3 mm copper grid covered with a continuous carbon film and dried at
room temperature. Subsequently, TEM characterization was performed on a JEOL JEM 2100 transmission electron microscope operating at 200 kV.

Fluorescence spectra were recorded in VARIAN CARY Eclipse with an excitation and emission slit width of 5 nm. Predefined quantities of the hydrogels (0.071 g) were weighted properly and dissolved in a 20 ml water and the solutions were then subjected to ultrahigh sonication. The dispersed solutions so obtained were used for analysis with proper dilution. A 20 ml stock solution of FA of $2.27 \times 10^{-6}$ M concentration was prepared. Aliquot from the stock solution was added in a sequential manner (40 µl-525µl) to the hydrogel solution (2ml) for sensing studies.

TRPL study was carried out using Edinburg Instruments FSP920, picoseconds Time-resolved cum Steady State Luminescence Spectrometer under standard conditions to elucidate the mechanistic details of fluorescence signal output. In the present context, an LED source of wavelength 290 nm was used as the excitation source.

The mechanistic details of the fluorescence process was investigated with CV studies. CV measurements were performed on GAMRY reference 3000 potentiostat/galvanostat instruments with a typical three electrode system consisting of a platinum working electrode, an Ag/ Ag+ reference electrode and a platinum wire counter electrode with 0.5 M KCl in water as an electrolyte.

3A.3 Results and Discussions

3A.3.1 Reaction scheme
Scheme 3A.2 Schematic representation of reactive pathways for the probable structures of the sensors-PT and PTQ

3A.3.2 Characterization

TEM of CdTe QDs:

The high resolution (HR) TEM images presented in [Figure 3A.1 (a)], which, illustrate that the QDs are spherically shaped with an average size of 5.9 nm. The HRTEM highlights their lattice fringes and the interplanar spacing was measured to be 0.729 nm [Figure 3A.1 (b)] which corresponds well to our XRD data.

Figure 3A.1 TEM image (at 20 nm scale bars) of CdTe-MSA prepared by one pot synthesis. (b) HRTEM image (at 5nm scale bars) showing lattice fringes of QDs.

EDAX measurement of CdTe QDs:

EDAX study has been done (Figure 3A.2) and it confirms the presence of S element which comprises S in MSA capping and S in the crystal.
FTIR analysis of CdTe QDs

The FTIR spectrum of CdTe/MSA QDs confirmed the presence of MSA capping on CdTe QDs surface. The stated FTIR spectrum is depicted below (Figure 3A.3).

Similarly, FT-IR analysis was carried out for gels. For PVA, peak position 3272 cm\(^{-1}\) corresponds to O-H stretch (H-bonded), 2908 cm\(^{-1}\) corresponds to sp\(^3\) C-H stretch (aliphatic), 1418 cm\(^{-1}\) corresponds to C-C stretch, 1091 cm\(^{-1}\) corresponds to C-O stretch. For PT, along with these peaks, there exist a peak for C=O (COOH) at 1658 cm\(^{-1}\). As NH\(_2\) of tryptophan in PT is hydrogen bonded so, N-H stretching overlaps with O-H stretching which gives corresponding peak at 3272 cm\(^{-1}\). For PTQ, disappearance of
peak 1648 cm\(^{-1}\), indicates the C=O group of MSA capped on CdTe undergoes strong interaction with PT via H-bonding. The results are in close agreement with the previous reports (Figure 3A.4).

**Figure 3A.4** FT-IR (ATR) of hybrid hydrogels PT and PTQ with reference PVA

**Thermal Studies**

TGA thermograms revealed the thermal stability of the synthesized hybrid hydrogels (Figure 3A.5). It was found that PT, PTQ hydrogels are stable upto (255- 290 °C). The complete degradation of PT occurs at 550°C while for PTQ, it occurs at 650°C.

**Figure 3A.5** Plots corresponding to thermal analysis of PT and PTQ hydrogels (A) DSC thermogram; and (B) TGA thermograms.

**AFM analysis of hybrid gels**
AFM analysis of synthesized hydrogels were carried out and the gels were found to possess high surface roughness. The AFM micrographs along with roughness profile of PT and PTQ are depicted below (Figure 3A.6).

**Figure 3A.6** AFM micrographs of PT and PTQ
Morphological studies of the gels were carried out using FESEM (Figure 3A.7). The FESEM micrographs showed striking morphological differences between undoped (PT) and doped (PTQ) system. FESEM micrograph of PTQ showed the formation of aggregated species as shown below.

![FESEM micrographs of A. PT and B. PTQ](image)

**Figure 3A.7 FESEM micrographs of A. PT and B. PTQ**

**GPC analysis of hybrid gels**

GPC analysis of the hydrogels are tabulated below (Table 3A.1) show in the molecular weights and polydispersity of the hybrid gels-PT and PTQ.

**PT**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>M_n (gmol⁻¹)</th>
<th>M_w (gmol⁻¹)</th>
<th>M_p (gmol⁻¹)</th>
<th>M_z (gmol⁻¹)</th>
<th>M_{z+1} (gmol⁻¹)</th>
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<td>1</td>
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<td>35086</td>
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<td>40443</td>
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**PTQ**

<table>
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<th>Sl. No.</th>
<th>M_n (gmol⁻¹)</th>
<th>M_w (gmol⁻¹)</th>
<th>M_p (gmol⁻¹)</th>
<th>M_z (gmol⁻¹)</th>
<th>M_{z+1} (gmol⁻¹)</th>
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<td>61333</td>
<td>59619</td>
<td>62201</td>
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<tr>
<td>2</td>
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<td>36603</td>
<td>41298</td>
<td>37418</td>
<td>38125</td>
<td>1.026255</td>
</tr>
</tbody>
</table>

**Table 3A.1 GPC for the prepared hydrogels**

**3A.3.3 Sensing Study**
The characteristics of the hybrid hydrogels-PT and PTQ were evaluated at pH ~ 7. Upon excitation at 250 nm, the emission spectra of PT and PTQ were observed at 363 nm. The absorption and emission properties of PTQ resembled PT with significant enhancement of intensity for PTQ compared to PT (Figure 3A.8). Photoluminescence (PL) studies were carried out for the hybrid hydrogels-PT with FA at \( \lambda_{ex} \) 250 nm [Figure 3A.9 (A)] together with PTQ with FA [Figure 3A.9 (B)]. It was observed that fluorescence quenching occurs with incremental addition of FA. The quenching efficiencies (QEs) were calculated; and PTQ (QE 47.37%) was found to be more efficient quencher compared to PT (QE 42.92%). However such behavior was neither observed with the QDs alone nor with PVA-QDs hydrogel with FA (Figure 3A.10). The PL of FA and QDs was provided as a reference (Figure 3A.11). Also, photostability study was carried out under optimized conditions where Xe flash lamp was used as an excitation source; and excitation and emission wavelengths were fixed at 250 nm and (290-450) nm respectively. Under such conditions, PTQ was found to be photostable compared to PT (Figure 3A.12).

![Figure 3A.8 PL spectra of PTQ, PT and PQ](image_url)
Figure 3A.9 PL plot of (A) PT+FA; and (B) PTQ +FA

Figure 3A.10 PL plot of (A) QDs+FA; and (B) PQ +FA

Figure 3A.11 The PL plots of FA and QDs showing their characteristic peaks respectively
Further, in order to investigate the nature of quenching, Stern-Volmer (SV) plot was evaluated. The linear Stern-Volmer plot suggested the probability of static quenching mechanism [121] (Figure 3A.13).

Mechanistic details
Theoretically, fluorescence quenching arises from any one of the following processes—non-fluorescent ground state complex formation, aggregation, energy transfer or electron transfer.

In the present context, fluorescence quenching with no shift in PL after the addition of FA, rules out the probability of quenching due to ground state complex formation. Also, the possibility of aggregation has been ruled out as there is no characteristic change in DLS. Here, the mechanism is proposed to be a combination of electron transfer and energy transfer between the fluorophore PTQ and the analyte FA.
In this regard, the details of electron transfer and energy transfer process needs to be investigated. There are primarily two types of electron transfer processes—Photoinduced Electron Transfer (PET) and Intramolecular Charge Transfer (ICT).

PET appears to be the most elegant, sensitive and effective way to report the presence of analyte. [121] It is also an extensively investigated process to understand quenching processes. It is predicted by an electron transfer process with an absence of spectral shift. But, ICT is an electron transfer process accompanied by spectral shift. [30] As there is fluorescence quenching without spectral shift, so ICT is ruled out and PET process is expected to take place.[193] The experimental evidence for PET is given by Cyclic Voltammetry (CV) studies (Figure 3A.14). It is found that Oxidation potential changes drastically upon incorporation of analyte FA, from 1.40 V to 7.83 V.

**Figure 3A.14** CV of FA, PTQ and PTQ+FA

PL study was carried out upon exciting at a wavelength of 250 nm. At this wavelength, absorption of photons takes place which result in electronic excitation from HOMO to LUMO of the donor PTQ. After that electron jumps from LUMO of the donor to HOMO of the acceptor resulting in the formation of ground state charge transfer complex as confirmed by TRPL studies with no emission (Figure 3A.15).

**Figure 3A.15** TRPL plot for PTQ (τ 7.703 ns) and PTQ+FA (τ 7.719 ns)
Further, the thermodynamic feasibility of the process has been investigated by well-known Rehm-Weller equation

\[ \delta G_{et} = E_{1/2}^{(ox)} - E_{1/2}^{(red)} - E_S + C \]

where, \( E_{1/2}^{(ox)} \) is the oxidation potential of PTQ, \( E_{1/2}^{(red)} \) is the reduction potential of FA, \( E_S \) is the excited state energy of fluorophore, and \( C \) is the coulombic term.[194] The negative \( \delta G_{et} \) value indicates the process is thermodynamically feasible. In addition to the predominant PET, energy transfer process is also found to be a reason behind quenching.

There are primarily two types of energy transfer processes—Fluorescence Resonance Energy Transfer (FRET) and Inner Filter Effect (IFE). Both the processes exhibit efficient spectral overlap between the absorbance spectra of acceptor and fluorescence spectra of donor.[195] The difference between the two processes can be drawn from Lifetime based studies, where IFE exhibits no lifetime change while FRET exhibits lifetime change before and after the addition of the analyte.[196] In the present context, there exist efficient overlap between the donor PTQ and the acceptor FA (Figure 3A.16); and invariance of emission lifetime before the addition of analyte FA (\( \tau \) 7.703 ns) and after the addition of analyte (\( \tau \) 7.719 ns), so IFE is found to be operative.

![Figure 3A.16 PL-UV overlap](image)

The utilization of blue QDs in our system has been justified from the fact that the physical size of QDs is not important for in vitro biosensing applications particularly
when FRET is absent. [197] Thus, PET together with IFE between PTQ and FA can be used to transfer discrete and stoichiometric recognition events into an altered fluorescence signal of fluorophore. The schematic representation of PET process is given below (Scheme 3A.3).

Scheme 3A.3 Schematic representation of PET process of the sensor system

Limit of Detection of the Sensor

It has been observed that fluorescence quenching takes place upon incremental addition of analyte FA, so sensitivity can be characterized from the intercept of the plot of fluorescence quenching against various concentrations of FA. The calibration curve has been constructed by plotting the fluorescence quenching at different concentrations of FA (Figure 3A.17).
From the calibration curve, Limit of Detection (LOD) was determined using the formula LOD = 3.3σ/S, where σ is the standard deviation of response and S is the slope of calibration curve and found to be 1.3 pM i.e. 0.57 pg/ml (where σ is 0.94 and S is 2.39). [194] The value of LOD deduced is much smaller than the earlier reported work. [175, 179-180] Normally, the concentration of FA in human blood serum is 3ng/ml. So, our sensor PTQ is sensitive and stable enough to be used for biological applications for detecting the amount of FA in blood serum.

3A.3.4 Selectivity study

PT and PTQ selectivity for FA were thoroughly studied. PL titrations were performed with certain aminoacids and biomolecules viz. aspartic acid (AA), proline (PR), threonine(TH), serine (SE), histidine (HS), vitamin B\textsubscript{12} (CO), cytosine (CY)and valine (VA) which are of immense importance in the design of sensor. It was found to have better selectivity for FA by PTQ compared to PT (Figure 3A.18). This might be due to non-availability of indolechromophoric group of tryptophan in PTQ for interaction with FA due to steric crowding resulting from incorporation of QDs. However, in PT, the indole group which is readily available undergoes significant interactions with biomolecules, resulting in non-selectivity towards FA. Thus, PTQ biosensor can be used even in the presence of high percentage of other biomolecules.
3A.3.5 Comparative study

A comparative analysis of our sensor with the reported FA sensors (including commercially used ELISA kit) were carried out (Table 3A.2). It was found that the sensitivity range and LOD of our sensor is better than the reported fluorescence based sensors and ELISA for FA.

<table>
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<tr>
<th>Sl. No.</th>
<th>Sensing Material</th>
<th>Buffer/PH</th>
<th>Sample</th>
<th>FA conc. range</th>
<th>LOD</th>
<th>Ref</th>
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<tr>
<td>1.</td>
<td>Cyst- AuNP</td>
<td>Borate / 9</td>
<td>Aqueous</td>
<td>4.6×10⁻⁷ M-9.1×10⁻⁶ M</td>
<td>2.9×10⁻⁷ M</td>
<td>180</td>
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<tr>
<td>2.</td>
<td>AuNCs</td>
<td>Borate / 9</td>
<td>Aqueous</td>
<td>1.1×10⁻⁷ M-2.3×10⁻⁶ M</td>
<td>6.5×10⁻⁸ M</td>
<td>180</td>
</tr>
<tr>
<td>3.</td>
<td>BSA- AuNCs</td>
<td>PBS/7.4</td>
<td>Aqueous</td>
<td>120.0 ng ml⁻¹-33.12 μg ml⁻¹</td>
<td></td>
<td></td>
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<tr>
<td>4.</td>
<td>ZnS:Cu/ZnS QDs</td>
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<td>Aqueous</td>
<td>-</td>
<td>-</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>ZnS:Mn/ZnS QDs</td>
<td>-</td>
<td>Aqueous</td>
<td>-</td>
<td>-</td>
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<tr>
<td>5.</td>
<td>ELISA</td>
<td>PBS</td>
<td>Bio-fluid</td>
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<td>&lt; 27.4 pg ml⁻¹</td>
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<td>6.</td>
<td>PTQ</td>
<td>PBS</td>
<td>Aqueous</td>
<td>0.09 pM- 1.19 pM</td>
<td>0.027 pg ml⁻¹</td>
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Table 3A.2 Comparative analysis of folic acid sensors

3A.3.6 Real time applications

Detection of FA in real blood samples via Fluorescence ‘Turn Off’
Blood comprises of two major components—blood plasma and blood serum. Blood serum does not bear a blood cell nor a clotting factor while, it includes all proteins not used in blood clotting and all electrolytes, antibodies, antigens, hormones and any exogenous substances like drugs and micro-organisms. It has been already reported that there exist primarily two assays for FA detection—red cell folate assay and serum folate assay. [198] The former is more complex to perform than the serum folate assay as it requires multistep in sample handling before analysis.[198] Thus, the precision of former is less compared to later.[198] Also, the former assay cannot be employed in patients with certain vitamin deficiency and those who suffer from macrocytosis. [198] This illustrates that the measurement of serum folate is an ideal technique to determine FA in cancer patients. [198] It has been reported that the ideal concentration of serum folate in a healthy individual is >3 ng/ml. [199] However, this serum folate concentrations vary significantly in numerous disorders.

It is a well-established fact that serum folate proteins like albumin, globulin causes strong interference in the measurement of serum folate. So, we have carried out some pre-treatments to denature serum folate protein and subsequently removed those interfering proteins from the blood serum. The blood serum devoid of serum proteins was then used for further analysis with our sensor PTQ. PL study of the effect of PTQ with serum samples of A, B and C loaded with FA were carried out by exciting at a wavelength of 290 nm and maintaining the slit widths at 5. It was observed that upon addition of PTQ at a sequential manner to the serum samples, no change in PL intensity were observed in three samples. The probable reasons can be attributed to absence of over expressed folate receptors in serum samples of A, B and C. The PL plots are given in Figure 3A.19.
Figure 3A.19 PL plot of effect of PTQ on A. Sample A (Mice blood serum with FA); B. Sample B (Drug loaded mice blood serum with FA) and C. Sample C (Human blood serum with FA).

It was found that among the four samples A, B, C and D; only D interacted efficiently with our sensor PTQ. Thus, PTQ was able to sense FA present in D via fluorescence quenching at 363 nm. A plausible reason for the same can be attributed to the presence of over expressed folate receptors in D (1, Patient a; 2, Patient b), which readily binds FA. This will further help to sense folate receptors which are closely associated with cancer. A schematic representation of the process along with the PL plots are depicted in (Figure 3A.20). The Limit of Detection (LOD) was calculated using the procedure stated above [194] and it was found to be 42.29 ng/ml for patient a and 365 ng/ml for patient b (Figure 3A.21).
Figure 3A.20 Blood serum sample preparation and the PL plots of PTQ hydrogel with FA treated real blood serum samples of two cancer patients A. Patient a (acute myeloid leukemia); and B. Patient b (ovarian cancer).

Figure 3A.21 Calibration curves for A. Patient a; and B. Patient b

3A.4 Conclusion

In a nutshell, we have synthesized a photostable hydrogel PTQ using a simple route for FA sensing. Fluorescence quenching behavior was observed in presence of FA. It
exhibited better LOD than commercially utilized kits for FA. In addition, real time application of the sensor PTQ was carried out and it was found to be efficient for FA detection in the blood serum samples of acute myeloid leukemia and ovarian cancer patients. This work will provide a new platform to design environmentally benign and nanomaterial doped hybrid hydrogels for biological, optoelectronic and photonic applications. Also, it will contribute to the rapidly growing field of biophotonics in cancer research.
Part B: PVA-formaldehyde based polymeric composite sponge as sulphur dioxide vapour sensor

3B.1 Introduction

In recent years, the development of gas sensing materials has emerged as one of the forefront areas of research within material science. Designing new gas sensors for toxic gases, pollutants, volatile organic compounds (VOCs) etc. are of immense importance in terms of human health, safety, amenity, environmental reservation and so on.[200] But, the development of novel materials for such gas sensing applications is itself a challenge. A newly developed material can be used as a gas sensor if it possess efficient sensing performance (sensitivity, selectivity and rate of response) and reliability (drift, stability and interfering gases).[200] In this regard, polymeric composite materials containing conductor or semiconductor fillers has escalated special attention particularly due to their flexibility and tailorable properties.[201] These materials possess superior thermal, mechanical, electrical and sensing properties compared to their virgin counterparts.[201] Recent years have witnessed numerous gas sensing materials viz. metal-oxide semiconductors, intrinsically conducting polymers, conducting polymer composites, metal-oxide polymer composites etc.[202-210] However, semiconducting polymeric composites are highly desirable as sensors in terms of their potential multifunctional nature in which they can serve simultaneously as a filtering device as well as a means to quantitatively estimate volatile organic compounds (VOCs).[211] Thus, designing novel polymeric composites can be considered as an emerging area of material development for effective gas sensing applications.

The need for efficient monitoring of VOCs and other hazardous gases associated with health and environment has led to the emergence of sensor technologies.[212] Sensors are based on various transduction mechanisms-electrical, optical, mass and heat.[213] However, sensors based on electrical transduction mechanism are advantageous particularly due to miniaturization and portability.[35] The electrical sensors are further subdivided into conductivity, impedance, potentiometry and magnetoelasticity based sensors. Among these, electrical impedance technique is a powerful technique particularly for characterizing electrical properties of materials via application of small sinusoidal ac voltage (2-10 mV).[214] In this technique, two
components of current is determined—the in phase response determines the real component of impedance (resistive) while out of phase current determines the imaginary component (capacitive).[215] This electrical impedance technique provides useful information regarding solid-gas interaction.[214] In addition, the construction of equivalent circuit from the impedance analysis provide an understanding of gas sensing behaviour.[214] Literature studies reveal the utilization of impedimetric sensors for vapour phase sensing of analytes.[86, 216-217] The cost effectiveness, highly sensitivity and ease of miniaturization compared to other well-known techniques like Gas Chromatography, Optical and Raman Spectroscopy has led to the emergence of electrical impedimetric technique as an ideal transduction technique for vapour sensing. [216-217]

Amongst various toxic gases and environmental pollutants, sulphur dioxide (SO$_2$) is considered as one of the most environmentally hazardous gases.[218] Occupational Safety and Health Administration (OSHA) has set a normal exposure limit of 5 ppm, National Institute for Occupational Safety and Health (NIOSH) has set 100 ppm as an exposure limit and The American Conference of Governmental Industrial Hygienists (ACGIH) has set a normal exposure limit of 0.25 ppm for short term exposure while Environmental Protection Agency (EPA) has set an exposure limit of 75 ppb.[219] The exposure above the permissible limits cause various types of health disorders like dermatitis, urticarial, angioedema, diarrhoea, abdominal pain, bronchoconstriction, and anaphylaxis.[204] With the rise of atmospheric pollution in developing and industrialized cities around the world and the presence of SO$_2$ as an accompanying gas in mineral oil, fossil, shale gas extraction and food materials like wine has enhanced the probability of human exposure to hazardous gases like SO$_2$ above the permissible limits.[220-221] It has been reported in the literature that SO$_2$ contents in oil and gasoline are indirectly monitored by means of IR spectroscopy, gas chromatography, optical sensors, amperometric sensors, Raman spectroscopy etc.[222-228] But, for all these methods, long and complicated sample preparation procedures are required. Thus, the current need emphasises on the development of cost effective material together with a low cost transduction mechanism for solid state SO$_2$ vapoursensing.
In the light of above, we have tried to prepare polymeric composite materials for impedimetric sensing of sulphur dioxide vapours. Polyvinyl alcohol formaldehyde (PVF) is chosen as a base material for the preparation of polymeric composites as it possesses many advantages over other polymers namely low densities, hydrophilic networks, excellent water absorption capacities, biocompatibility etc. [229] The presence of numerous hydroxyl groups in PVF leads to tuning of its surface properties for varied applications. [229] Recently, the potential application of polymeric composites like PVF has been investigated. Kim et al. prepared PVF 2 coated polyaniline sensor for VOCs.[229] Few other groups has utilized PVF sponges for water super absorption.[230] Another group has exploited PVF for environmental applications like absorbents for oil spills. [231] Herein, we have developed a series of polymeric composites based on PVF namely PVF-Single Walled Carbon nanotubes (PVFCNTs), PVF-Carbon nanoparticles (PVFCNPs), PVF-Thiophene (PVFTH), PVF-Cytochrome (PVFCY) and PVF-Coumarin-6 (PVFCOU) followed by their detailed characterization and applications toward SO$_2$ gas sensing. The schematic representation of the present work is depicted in Scheme 3B.1.

![Scheme 3B.1 Schematic representation of the present system](image-url)
3B.2 Experimental Section

3B.2.1 Materials
Polyvinyl alcohol (LobaChemie), Formaldehyde (Merck), Polyethylene glycol-100 (Aldrich), concentrated sulphuric acid (Ranbaxy), Single Walled Carbon Nanotubes (SWCNTs) (Aldrich), Coumarin-6 (Aldrich), Carbon nanopowder (Aldrich), Thiophene (Merck), Cytochrome C (SRL), Sodium sulphite anhydrous (Merck), Hydrochloric acid (Merck) and Ethanol (Merck). Milli Q water was used in our experiments. All other reagents and solvents used were of analytical grade and used without further purification.

3B.2.2 Synthesis of polymeric composite sponges

Synthesis of PVF sponge
PVF composite was prepared using a simple modification of a reported protocol. [232] Briefly, 50 g of PVA was dissolved in 450 g of deionized water by vigorous stirring with a magnetic stirrer at 95 °C until completely dissolved. A PVA solution of 10% weight was obtained. It was followed by the addition of formaldehyde (10 ml) and polyethylene glycol-100 [PEG] (1.5 g) into hot PVA solution with vigorous stirring. The liquid froth so obtained after 5 min was added to 30 ml of 60 % H$_2$SO$_4$. The raw sponge sample was taken in a petridish and kept in an oven at 50 °C for 5 h. This was followed by washing the samples with deionized water for at least 5 times to remove the unused reactants. The sample so obtained was finally dried for 1 h at 60 °C. The product yield was found to be 10.523 g.

For the synthesis of conjugates, a similar procedure was adopted where dopants are added prior to the addition of conc. H$_2$SO$_4$. Here, conc. H$_2$SO$_4$ acts as a cross-linking agent between free OH and the analytes as reported earlier. [233]

Synthesis of PVF-Coumarin6 composite sponge (PVFCOU)
50 g of PVA was dissolved in deionized water by vigorous stirring with a magnetic stirrer at 95 °C until completely dissolved followed by the addition of formaldehyde (10 ml) and polyethylene glycol-100 (1.5 g) into hot PVA solution with vigorous stirring.
0.25 mg of coumarin-6 was added to the froth followed by addition of 15 ml conc. H$_2$SO$_4$ (60 %) at room temperature. The raw material was oven dried for 5 h. The unreacted material was thereafter removed by washing 5 times with deionized water. The sample so obtained was finally dried for 1 h at 60 °C.

The sponges PVFCNPs, PVFCNTs, PVFCY and PVFTH were prepared by adopting same procedure by adding CNPs / CNTs/CY /TH to the respective solutions.

3B.2.3 Physical Measurements

FT-IR Spectra were carried out in the ATR mode with a NICOLATE 6700 FT-IR spectrophotometer in the range 650-4000 cm$^{-1}$. TGA analysis were performed using Perkin Elmer TGA 4000 instrument at a heating rate of 10°C per minute with a nitrogen flow rate at 20 ml/min. FESEM studies were carried out with SIGMA-VP (ZEISS) SEM at an accelerating voltage of 5 kV in SE2 mode. Impedance (Z) studies were done with HIOKI 3532-50 LCR HiTESTER.

3B.2.4 Sensing experiments

Cost effective approaches are usually adopted in the mechanical and electrical design of electrical impedance techniques.[234] These methods basically encompass three implementation approaches-non-intrusive electrode pair sensors flush-mounted in a pipe wall, impedance tomography sensor that use sets of circumferential electrodes and intrusive wire mesh sensors that detect phase at discrete locations.[234] Here, we have developed simple electrodes derived from the concept of intrusive wire mesh sensors. Two stainless steel electrodes (SSE) with fine pores and square geometry are designed keeping in mind the ease of development mechanically. The SSE acts as blocking electrode and hence the equivalent circuit of the test cell is depicted below in Scheme 3B.2.[235, 236] In the circuit diagram, C$_g$ is geometric capacitance that appears parallel and which is the consequence of the charges on the electrodes. However, its magnitude is small compared to the double layer capacitance C$_{dl}$ as the intercharge separation is much larger.

The electrical properties of the composites were studied in detail via impedance measurements. A temperature range (35-85) °C was set for measuring bulk electrical property of the composites. Two symmetric stainless steel electrodes (SSE) of
dimension 1 cm× 1 cm each at room temperature were taken and a pellet of diameter 10 mm was sandwiched between the two plates at a relative humidity of 60%. The cell geometry was maintained as SSE|SF/SFC|SSE. Similarly, I-V measurements were carried out within the temperature range of (35-85) °C and frequency 1 KHz using HIOKI 3532-50 LCR HiTESTER. The schematic representation of the circuit employed and the experimental set up for the electrical studies is given in Scheme 3B.2.

Scheme 3B.2 Schematic representation of (A) the circuit and (B) experimental set up for the electrical studies

3B.3 Results and Discussions

3B.3.1 Reaction scheme

The synthesis of PVF from PVA is carried out via an acid mediated process by reacting PVA with formaldehyde (HCHO) as reported in the literature. [237] The hydroxyl
group of PVA interacts with the carbonyl group of HCHO in presence of PEG followed by addition of sulphuric acid in catalytic amounts. PEG is added as a porogen in PVF, which imparts porosity in PVF. [238] The process leads to the formation of a hydrophobic PVF from hydrophilic PVA.

Composite materials have gained significant interest due to their remarkable changes in the properties-electrical, thermal etc. [239-240] In the present context, inorganic/organic moieties are incorporated into the polymer PVF for the generation of composites resulting in the variation of the electrical properties. [241]

The inorganic moieties, SWCNTs comprise a single graphene cylinder with an aspect ratio > 1000. [242] Composite materials based on CNTs-polymer are expected to have excellent functional properties of CNTs and good processability characteristics of polymer.[243] Here, the hydrophobic SWCNTs are ultrasonically agitated (20-24 kHz) in a non-aqueous media and blended insitu with PVF in absence of cross linker to hydrophobic PVF to form the composite PVFCNTs.

Similarly, organic moieties like CNPs are impregnated in situ to PVF resulting in the formation of the polymeric composite PVFCNPs. A similar strategy is adopted for PVFTH, PVFCOU and PVFCY. The synthetic pathway for the development of polymeric composites is shown in **Scheme 3B.3**.
3B.3.2 Characterization of the composites

Physical characteristic of polymeric composites (Colour, form and yield)

The synthesized polymeric composites are found to be amorphous in nature and exhibited distinct colors-white (PVF), grey (PVFCNTs), black (PVFCNPs), brown (PVFTH), deep orange (PVFCOU) and pale white (PVFCY). The highly amorphous nature of these composites make these materials desirable for gas sensing applications. It is reported in the literature that amorphous surfaces are ideal for adsorption or absorption types gas sensing studies. [244] The images of the air dried synthesized PVF based composites are depicted in Figure 3B.1.

![Figure 3B.1 Synthesized PVF based composite sponges](A) PVF; (B) PVFCNTs; (C) PVFCNPs; (D) PVFTH; (E) PVFCY and (F) PVFCOU

Further, the composites are obtained in high yields-PVFCNTs (11.215 g), PVFCNPs (10.342 g), PVFTH (11.432 g), PVFCY (10.674 g) and PVFCOU (9.652 g). The % yields of the synthesized composites are found to be PVFCNTs (89.75%), PVFCNPs (97.33 %), PVFTH (99.2%), PVFCY (83.76 %) and PVFCOU (92.63%).

FT-IR Analysis

The configurational details of the synthesized composites are done with the help of FT-IR. FT-IR stretching frequencies provides information regarding nature of interaction between PVF and CNTs/CNPs/TH/CY/COU. PVF exhibits the characteristic stretching frequencies- 3452 cm\(^{-1}\) (H-bonded OH), 2889 cm\(^{-1}\) (C-H), 1714 cm\(^{-1}\) (C=O) and 1004 cm\(^{-1}\) (C-O-C). However, for PVFTH, H-bonding interaction between S of Thiophene with free OH of PVF is clearly visible which corresponds to stretching frequency 2339 cm\(^{-1}\) (S-H) while for PVFCOU, there exist electron transfer from S of COU toC=O which correspond to the shift in stretching frequency to 1644 cm\(^{-1}\) (C=O) from 1714 cm\(^{-1}\) (C=O) of PVF. In contrast, PVFCNTs, PVFCNPs and PVFCY do not exhibit any characteristic changes which implies PVF is attached to CNTs/CNPs/CY via vander
waals forces. The stretching frequencies depicted above corresponds to those reported in the literature.[194] The FT-IR spectra are depicted below in Figure 3B.2.

![Figure 3B.2 FT-IR spectra of the synthesized composite sponges (A) Expanded range and (B) Narrow range](image)

**FESEM**

Surface morphological analysis of composites was carried out with the help of FESEM. Interesting observations were drawn from the FESEM micrographs in terms of surface roughness and porosity, which play pivotal role in gas sensing applications. [245] It was observed that PVF exhibited highly amorphous macroporous surface with macropores measuring (6-12) µm. Upon doping with CNTs/CNPs/TH/CY/COU, the surface roughness and porosity of PVF is affected. From the FESEM micrographs, the polymeric composites are found to exhibit amorphous nature and porosity is found to follow the order PVF>PVFCOU>PVFTH>PVFCY>PVF CNPs>PVF CNTs. The probable reason for such striking morphological changes may be attributed to the nature of interactions existing between PVF and dopant. The FESEM micrographs of the synthesized composites are depicted in Figure 3B.3.
**Electrical Analysis**

In order to exploit materials for electrical sensing of analytes, the first step is to investigate the nature of the materials—conducting, semiconducting or insulator. The conducting and semiconducting materials are particularly important for designing chemiresistive materials as sensors. The literature studies shows that for conductors, electrical conductivity $\sigma > 10^3$ S/cm, for semiconductors $10^{-8}$ S/cm $< \sigma < 10^3$ S/cm and for insulators $\sigma < 10^{-8}$ S/cm.$[246]$ In order to measure bulk electrical conductance of the
polymer composites, thermal analysis of the composites are done (Figure 3B.4). It is found that all the composites are stable and bulk electrical conductance can be readily measured up to 85 °C. Thus, Arrhenius plots of the composites are constructed for a temperature range of (35-85) °C and σ values at 35 °C were deduced in accordance with the known method.[247] It is observed that σ values for the ac conductivity at 35 °C are found to be $9.00 \times 10^{-9}$ S/cm (PVF), $6.74 \times 10^{-9}$ (PVFCNTs), $1.14 \times 10^{-3}$ (PVFCNPs), $5.00 \times 10^{-9}$ (PVFTH), $9.41 \times 10^{-4}$ (PVFCY) and $1.98 \times 10^{-5}$ (PVFCOU). The values indicate that PVF, PVFCNTs and PVFTH exhibit insulating property while PVFCOU/PVFCNPs/PVFCY exhibits semiconducting property. The Arrhenius plots of the synthesized composites are depicted in Figure 3B.5. FT-IR studies clearly demonstrates that in PVFCOU, PVF interacts with COU via electron transfer from sulfur to ‘C=O’ of PVF as found from FT-IR stretching frequencies. In addition, FESEM micrograph of PVFCOU depicts decrease in porosity of PVFCOU compared to PVF resulting due to trapping of COU in PVF. The decrease in conductivity of PVFTH arise due to molecular entrapment leading to structural distortion while for PVFCOU, entrapment with no structural distortion takes place. But, for PVFCNTs, the decrease in conductivity results from the presence of non-directional non-covalent interactions leading to structural distortions. Such structural distortions hinders the free mobility of electrons leading to decrease in conductivity.[248]
Figure 3B.5 Arrhenius plots of the synthesized composites
3B.3.3 Sensing Study

Experimental Set up

In order to carry out sensing studies, a simple experimental set up has been constructed for vapour generation maintaining a flow rate of 20 ml/min. The chemical reaction involved in the generation of SO$_2$ vapours is shown below in equation 1.[249]

$$\text{Na}_2\text{SO}_3 (s) + 2 \text{H}_2\text{SO}_4 (aq) = 2 \text{NaHSO}_4(s) + \text{H}_2\text{O}(aq) + \text{SO}_2$$

The experimental set up used in the present study is given in Figure 3B.6.

![Experimental set up for SO$_2$ vapour generation and conductivity measurements by PVF based composites](image)

**Figure 3B.6** Experimental set up for SO$_2$ vapour generation and conductivity measurements by PVF based composites

Arrhenius plots of sponges after exposure to SO$_2$ vapors

The effect of exposure of SO$_2$ vapors for half an hour maintaining a flow rate of 20 ml/min towards synthesized composites are studied in detail for a temperature range (35-85)$^\circ$C. The σ values after SO$_2$ exposure are found to be $5.93 \times 10^{-3}$ S/cm (PVFSO$_2$), $4.22 \times 10^{-9}$ (PVFCNTsSO$_2$), $1.78 \times 10^{-3}$ (PVFCNPsSO$_2$), $6.77 \times 10^{-3}$ (PVFTHSO$_2$), $4.32 \times 10^{-3}$ (PVFCYSO$_2$) and $4.26 \times 10^{-9}$ (PVFCOUSO$_2$). The σ values changes from insulating to semiconducting in PVFSO$_2$ and PVFTHSO$_2$ while semiconducting to insulating in PVFCOUSO$_2$ (Figure 3B.7). These results can be correlated with FT-IR before and after sensing (Figure 3B.8). Further, these results provide interesting
corelation with EDAX studies which showed a considerable percentage of sulfur in PVFTHSO$_2$ (6%) and PVFCOUSO$_2$ (4.29%) (Figure 3B.9). The switching from insulating behaviour of PVF ($9.00 \times 10^{-9}$ S/cm) to semiconducting PVFSO$_2$ ($5.93 \times 10^{-3}$ S/cm), insulating PVFTH ($5.00 \times 10^{-9}$) to semiconducting PVFTHSO$_2$ ($6.77 \times 10^{-3}$), semiconducting PVFCOU ($1.98 \times 10^{-5}$ S/cm) to insulating behavior PVFCOUSO$_2$ (S/cm) can be explained from FT-IR spectra (Figure 3B.8). FT-IR spectra of PVFSO$_2$[Figure 3B.8 (A)], PVFTHSO$_2$[Figure 3B.8 (B)] and PVFCOUSO$_2$ [Figure 3B.8(C)] are closely monitored. Figure 3B.8(C) clearly depicts the stretching frequency corresponding to S=O at around (1000-1200)cm$^{-1}$, S-H stretching (2357 cm$^{-1}$), shifting of C-O-C stretching from 1168 cm$^{-1}$ to 1180 cm$^{-1}$; and shifting of O-H stretching from 3459 cm$^{-1}$ to 3435 cm$^{-1}$. The variation in stretching frequencies is indicative of the presence of chemical interaction between PVFCOU and SO$_2$. Similar observations are found for Figure 3B.8(A) and Figure 3B.8(B). However, for PVFCNTsSO$_2$, PVFCNPsSO$_2$ and PVFCSO$_2$, no significant difference in stretching frequencies are observed except the characteristic stretching frequency corresponding to S-H (Figure 3B.10) PVFCOU which bears an amorphous nature, highly non-uniform surface and macroporosity; upon exposure to SO$_2$, aggregation and trapping of SO$_2$ particles within the pores takes place and the surface roughness also increases, leading to the complete coverage of pores in PVFCOUSO$_2$. For PVFCNTs, low SO$_2$ sensing can be attributed to lack of macroporosity, where vapors were aggregated only at the surface in PVFCNTsSO$_2$. It has been observed that for PVFCNPs, exposure to SO$_2$ causes aggregation of vapors on its surface with no effect on macroporosity in PVFCNTsSO$_2$. 
Figure 3B.7 Arrhenius plots for synthesized PVF sponges.

Figure 3B.8 FT-IR plots before and after sensing SO$_2$ for (A) PVF; (B) PVFTH; and (C) PVFCOU.
Figure 3B.9 EDAX profiles of (A) PVFTH and PVFTHSO_2; and (B) PVFCOU and PVFCOUSO_2

Figure 3B.10 FT-IR spectra of (A) PVFCNPs and PVFCNPsSO_2; (B) PVFCNTs and PVFCNTsSO_2; and (C) PVFCOU and PVFCOUSO_2

Transport number measurements

The total electronic and ionic contribution to the conductivity is calculated from the standard Wagner polarization technique.[250] In this technique, voltage is applied to the cell resulting in either electronic conduction or ionic conduction. For electronic samples, current varies at a constant rate with time while for ionic samples, the current passing through a blocking electrode falls rapidly with time and then reaches an equilibrium state.[232] Herein, the sample is prepared in the form of thin film which is placed between two stainless steel anvils acting as a blocking electrodes. A step potential of 500 mV is applied across the electrodes and the resulting current is monitored as a function of time. The electrical switching behavior from insulator (PVF, PVFTH and PVFCOU) to semiconductor were observed upon exposure to SO_2 (PVFSO_2, PVFTHSO_2 and PVFCOUSO_2). The nature of semiconducting behavior is understood in
detail from transport number measurements. It is observed that for PVFSO$_2$ and PVFTHSO$_2$, ionic conduction is prevalent. The plots are depicted below in Figure 3B.11.

![Figure 3B.11 Effect of change in polarity on passage of current for (A) PVFSO$_2$; (B) PVFTHSO$_2$.](image)

**Figure 3B.11** Effect of change in polarity on passage of current for (A) PVFSO$_2$; (B) PVFTHSO$_2$

**Current (I)- Voltage (V) Studies of the foams PVF, PVFTH and PVFCOU before and after exposure to SO$_2$ vapors**

The measurement of transport number and the nature of variation of current with time for the composites led us to adopt an ac mode of conductivity measurements. From the I-V curves (Figure 3B.12), it can be concluded that the current increases gradually with increase in SO$_2$ concentration from 100 ppt-400 ppt [SO$_2$ (1) = 100 ppt, SO$_2$ (2) = 200 ppt, SO$_2$ (3) = 300 ppt; SO$_2$ (4) = 400 ppt] for PVF and PVFTH while it decreases gradually for PVFCOU with increase in concentration of SO$_2$ vapours. The probable reason behind such behaviour can be attributed to the ease of free electron diffusion from the electron rich SO$_2$ vapours to the polymer composites-PVF, PVFTH and PVFCOU. The molecular diffusion follows the order PVF>PVFTH>PVFCOU. These results can be correlated with FESEM images, where porosity follows the order PVF>PVFTH>PVFCOU. It is reported in the literature that free electronic movement of gas molecules efficiency is directly correlated with mean free path of gas molecules which is directly proportional to pore size.[251] Thus, electronic conduction of PVFSO$_2$ increases followed by PVFTHSO$_2$. However, PVFCOU has smaller pore size leading to restriction in free electronic movement. This leads to decrease in conduction in PVFCOUSO$_2$. 

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The variation of current flow with applied voltage at 1 kHz for the foams before and after SO$_2$ exposure were studied in detail.

**3B.3.4 Selectivity study**

Selectivity studies of PVFCOU composite is carried out in presence of chemicals like hydrogen sulphide (H$_2$S), ammonia (NH$_3$) and formaldehyde (HCHO) (Figure 3B.13). It is observed that a ‘Turn off’ effect is evident for SO$_2$ for the voltage in the range of (2-5) V. However, “Turn on” effect is observed for the analytes-H$_2$S, NH$_3$ and HCHO with discrimination in the range (0-2) V while the gases can’t be distinguished in the range (2-5) V as all these gases attain saturation.

**Figure 3B.12** I-V curves of (A) PVF and PVFSO$_2$ and (B) PVFTH and PVFTHSO$_2$

**Figure 3B.13** Selectivity studies of PVFCOU
3B.3.5 Effect of humidity

The effect of humidity has major implications in real time sensor efficiency.[252] The effect of humidity on sensing efficiencies are also studied at 20%, 40%, 60%, 70% and 80% humidities. It is observed that the sensing efficiency of our sensor PVFCOU is unaffected by the variation in humidity. The plot for the same is depicted below (Figure 3B.14).

![Sensing studies at different humidities (20-80) %](image-url)

**Figure 3B.14 Sensing studies at different humidities (20-80) %**

3B.3.6 Mechanistic Insight

It has been observed from these studies that PVCOU exhibit better selectivity towards SO$_2$ sensing compared to PVF and PVFTH. So, PVFCOU is the sensor of...
our choice. It is observed from the I-V studies that PVFCOU has a nonlinear I-V characteristics. It follows ohmic current upto 3.5 V whereas non-ohmic from 3.5-5 V. Such kind of non-linear I-V behaviour is a typical feature of inhomogeneous systems impregnated with conductive particles or clusters as fillers.[201] Here, Coumarin-6 has been incorporated as filler. It is a laser dye and have narrow bandgap, so, it bears conductive nature. An electrical conductivity switching is observed in PVFCOU upon exposure to SO$_2$. It is found that the conductivity value of PVFCOU decreases gradually with the increasing concentration of SO$_2$. The reason behind the switching behaviour is studied in detail. The interaction is supposed to be via chemisorption of SO$_2$ vapours on PVFCOU (Figure 3B.15). Chemisorption is an adsorption phenomena arising due to chemical interactions between the adsorbate molecules and the surface functional groups of adsorbents.[253-254] Here, PVFCOU can be regarded as an amorphous porous organic polymer composite. It has been reported in the literature that porous organic polymers are widely used for gas sensing applications particularly due to their surface area and pore structure.[255] The evidence regarding chemical interactions leading to chemisorption are supported by FT-IR plots and FESEM images collected for PVFCOUSO$_2$ at 35 $^\circ$C.

Figure 3B.15 Mechanistic insight into PVFCOU interaction with SO$_2$ vapours

3B.3.7 Time interval measurements

Response time is an important characteristics of a sensor where, lower the response time better is the sensor efficiency.[256] From the time interval measurements, it is found that PVACOU responds instantly to the vapour of SO$_2$. The sensor exhibits two order change upon exposure to SO$_2$ at (0-3) sec. The response time of PVFCOU towards SO$_2$ vapors is found to be 3 s for SO$_2$ concentration of 400 ppt. The time interval
measurement plot of the same is given in Figure 3B.16. Further, reproducibility of the sensor PVFCOU is studied and it is found that the sensor can be utilized up to three cycles (Figure 3B.17).

![Figure 3B.16 Time interval measurement plot of PVFCOU and PVFCOUSO₂](image)

![Figure 3B.17 Repeatability and reproducibility curves for SO₂ sensing by PVFCOU for a consecutive three cycles](image)

3B.3.8 Calibration curve of PVFCOU

In order to calculate limit of detection (LOD) of our sensor towards SO₂ vapors, a calibration curve is constructed by plotting $\Delta R/R_0$ (Y-axis) against $[SO_2]$ (X-axis).[257] LOD is calculated in accordance with the formula $LOD = 3 \sigma/S$, where ‘σ’ is the standard deviation of response and ‘S’ is the slope.[258] In accordance with this
technique, the LOD of our sensor is found to be 1.15 ppb. The calibration curve for the sensor PVFCOU is given in Figure 3B.18. Though many SO₂ sensors based on various transduction principles like electrochemical, optical and piezoelectric are reported in the literature, SO₂ sensor based on ac electrical impedance has not yet been reported.[259-261] Thus, PVFCOU emerges as a novel polymeric composite sensor based on ac electrical impedance with a good LOD for SO₂ vapours.

![Figure 3B.18 Calibration curve for PVFCOU](image)

**3B.4 Conclusion**

The present study demonstrates the utilization of ac electrical impedance technique for SO₂ vapour detection for the first time. The sensing material is developed by a simple one pot approach via utilization of ‘green’ precursor materials – coumarin-6 and PVA. A simple impedimetric technique has been utilized in the present study. The composite PVFCOU is found to exhibits response time of 3 s for 400 ppt SO₂ and a LOD of 1.15 ppb SO₂ at an ambient condition. Further, the interaction is found to be of chemical nature as evidenced from FT-IR. The development of such cost effective materials together with its integration to some portable electrical devices can open up a new area for highly efficient multiple vapour detection system within a single frame.
**Part C: PVA based impedimetric sensor for the detection of carcinogen formaldehyde**

**3C.1 Introduction**

Formaldehyde is an active component of formalin (aqueous solution of formaldehyde stabilized by methanol), which is widely used as preservative in processed food materials like fishes and during transportation of fishes particularly in third world countries like Bangladesh, Srilanka and India.[262] It is also widely used as an intermediate during the production of consumer goods like shampoos, soaps, detergents, sterilizing agent in pharmacology and medicine.[263] It has been classified as mutagen and carcinogen and thus its intensive use is found to exhibit detrimental effects in human health and environment.[262,263]

Researchers around the globe have investigated a number of sensor approaches for the detection of formaldehyde. Kettrup et al. has developed voltametric devices and chemosensors based on graphite paste electrode modified by palladium.[264] Xie et al. has developed a polarographic method by direct determination of formaldehyde with Girard T-reagent.[265] Few other optical, amperometric and potentiometric biosensors has also been designed by some research groups. [266-268] But, most of these techniques suffer from many drawbacks like sophisticated instrumentation, tedious sample preparation and expensive precursor materials for sensing material development. Recently, fluorimetric sensors have been developed for sensing and bioimaging applications. [269-271] However, fluorimetric techniques are not suitable for the development of portable and cost effective devices for analyte detection. In this regard, electrical impedance spectroscopy has clear advantages. The portability and ease of sample preparation are two distinct advantages compared to other techniques. [272-273, 35]

Literature studies reveal that Polyvinyl alcohol (PVA) is widely used as a desirable material for sensor development particularly due to its low cost, thin film forming properties etc. [258, 274-278] Coumarin-6 incorporation in polymer matrix for fluorimetric analysis is also widely reported. [279-282] Herein, we have prepared PVA-Coumarin-6 nanocomposite material (PC) for impedimetric analysis of formaldehyde via utilization of capillary action phenomena of cellulosic paper.

**3C.2 Experimental Section**
3C.2.1 Materials

Polyvinyl alcohol (Loba Chemie), Coumarin-6 (Aldrich), Formaldehyde (Merck), 4-Vinyl pyridine (SRL), Benzene (Merck) and 1-Vinyl-2-Pyrolidone (SRL). Milli-Q water is used during our experimental analysis. All other reagents used in our analysis are of analytical grade and used without further purification.

3C.2.2 Synthesis of PVA-Coumarin-6 thin films

Pure and doped PVA are prepared by solution casting technique as reported in the literature. [282] Briefly, 2.5 g of PVA and coumarin-6 are dissolved in 100 ml of water and ethanol respectively. These solutions are then magnetically stirred till the constituents are completely mixed. This is followed by the addition of Coumarin-6 (0.1, 0.3, 0.5, 1) % to PVA solution separately. The homogeneous solutions so obtained are poured in petridish and left on for drying overnight to yield samples in the form of thin films of thickness 0.3 mm. Such films are then used for different analysis. The chemical structures of the reactants and probable structure of the sensor PC (PVA and Coumarin-6 are held together by H-bonding interaction) are given below in Figure 3C.1.

![Figure 3C.1 The probable structure of the sensor PC](image-url)

3C.2.3 Instrumental techniques
Fourier Transform Infrared Spectrophotometer (FT-IR) Spectra has been done in the Attenuated Total Reflectance (ATR) mode with a NICOLATE 6700 FT-IR spectrophotometer in the range 650-4000 cm\(^{-1}\). Thermogravimetric Analysis (TGA) are performed using Perkin Elmer TGA 4000 instrument at the heating rate of 10°C per minute with a nitrogen flow rate at 20 ml/min. Field Emission Electron Microscopy (FESEM) studies are done with SIGMA-VP (ZEISS) SEM at an accelerating voltage of 5 kV in SE2 mode. Further, Impedance (Z) studies are carried out with HIOKI 3532-50 LCR HiTESTER.

### 3C.2.4 Fabrication of low cost device for detection of analyte in solution state

A novel concept for the detection of analyte in solution state has been proposed particularly keeping in view the cost effectiveness of the analytical system. [271] Two stainless steel plates of dimension 1 cm\(^2\) × 1 cm\(^2\) with uniform porosity are chosen as non-reactive conducting electrodes. A thin film of the sensor material (thickness 0.3 mm) is sandwiched between the plates. A commercially available paper clip with insulating polymer coating is utilized to hold the plates together with the sensor material in between the plates. The terminals of the plates are further connected to the impedance analyzer. A filter paper of dimension 1 cm × 1 cm is utilized as a cellulosic strip for the flow of analyte to the sensor material. Aliquots of analyte are added to the cellulosic strip for the sensing purpose. In a nutshell, a cost effective analytical system is designed by utilizing two porous stainless steel plates (1 cm\(^2\) × 1 cm\(^2\)), an insulated paper clip, the sensor material (PC), a cellulosic strip and aliquot of analyte. The analytical set up for sensing formaldehyde is given below in Figure 3C. 2.
Figure 3C. Low cost device fabrication for detection of analytes in solution state

3C.2.5 Hemocompatibility (Interaction with human blood cells)

% hemolysis assay

Blood is collected from a healthy human volunteer in heparinised tubes and stored at 4 °C and used within a week of collection. The fabricated sensor materials weighing 1 mg, 3 mg and 5 mg are incubated for 1 h at 37 °C with 10 ml of human red blood cells (RBC) in 1:9 dilutions with normal saline (0.9%). Blood in normal saline without test materials is considered as a negative control and with distilled water was considered as positive control. After the incubation period, the reaction mixture is centrifuged at 1500 rpm for 10 min and the supernatant is carefully collected and the concentration of free haemoglobin is measured at 540 nm using UV-Vis spectrophotometer. The percentage hemolysis is calculated as per the following equation [283].

\[
\text{% Hemolysis} = \frac{(\text{Abs Sample} - \text{Abs -ve control})}{(\text{Abs +ve control} - \text{Abs -ve control})} \times 100
\]
3C.2.6 Cytocompatibility assay (Interaction with mammalian cells)

Cytocompatibility assay (Interaction with mammalian cells)

Cytocompatibility potential of the fabricated material is evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay.[289] L929 mouse fibroblast cells are procured from National Centre for Cell Sciences (NCCS), Pune and maintained according to supplier’s guidelines. 100 μl DMEM (Dulbecco’s Modified Eagle Medium, supplemented with 10 % fetal bovine serum) culture medium containing L929 cells in a density of 1 ×10⁴ cells per well, are incubated in 96-well cell culture plate. After 24 h incubation, cultured well is treated with a series of different concentrations (100, 250 and 500 μg/ml) of the fabricated materials in culture media (serum free Eagle’s minimal essential medium) and further incubated for 24 h, 48 h and 72 h. After completion of the stipulated incubation period media is removed and treated with MTT dye (0.5 mg/ml) and incubated for 4 h at 37 °C. Finally, blue formazan precipitate is dissolved via addition of 100 μl dimethyl sulfoxide (DMSO) to each well and absorbance is measured at 570 nm using a multimode reader (Thermo Scientific, USA). The percentage viability is calculated by using the following equation:

\[
\frac{N_t}{N_c} \times 100
\]

Where \(N_t\) is the absorbance of the treated cells and \(N_c\) is the absorbance of the untreated cells. All the samples are done in triplicate.

3C.2.7 In vivo biocompatibility study

Animals and maintenance

Six male adult wistar rats weighing 180-200 gm were used for this study. All the animals were housed in polypropylene cages at animal house of Institute of Advanced study in Science and Technology (IASST), Guwahati (India). Animal house conditions were maintained at 24 °C ± 1 °C, with relative humidity of 45–55% and 12:12 h dark/light cycle. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of IASST, Guwahati (IASST/IAEC/2014-15/774). Animals were acclimatize for 5 days to the laboratory environment before performing the experiment.
and fed with standard pellet diet (Provimi Animal Nutrition Pvt. Ltd., India) and water ad libitum throughout the experimental period.

**Surgery protocol**

All the animals were anesthetized with ketamine hydrochloride and xylasine cocktail (80/10 mg/kg) to perform the surgery. Further animals were transferred to the surgery table and hair on the ventral region was removed. 2 mm incision was made and the PC was implanted inside the animal’s body (Under the skin) and incision was closed. The wound was applied with antibiotic lotion to avoid infections. Biocompatibility experiment was continued for 14 days and implant along with surrounded tissue was harvested on 7th and 14th day of the surgery to perform the histopathology analysis (n=3).[284,285]

**Histopathology analysis**

The harvested tissue samples were fixed in 10% buffered formaldehyde and processed for histological studies. The processed tissue was embedded in the paraffin followed by 5 mm thickness sections were prepared by using microtome. Further the sections were stained with hematoxylin and eosin to observe the histological changes under bright field microscope. [284, 285]

**3C.2.8 In vitro degradation analysis**

**Preparation of Simulated Body Fluid (SBF)**

SBF with ion concentrations in mM: Na\(^+\) 142, K\(^+\) 5.0, Mg\(^{2+}\) 1.5, Ca\(^{2+}\) 2.5, Cl\(^-\) 148, HCO\(_3\)^- 4.2, HPO\(_4^{2-}\) 1.0, SO\(_4^{2-}\) 0.5) nearly equal to those of human blood plasma was prepared by dissolving NaCl, NaHCO\(_3\), KCl, K\(_2\)HPO\(_4\), MgCl\(_2\) . 6H\(_2\)O, CaCl\(_2\), and Na\(_2\)SO\(_4\) in ion-exchanged water in a polystyrene bottle, according to the method described previously.[286] This solution was buffered at pH 7.4 with tris(hydroxy-methyl)aminomethane ((CH\(_2\)OH)\(_3\)CNH\(_2\)) and 1M hydrochloric acid (HCl) at 37°C.

Phosphate buffer saline (pH 7.2) was purchased from HIMEDIA (M1452).

**Weight loss during degradation**

*In vitro* degradation by means of the weight loss was calculated by comparing the dry weight (\(W_d\)) of the remained sample after incubation with SBF and PBS solution separately for a predetermined time with the original dry weight (\(W_0\)) of the sample as
the eq. (1). At predetermined intervals of 0, 3, 6, 12, 24 and 48 days; samples were taken out, purged with distilled water and subsequently dried until absolute desiccation, then weighted.

\[
\text{Weight loss (\%) = } \frac{W_0 - W_d}{W_0} \times 100 \quad (1)
\]

3C.3 Results and Discussions

3C.3.1 Characterization

Conformational and configurational analysis

The FT-IR of parent components PVA and coumarin-6 were carefully analyzed. PVA showed O-H stretch (H-bonded) at 3272 cm\(^{-1}\), sp\(^3\) C-H stretch at 2908 cm\(^{-1}\) (aliphatic), C-C stretch at 1418 cm\(^{-1}\) and C-O stretch at 1091 cm\(^{-1}\).\(^{[258]}\) However, upon doping PVA with coumarin-6, shifting of base peaks of PVA were observed which imply probable interaction between PVA and Coumarin-6. The FT-IR spectra of PC with different concentrations of coumarin-6 (0.1\%, 0.3\%, 0.5\% and 1\%) is given below in Figure 3C.3.

![Figure 3C.3 FT-IR spectra of PC having different compositions of Coumarin-6](image)

**Figure 3C.3** FT-IR spectra of PC having different compositions of Coumarin-6
Current (I)-Voltage (V) Studies

I-V studies of the synthesized polymeric composites were carefully studied by passing alternating current at a fixed frequency 1 kHz at room temperature. The I-V curve (Figure 3C.4) showed linear rise in current with the rise in voltage for the polymeric composite PC, thus showing ohmic behaviour while the conductivity in other polymeric composites remain unaltered with the rise in voltage. The probable reason behind the ohmic behaviour in PC may be attributed to homogeneous distribution of coumarin-6 into the PVA matrix. Coumarin-6 is a solid state laser dye with a lasing peak at 534 nm which bears interesting properties like lack of toxicity and versatility.[287] Since it has an emission at longer wavelength, so theoretically the band gap is lower in coumarin-6. [194] The homogeneous incorporation of coumarin-6 in PC probably leads to free electronic movement resulting in ohmic behaviour.

![Figure 3C.4 I-V curve of the composites-PC 0.1, PC 0.3, PC 0.5 and PC](image)

Bulk electrical conductivity measurements

The measurement of bulk electrical conductivity is an important aspect with respect to impedimetric sensor design. The bulk electrical conductivity of PC was studied in detail for a temperature range of (35-85) °C. TGA studies indicated that PC is thermally stable
upto 170 °C (Figure 3C.5). Nyquist plot of PC was also was constructed in order to find out the bulk resistance of PC (Figure 3C.6). The log (σT) versus 1/T(Arrhenius plot) was constructed and depicted in Figure 3C.7. The a.c. conductivity values at 35 °C for PC was found to be 1.5× 10^{-7}, which implies its semiconducting nature. The conductivity of PC changed non-linearly with increase in temperature, it first showed an increase followed by gradual decrease. The probable reason behind such behaviour can be explained from the contribution of ionic and electronic polarization. [288] The increase in conductivity (polarized) with increase in temperature from (35-55) °C can be attributed to the enhancement of ionic polarization and the decrease from (55-85) °C can be explained via enhancement of electronic polarization. [288]

![Comparative TGA plot of synthesized materials with precursors](image)

**Figure 3C.5** Comparative TGA plot of synthesized materials with precursors (Inset: PVACOU1 = PC)
Hemocompatibility studies

Human RBC constitutes 40-50 % (v/v) of human whole blood and widely used for biosafety evaluation studies of various materials of biomedical interests. In the present study, we have evaluated the effect of the prepared sensor material (PC) in different weights on the lysis and morphological changes of the RBCs. The tested material was found to be non-toxic at different concentrations toward RBC upon treatment for 1 h
For blood contacting biomaterials, the hemolysis percentage should be below 5% whereas in our case the treated material with highest concentration showed hemolysis less than 1%.

**Effect on RBC morphology**

RBCs are biconcave disk shape and they are very sensitive to interaction with membrane active substances. The morphology of RBCs were unaltered upon interaction with test material whereas the cells in the positive control group were disrupted completely (Figure 3C.8A-9E.).

![Figure 3C.8 FESEM micrograph of RBCs with different treatments (A) Positive control; (B) Negative control, (C-E) cells treated with sensor materials weighing 1 mg, 3 mg and 5 mg respectively, (F) Graph showing percentage hemolysis of RBC with different treatments](image)

Note: Inset: DW: Distilled water; NS: Normal saline

**Effect of sensor material on mammalian cells**

Prior to any in vivo applications of the fabricated sensor material PC, high dose tolerance exhibition by a mammalian cell line is of outmost necessity. In this study, the tested materials exhibited negligible cytotoxicity on the mitochondrial activity of L929 mouse fibroblastic cell line at different concentrations (Figure 3C.9). The minimal cell death exhibited by the test material may arise due to disturbances created by the sensor material PC in the physiological environment of incubated cells.
Figure 3C.9 Effect of test material at different concentrations on percentage cell viability of L929 mouse fibroblast cell lines.

In vivo biocompatibility assay of the sensor PC
To evaluate the in vivo biocompatibility, PC was implanted into the animal system (Sub plantar). No mortality of the animals were observed after implanting the PC during the experimental period. After 7th and 14th day of the implantation, histopathology studies were performed which were depicted in Figure 3C.10. The pathology results explained that at 7th day inflammatory cell infiltration was observed with no traces of the PC. But, with tissue sample day 14th exhibited prominent muscle cells with complete lack of inflammatory cells. From this experimental evidence, we can conclude that our fabricated sensor material PC possesses biocompatibility and rapid biodegradation property.

Figure 3C.10 Histopathology analysis of sensor implant and surrounded tissue at (A) 7th day and (B) 14th day of surgery. Abbreviations: I: Inflammatory cells; M: Muscle cells.

Degradation behaviour in PC within 48 days
The weight loss of PC films in SBF and PBS on the 1st, 3rd, 6th, 12th, 24th and 48th day were shown in Table 3C.1. The weight loss velocity in SBF was much faster than that in PBS. In SBF, the weight loss velocity was faster in the first day, and then exhibited a
gradual enhancement. The instant weight loss on the first day was caused by the ethanol liberation and dispersion of loosely bound Coumarin-6 in the buffer solutions. It has been demonstrated that the ethanol always dissolved into the water in the first few hours. From day 1 to day 48, the decrease of the weight loss was very slow in case of SBF and PBS but the weight loss was completely stopped after 7 days in case of PBS. This phenomenon demonstrated that the degradation and dissolution rate of PVA was very slow in SBF under in room temperature.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Weight loss % (SBF)</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>6.9</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>7.4</td>
<td>6.5</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>6.9</td>
</tr>
<tr>
<td>24</td>
<td>10.3</td>
<td>7</td>
</tr>
<tr>
<td>48</td>
<td>15.9</td>
<td>7</td>
</tr>
</tbody>
</table>

*Table 3C.1 The weight loss of PC films in SBF and PBS (Day 1-Day 48)*

### 3C.3.2 Sensing Studies

**Packaging, fabrication and sensing set up of sensor material**

A cost effective packaging was realised for sensing material comprising of thin film of PC (1 cm²), reusable uniformly porous stainless steel plates of dimension (1 cm × 1 cm), a polymer coated paper clip (commercially available) and a disposable paper bag.

Sensing material (PC) was sandwiched between two plates with the paper clip as reported previously.[86] PC was kept in contact with cellulosic filter paper which is impregnated with formaldehyde via capillary action. The terminals of the plates were then connected with an impedance analyzer for measuring sensitivity towards formaldehyde (*Figure 3C.11*).
Selectivity studies

In order to carry out selectivity studies, cellulosic filter paper is utilized for making a contact between PC and analytes. It was observed that the utilization of cellulosic filter paper do not alter the current conductivity of the sensor PC. Selectivity studies were carried out with well known chemicals like 4-vinyl pyridine, 1-vinyl-2-pyrolidone and benzene. Further, it was observed that these chemicals have negligible impact on the sensitivity of PC (Figure 3C.12). However, enhancement in conductivity was observed from 1.3 mA to 7 mA in presence of formaldehyde.
Mechanistic insight

The probable reason behind high current conduction of PC in presence of formaldehyde was studied in detail. It is well known that the electron transport at room temperature leads to increase in current conductivity due to the presence of good electron acceptor.[289] Herein cellulose modified formaldehyde acts as an acceptor while PC acts as a donor. The mechanistic pathway is depicted in Figure 3C.13.
Reversibility studies

Reversibility study of the sensor PC was carried out for five consecutive days (Figure 3C.14). It was observed that the sensing efficiency of PC towards formaldehyde is of irreversible nature. Thus, the sensor PC can be used only once for sensing formaldehyde. Since, PC can be used only one time, so biodegradability and biocompatibility of the PC is of utmost importance. It is found that the PC is biodegradable as well as biocompatible, which makes it an ideal sensor material for formaldehyde.
Limit of Detection analysis

The calibration curve was constructed by plotting mean current passing through the sensor material with increasing amount of formaldehyde. The Limit of Detection (LOD) was found out experimentally from the intersection of the two tangents in the calibration curve (Figure 3C.15).[125] The LOD of 3.51 ppm was found out which depicts the capability of our sensor to detect formaldehyde in the ppm range.
In addition, time interval measurement was carried out and the response time of the sensor PC towards formaldehyde was measured and found to be 30 s. The time interval measurement plot is given below in Figure 3C.16.

Comparative studies
A comparative analysis was carried out with reported works for formaldehyde sensing. Han et al. reported CdO activated Sn-doped ZnO for formaldehyde sensing with a LOD 10 ppb.[290] In another study, Wang et al. reported functionalized TiO₂ fibers on quartz crystal microbalance and it was found to exhibit a LOD of 1 ppm.[291] However, in the present context, our biodegradable and cost effective sensor PC was found to exhibit a LOD of 3.51 ppm which is comparable to the reported works.

3C.3.3 Real time applications

Real time applications of sensor is particularly important keeping in view of the practicability of sensors. The carcinogenic health effects of formaldehyde led us to detect formaldehyde in blood serum sample. It was found that our sensor is sensitive for detection of formaldehyde in blood serum and exhibited a LOD of 1.72 ppm (Figure 3C.17). This suggests that our sensor is applicable for real time application.

![Figure 3C.17(A) I-V sensing of the sensor with HCHO in blood serum; (B) Calibration curve](image)

3C.4 Conclusion

In this work, we have demonstrated the synthesis of PVA based thin film-PC as a sensor for the detection of formaldehyde in solution state. PVA-Coumarin-6 (PC) thin film was prepared by employing a cost effective simple one pot approach. The sensor was found to exhibit in cytocompatibility, hemocompatibility, invivo-biocompatibility and biodegradability. The sensing of formaldehyde was carried out with cost effective strategy using impedance spectroscopy. Two reusable stainless steel plates with uniform
pores were employed as electrodes, a thin film of sensing material (PC) was sandwiched between the electrodes and a cellulosic strip was utilized for making contact between formaldehyde solution and PC. A LOD of 3.51 ppm and a response time of 30 s were achieved. PC also exhibited good selectivity and was unaffected by alteration in humidity. Further, real time sensing application was carried out by sensing formaldehyde in blood serum and a LOD of 1.72 ppm was achieved. In a nutshell, such works demonstrate the potential of development of cost effective materials and sensing system for the detection of hazardous chemicals.