CHAPTER THREE

THE MATERIALS AND THE METHODS USED IN THE EXPERIMENTS
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In this chapter, the liquid systems that have been investigated in the present study and brief description of the materials and the methods used in these experiments are given.

3.1 LIQUID SYSTEM AND MATERIALS:

Fluorescence quenching studies have been performed for the following three 4-azidomethyl coumarin derivatives in different solvents and solvent mixtures at room temperature. Aniline is used as an external quencher in the fluorescence quenching studies. Later estimation of ground state and excited state dipole moments have also been performed in different solvents and the solutes are

1) 5, 6 benzo-4-azidomethyl coumarin,
2) 7, 8 benzo-4-azidomethyl coumarin,
3) 6-methoxy -4- azidomethyl coumarin

These 4- azidomethyl coumarin derivatives were synthesized as explained here. 4- Bromomethyl coumarin (0.01 mol) was taken in acetone 20 ml in a round bottom flask. To this sodium azide (0.78 gm, 0.012 mol) in 3 ml water was added drop wise with stirring. The stirring was continued for 10 hours. Then the reaction mixture was poured into ice cold water. The separated solid was filtered and recrystallised from suitable solvent. The various solvents used in the present study are n-hexane, n-heptane, cyclohexane, toluene, benzene, 1,4-dioxane, acetonitrile, ethyl acetate, diethyl ether, dichloromethane, acetone, 1,1,2,2- tetrachloroethane, tetrahydrofuran, ethylalcohol, dimethylsulfoxide, 1-hexanol, tetrachloroethylene, butanol, methanol, carbon tetrachloride, and propanol. All these are the scintillator solvents of spectroscopic grade and obtained from S.D-fine chemicals. The quencher
aniline was also obtained from S.D-fine chemicals and was double distilled and tested for its purity before use.

The molecular structures along with the molecular weight and names of three solute molecules are given below

![Molecular Structures](image.png)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,6-benzo-4-azidomethyl coumarin</td>
<td>251</td>
</tr>
<tr>
<td>7,8-benzo-4-azidomethyl coumarin</td>
<td>251</td>
</tr>
<tr>
<td>6-methoxy-4-azidomethyl coumarin</td>
<td>231</td>
</tr>
</tbody>
</table>

Fig. 3.1 Molecular structure of the solute molecules.

A) SAMPLE PREPARATION:

Solutes and quenchers were weighed using a semi microbalance having an accuracy of 0.01 mg. The solutions were prepared in respective solvents keeping the solute concentration fixed at $1 \times 10^{-4}$ M/L and the quencher concentration [Q] of aniline was varied from 0.00-0.10 M/L. Each time fresh homogeneous solutions were placed in quartz cell with airtight stopper to record both absorption and fluorescence spectra. In case of ground and excited state dipole moment studies, the concentrations of solutes used were $2.5 \times 10^{-4}$ M/L, $5 \times 10^{-4}$ M/L, $7.5 \times 10^{-4}$ M/L and $10 \times 10^{-4}$ M/L to record the dielectric constants and refractive indices of these concentrations.

3.2 EXPERIMENTAL DETAILS:

The absorption spectra were recorded using UV/VIS Spectrophotometer Model 150-20. The fluorescence spectra were recorded using Fluorescence Spectrophotometer Model F–2000. The fluorescence lifetimes were measured using time correlated single photon counting technique (TCSPC). The dielectric constants
of solutions were measured using Forbes Tinsley (FT) 6421 LCR Data Bridge. The refractive indices of solutions were measured using ATAGO 3T Refractometer. The details of all these experimental techniques are discussed below.

3.3 UV/VIS SPECTROPHOTOMETER:

The optical system of the UV/VIS Spectrophotometer Model 150-20 is shown in Fig.3.2. It is a sensitive instrument for the study of absorption spectrum in ultraviolet and visible region. Its light source compartment contains a tungsten lamp for visible region, and deuterium lamp for ultraviolet region. The light emitted from the source passes through the stray light cut filter F and slit S₁, and enters into the Seay-Namioka mount monochromator utilizing a concave diffraction grating (having a grating constant of 1/600 nm, a blaze wavelength of 250 nm, and a diffraction area of 20 nm x 25 nm). In the monochromator, the light of desired wavelength is obtained and it is made to pass through the exit slit S₂ and toroidal mirror M₂, and then reaches the rotating mirror M₃ where it is divided into two light beams; one is used for reference and the other is for sample. These two light beams, after passing through the sample compartment, alternatively irradiate the R928 ultra high sensitivity detector where they are converted into two electric signals. The electric signals converted from the optical signals are amplified by the preamplifier, and then using ADC, digital signal was obtained. Thereafter, they are automatically processed by the arithmetic programs built in the control unit. The measured results are displayed on the CRT screen and recorded on the graph printer. The signals are processed by Hitachi's unique differential feedback system capable of measuring even negative absorbance. The Model 150-20 takes in a zero signal every time, the rotating mirror in turn makes retaining zero level at all times accurately.
3.4 FLUORESCENCE SPECTROPHOTOMETER:

The Fluorescence Spectrophotometer Model F–2000 block diagram as shown in Fig 3.3. Figure 3.4 shows the optical system configuration of the Fluorescence Spectrophotometer Model F–2000. The radiation coming from the Xenon lamp is converged at entrance lenses L1 and L2. Only the light dispersed by the excitation concave grating (excitation beam) enters the exit slit S2. The excitation beam from exit slit S2 is reflected by concave mirror M1 and it splits into two by the beam splitter BS. One of the two excitation beams goes to the monitor detector for its measurement and the other beam (most of excitation) passing through BS is converged to the sample cell through lens L3. The fluorescence coming out of the sample is restricted
into entrance slit $S_3$ of the emission monochromator through lenses $L_4$ and $L_5$. The fluorescence dispersed by the emission concave grating passes through the exit slit $S_4$ and is converged at the photomultiplier via concave mirror $M_2$ for intensity measurements. The emission slit serves also as shutter to protect the photomultiplier and it automatically closes, upon opening the lid of the sample compartment. All the driving components, that is wavelength drive motors and slit control motors are operated by signals sent from the computer.

Fig.3.3 Block diagram of Fluorescence Spectrophotometer [model F 2000].
On the other hand, output signals from the monitor detector and fluorescence detector (photomultiplier) are processed by the computer via the A/D converter and transmitted to the CRT or graphic plotter.

3.5 TIME CORRELATED SINGLE PHOTON COUNTING (TCSPC) TECHNIQUE

Fluorescence lifetime can be measured by various techniques. Some of the commonly used techniques are:

a) Phase shift technique,

b) Sampling method using sampling oscilloscopes and

c) Picosecond techniques using mode locked or synchronously pumped dye laser.

Among all, the best method for measuring the fluorescence lifetime is the phase shift technique i.e. Time Correlated Single Photon Counting (TCSPC) technique. Photophysics model of SPC nanosecond fluorescence spectrometer
available at R.S.I.C, I.I.T, Mumbai, India to measure the fluorescence lifetimes of the solutes with and without quencher.

These instruments utilize a nanosecond flash lamp for pulsed excitation and single photon counting technique for the measurement of fluorescence intensity as a function of time. A schematic diagram of the SPC instrument is given in the figure 3.5 and working of the SPC is described in the following paragraph.

Excitation pulse from a thytron gated hydrogen flash lamp is simultaneously used for two purposes; one to excite the sample and the other to an electrical start signal. The optical signal from the excitation source is transmitted to a START PMT through a fiber optical cable to generate the electrical start pulse, which is routed through a constant fraction discriminator (CFD) to start the input of a Time to Amplitude Converter (TAC) to initialize its charging operation. In the mean time the same optical pulse excites the sample, which subsequently gives the emission. The emitted photons are then detected by a STOP PMT to generate an electrical stop pulse. The collection rate of emitted photons in the STOP PMT is kept very low by adjusting the apertures in both the excitation and the emission monochromator slits. The stop pulse is then routed through another CFD and a variable delay line to the stop input of the TAC. On receiving the stop signal, the charging operation in TAC is stopped and it generates an electrical output (TAC-output) having amplitude proportional to the time difference between (Δt) the start and stop pulses arriving at the TAC. The TAC output pulse is then given to the input of a Multi Channel Analyzer (MCA). The MCA is having an analog to digital converter (A/D converter) which generates a numerical value corresponding to the amplitude of the TAC output and a count is stored in an appropriate address channel of the MCA, selected by the number generated in the A/D converter. The above cycle from excitation to data

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storage is repeated a large number of times to generate a histogram of number of counts against the channel number of the MCA. Since the collection of the emitted photons in the stop PMT is kept very low, the distribution of the count in MCA channels (the histogram) exactly represents the probability of emission of a single-photon after an excitation event and thus the fluorescence decay of the sample. The MCA can operate either in Pulse Height Analysis (PHA) or in Multi Channel Scaling.
(MCS) mode. For our experiments we used MCA in PHA mode. Then the data stored in the MCA channel is transferred into a LSI-11/23 (Plessey, UK) microcomputer for analysis, with available programs by the reconvolution technique using a nonlinear least square fit method. Plots of weighted residuals and values of reduced $\chi^2$ tests are used to judge the quality of the fits.

3.6 FORBES TINSLEY (FT) 6421 LCR DATA BRIDGE:

The dielectric constants of the solutions are usually measured as the ratio of capacitance with the dielectric and air as the media in a suitably fabricated cell of usually small capacitance. Since the solutions are dilute, the dielectric constants so measured are not expected to differ much from solution to solution. Therefore, an accurate determination of small changes in capacitance is required. These small capacitances can be measured with the help of Forbes Tinsley (FT) 6421 LCR Data Bridge at 10kHz frequency. The necessary dielectric sample holder consists of two concentric brass cylinders kept in position with small glass strips (to achieve electric isolation) and their leads are coated with gold for electrical contact. This assembly is kept in a glass beaker so that dilute solution can be filled into the cell. The capacitance of the empty cell (air) would be of the order of pico-farad (pF). Measurements of capacitance were made as detailed below.

Capitance of cylindrical cell with air as dielectric media $= C_1$

Capitance of cylindrical cell with solution as dielectric media $= C_2$

The dielectric constant ($\epsilon$) of a dilute solution can be determined by the ratio of change in capacitance with and without the sample i.e.

$$\epsilon = \frac{C_2}{C_1}$$
However, this expression does not take into account the capacitance due to connecting leads, hence the application of the above equation is limited. By taking into the account of capacitance of leads, the modified ratio for $\varepsilon$ may be given as follows

$$\varepsilon = \frac{(C_2 - C_x)}{(C_1 - C_x)}$$

where $C_x$ represents the capacitance of the connecting leads and it is of the order of 5.457 pF. The value of $C_x$ was determined by carrying out measurements on several standard solvents of known dielectric constants.

3.7 ATAGO 3T REFRACTOMETER:

The refractive indices of the dilute solutions are usually measured with the refractive index and air as the media in a prism cell. Since the solutions are dilute, the refractive indices so measured are not expected to differ much from solution to solution. Therefore, an accurate determination of small changes in refractive indices is required. The refractive index ($\eta$) of a dilute solution can be measured with the help of ATAGO 3T Refractometer. The necessary refractive index of sample solution drop kept on prism cell, soon after closing prism cell, then adjusting colour compressor knobe and scale refractive index of the dilute solution can be recorded of given solution. The value of refractive index of solution was determined by carrying out measurements on several standard solvents of known refractive indices. The value of refractive index of given solution is automatically displayed on the front panel of instrument.