CHAPTER II

EXPERIMENTAL TECHNIQUES
The principal instruments employed in the present investigation of steady-state measurements are UV-vis absorption spectrophotometer, spectrofluorometer, refractometer, LCR data-bridge and dielectric cell. Time-resolved measurements were carried out employing time correlated single photon counting and fluorescence up-conversion system. A detailed account of these instruments is given in the following sections.

II.1. ABSORPTION SPECTROPHOTOMETRY

A UV-vis absorption spectrophotometer (Hitachi, model U-2800) is used for the absorption measurements of the sample solutions at room temperature (298K) the optical system of which is shown in Fig.2.1. It is a double beam instrument, which features a half mirror to split in to reference and sample beams. The white light emitted from the source is fed to Seya-Namioka mount monochromator utilizing a concave diffraction grating (grating constant: 1/600 mm, a blaze wavelength: 250 nm and grating area: 20 mm x 25 mm) featuring high-energy efficiency and low stray light level, where it is transformed into a monochromatic beam. The beam sent from the monochromator is reflected by the toroidal mirror (M3) and then separated into reference and sample beams by the half mirror (M4). The two beams after passing through the sample compartment are focused by lenses, and are then irradiated into the detector where they are converted into electric signals. The electric signal converted from optical signal enters the LOG amplifier where it is LOG converted to provide absorbance data. This data is amplified, and is then converted in to digital signal to be processed as digital value. The processed results are displayed on the LCD and recorded onto the printer. U-2800 can also be controlled by the optional UV solution software through a
Figure 2.1: Optical layout of Hitachi model U-2800 UV-vis spectrophotometer
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Windows-based computer. All the measurements were done using this computer controlled software UV solutions 2.0. The concentration of the solutions was maintained in the range from $10^{-5}$ to $10^{-6}$ M.

II.2. FLUORESCENCE SPECTROPHOTOMETRY

The fluorescence spectrophotometer (Hitachi, model F-2000) was used for recording the fluorescence spectra of the selected probes in different solvents. In the steady-state measurements, the most commonly used light source is the high-pressure xenon lamp, giving output in the range of about 200-800 nm. Such lamps are generally useful because of their high intensity at wavelengths ranging upwards from 200 nm. The optical layout of the instrument shown in Fig.2.2 is equipped with monochromators to select both the excitation and emission wavelengths. The excitation monochromator contains two gratings, which increase the purity of the exciting wavelength. In addition, these monochromators use concave gratings, produced by holographic means, which further decrease stray light level.

The radiation from the xenon lamp is converged at the entrance slit $S_1$ of the excitation monochromator through the lenses $L_1$ and $L_2$. Only the light dispersed by the excitation concave grating enters the exit slit $S_2$. The excitation beam from the exit slit $S_2$ is reflected by the concave mirror $M_1$ to the beam splitter $BS$ where the beam splitter splits the light emerging from the excitation monochromator. A beam splitter, placed in the excitation light path, consists of a thin piece of clear quartz, which reflects about 4% of the incident light. This amount is generally adequate for a reference channel, which does not use a monochromator. One of the two-excitation beams directed to the monitor detector for its measurement, and the other beam passing through the
Figure 2.2: Optical system diagram for model F-2000 fluorescence spectrophotometer.
beam splitter is converged to the sample cell through the lens L3 and is used to excite the sample in the cuvette. The fluorescence emitted by the sample is normally collected at right angle to the excitation beam and is restricted into the entrance slit S3 of the emission monochromator through the lenses L4 and L5. The fluorescence dispersed by the emission concave grating passes through the exit slit S4 and is converged onto the photo multiplier through concave mirror M2 for intensity measurement.

Polarizers are present in both the excitation and emission light paths. Generally the polarizers are removable so that they can be inserted only for measurements of fluorescence anisotropy. Accurate measurement of fluorescence anisotropies requires accurate angular positioning of the polarizers. For this reason the polarizer mounts must be sturdy and accurately indexed to determine the angular orientation. A schematic diagram of fluorometer for anisotropy measurements is presented in Fig.2.3. The polarizer accessories (Hitachi, Model 650-0155 and 650-0156) installed in the sample compartment of the fluorescence spectrophotometer enable the measurement of steady-state fluorescence anisotropy.

II.3. LCR BRIDGE AND DIELECTRIC CELL

To determine the dipole moment of a probe UVITEX-OB (U-OB) in its ground state requires the measurement of dielectric constants of the solvent and dilute solutions with only small differences in concentrations. The change in capacitance with variation in concentration will be very small and thus accurate determination of minor changes of capacitance is essential. The dielectric constant of a medium is usually measured as a ratio of the capacitance of a condenser cell with and without the dielectric medium in it.
Among several methods available for the measurement of very small changes in capacitance, a method suggested by Ferre et al. [1] and by others [2-4] is widely used. In the present investigation, a digital LCR data bridge (10 KHz) of the type 6421 supplied by Forbes Tinsley Co. Pvt. Ltd., Aurangabad, India, having a resolution of 0.001 pf with a basic accuracy of ±0.01% with four terminal integral test jig connections, is used.

**Dielectric cell:** A dielectric cell (Fig.2.4) is used to measure static dielectric constant \( \varepsilon_{12} \), was fabricated in the laboratory. It comprises two concentric metallic cylinders kept in position with a small glass strip (to achieve electric isolation) between the cylinders and their leads are coated with gold. The entire cell is placed in a glass beaker so that dilute solution can be filled into the cell and the capacitance of the empty cell (with air) would be of the order of pico farads.

Measurements of the capacitance were made as followed:

Let, capacitance without cylindrical cell = \( C_1 \)

capacitance of cylindrical cell with air as a dielectric media = \( C_2 \)

and capacitance of cylindrical cell with solution = \( C_3 \)

The dielectric constant of a dilute solution (\( \varepsilon_{12} \)) can be determined by the ratio of change in capacitance with and without the sample, which is given by

\[
\varepsilon_{12} = \frac{C_1 - C_3}{C_1 - C_2}
\]

However, this expression does not take the capacitance due to connecting leads into account and as such, the application of this equation...
Figure 2.4: Schematic representation of the cell used for the determination of static dielectric constant
becomes limited. By taking the capacitance due to connecting leads ($C_x$) into account, we get

$$\varepsilon_{12} = \frac{(C_1 - C_g) - C_x}{(C_1 - C_g) - C_x}$$

In the present study, $C_x$ was determined by carrying out measurements on several standard liquids (polar and nonpolar) whose $\varepsilon$ are known and the value of $C_x$ thus found for the setup is 5.507 pF. Thereby, $\varepsilon_{12}$'s are the static dielectric constants of the graded solutions of U-OB in toluene at 25° C.

**II.4. REFRACTIVE INDEX MEASUREMENTS**

Refractive index ($n_{12}$) of U-OB solutions for sodium-D line to calculate ground state dipole moments using Guggenheim method [5] was measured using the thermostatically controlled Abbe’s refractometer (Atago 3T, Tokyo, Japan). Precision of the instrument was ±0.0001 units. This refractometer was fitted with hollow prism casings through which water was circulated. The temperature of the prism casings was observed with digital display (±0.1° C). The instrument was provided with two prisms placed one above the other in front of the telescope. Upon inserting a drop of the test liquid using a hypodermic syringe, the incident ray forms a line of demarcation between light and dark portions of the field. This was viewed with telescope, which moves with the scale. The instrument directly gives the value of $n$.

The refractive index measurements have been carried out at 25° C by circulating water from a thermostat. In order to obtain precise data, refractometer was calibrated frequently using a glass piece of known refractive index supplied along with the instrument [6]. It was also double checked by
measuring the refractive index of the pure water. A built-in sodium-D lamp was used as a light source and an average of triplicate measurement was considered in all the calculations.

II.5. TIME-RESOLVED FLUORESCENCE DECAYS

Steady-state measurements cannot always resolve the individual components, which may contribute to overall fluorescence. Measurements of the fluorescence decay time are able to do so. Two techniques used to resolve fluorescent components are: Time Correlated Single Photon Counting (TCSPC), and phase modulation techniques [7]. The TCSPC method based on the repetitive precisely timed registration of single photons of a fluorescence signal has been employed in the current study.

II.5.1. Time Correlated Single Photon Counting (TCSPC) technique

II.5.1.1. Principle of operation: The fluorescence decay of each fluorophore obeys first order kinetics, since fluorescence decay is a unimolecular process. This allows the resolution of different fluorescence components. TCSPC involves an elaborate instrument to measure the time between an excitation light pulse, generated by a pulsed light source such as a laser, and the arrival of a fluorescent photon at a microchannel plate-photomultiplier tube. The time-to-amplitude converter (TAC) is the device that achieves the time correlation between excitation and emission events. Upon receipt of a start signal, and after a certain fixed delay, a timing capacitor is charged linearly from a constant current source. The charging is discontinued upon the acceptance of a stop pulse and an output pulse is generated with amplitude derived from the final charge in the capacitor. Therefore, the output
pulse height is proportional to the time difference between start and stop pulses. If no stop pulse is received after a certain amount of time, charging is automatically discontinued and the capacitor is reset [7]. The measured times are digitized by the electronics and then output to a computer-controlled multichannel analyzer. This accumulates the photon counts in data channels assigned to different time points, typically 2048 at 10 ps/channel each. The resulting histogram illustrates the fluorescence decay of a sample. Each point on the decay profile is obtained from a single pulse. The excitation intensity is attenuated with a neutral density filter, so that only one fluorescence photon is detected per 100 excitation pulses. At this rate, approximately 8000 pulses are detected in 1 second for an 82 MHz excitation source. To optimize the signal to noise ratio, 10000 counts are collected in a typical curve. An intensity-time profile of the laser pulse, also known as the instrument response function (IRF), can be generated using the Stokes Raman scattering or Raleigh scattering of pure water at an appropriate wavelength [8].

II.5.1.2. **Experimental setup:** A schematic diagram of fluorescence lifetime (IBH, UK, Model 5000U) is shown in Fig.2.5. The second harmonic output from the Tsunami mode locked picosecond laser was used as the excitation source. The mode locked 375 nm laser pulses are focused on the sample and the fluorescence photons from the sample were collected at right angles to the excitation beam. These emitted photons were detected by MCP-PMT (R3809U, Hamamatsu) after passing through the emission monochromator. The output of the MCP-PMT was fed to a discriminator whose output serves as a stop signal for the TAC. The start signal for TAC is derived from a high-speed red sensitive silicon photo detector (DET 210, Thor Labs Inc.). The fundamental output (750 nm) from the Tsunami mode locked
Figure 2.5: Picosecond Laser and TCSPC setup
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Picosecond laser was focused on the photodiode. The photodiode signal is converted to a TTL signal by a pulse converter (model TB-01, IBH). The output TTL signal from the pulse converter is used as a start pulse for the TAC. The TAC output is fed to MCA card (Oxford Corporation, UK) and the data collection was carried out by a software (Data station 2000) provided by IBH. Repetitive laser pulsing and emitted photon collection produce a histogram of counts against voltage (time). This histogram represents the fluorescence decay of the sample under study. For recording the lamp profile, a scatterer was placed in place of the sample and the above procedure was repeated. The response time of this instrument is ~50 ps.

II.5.1.3. Picosecond laser source: The diode laser pumped continuous wave yttrium vanadate (NdYVO₄) laser (Millenium V, Spectra Physics, USA) was used to pump the Ti:sapphire rod in the mode locked picosecond laser (Tsunami, Spectra Physics). The diode laser contains two laser diode bars each having 19 diode elements. These diodes have an output at 809 nm with a power of 13 W, which are fibre coupled to pump an NdYVO₄ laser that produces an output at 1064 nm. The frequency doubled using a lithium triborate (LiB₂O₃) crystal to produce 5 W of green light at 532 nm. This NdYVO₄ laser now pumps the original Ti:sapphire laser, which is tunable over a wavelength range 720 to 840 nm. The regenerative mode locked pulses of the laser are obtained by Kerr effect and the pulse width of the mode locked Tsunami laser is < 2 ps operating at 82 MHz. The pulse width is measured using an autocorrelator (model 409-08, Spectra Physics), which employs second harmonic generation with background free configuration technique. The measured pulse is displayed on a high impedance oscilloscope (Scientific, 300 MHz) for real time viewing.
The pulse picker (model 3980, Spectra Physics) selects the pulses at a rate of 4 MHz from the 82 MHz trains of pulses from Tsunami laser.

A flexible harmonic generator (FHG) (Spectra Physics) is used to generate the second and third harmonic laser outputs. In this unit second harmonic generation is accomplished by focusing the laser from the pulse selector into LBO crystal. In order to generate third harmonic signal, both the fundamental and second harmonic beams should overlap perfectly with time and space in $\beta$-barium borate (BBO) crystal. With the standard optics, Tsunami generates 750 nm pulses as a fundamental output and the second harmonic output from the FHG at 375 nm is used as an excitation source for the samples. The fluorescence photons emitted from the sample are detected at right angles to the excitation beam, by a high gain Micro Channel Plate Photomultiplier Tube (R3809U MCP-PMT, Hamamatsu).

II.5.1.4. **Fluorescence decay analysis:** The measured fluorescence decay is the convolution of true fluorescence decay, excitation function and the instrument response function (IRF). The fluorescence kinetic parameters (lifetime, amplitude, etc.) are obtained by deconvoluting the excitation and the IRF from the measured fluorescence decay. The data analysis was accomplished by programming software known as DAS-6 provided by IBH. DAS program acts as a user-friendly interface for the creation of decay associated spectra. This is based on the reconvolution technique using iterative nonlinear least square methods. The reconvolution is preceded by a series of iteration until a chi-square ($\chi^2$) value is reduced. The quality of fit is normally identified by the reduced $\chi^2$, weighted residual and the autocorrelation function of the residual.
II.5.2. Fluorescence up-conversion system

II.5.2.1. Frequency conversion unit: Fig.2.6 shows a schematic layout of the CDP2015 frequency conversion unit (CDP Corp., USA). An input beam of Ti:sapphire fundamental frequency (FF, red line) with horizontal polarization hits second harmonic generator consisting of focusing lens L1, nonlinear crystal NC1 and collimating lens L2 that controls the divergence of fundamental beam.

There are two holes for the input beam in the frequency conversion unit box. Main input path contains two apertures A1 and A2 for easy unit alignment. Femtosecond second harmonic (SH) pulse is delayed in time relative to fundamental one due to group velocity dispersion of NC1. This delay should be compensated before sum frequency (third harmonic) generation. Dichroic beamsplitter BS1 transmits the fundamental beam of horizontal polarization and reflects the second harmonic beam. The fundamental beam goes through adjustable optical delay line, consisting of M2-M3 mirror pair, and directed to sum frequency generator of the FOG100 optical unit with the help of M4 and M5.

The second harmonic beam (blue line) is directed with BS1 to a periscope polarization rotator consisting of M6 and M7, and the beam level of 70 mm at BS1 is decreased to 60 mm at M6. Polarization rotator changes the beam polarization from vertical to horizontal changing the beam polarization by $90^\circ$ and putting up the beam again to 70 mm level. The second harmonic beam of horizontal polarization goes through a telescope, consisting of L3 and L4, which controls the beam divergence. Dichroic beamsplitter BS2 reflects the second harmonic beam and transmits the fundamental beam. Second harmonic generator of the FOG100 optical unit, consisting of L5, NC2 and L6, is used as
Figure 2.6: Optical scheme of the CDP2015 frequency conversion unit.
The horizontal polarization is represented by ( —i) and vertical polarization by (■) symbol.
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a sum frequency (third harmonic) generator. Beam splitter BS3 replaces standard FOG100 beamsplitter. BS3 reflects third harmonic (TH, dark red line) of vertical polarization and transmits fundamental and second harmonic beams.

II.5.2.2. Fluorescence up-conversion unit: Sum frequency generation (up-conversion) method is used in the femtosecond optically gated (FOG) fluorescence kinetic measurement system FOG100 to achieve a temporal resolution better than 100 femtoseconds [9]. This FOG100 system is the first complete measurement system for the femtosecond kinetic spectroscopy, designed to be matched with any type of femtosecond oscillator or amplifier operating at ≥1 KHz pulse repetition rate. The system includes optical and mechanical components (including optical delay line) installed on a breadboard, monochromator, selected photon counting PMT, electronic control unit and Lumex 3.1 software. The control unit is connected to a computer via serial port.

A general view of the FOG100 system configuration is shown in Fig.2.7. The system consisting of optical unit in which optical delay line and frequency doubler for operation with Ti:sapphire laser are installed. A monochromator (CDP 2022S), 380-mm single or 160-mm double is adapted to the PMT head and optical unit through PMT and monochromator adapter, respectively. The PMT head contains a selected photon counting PMT, preamplifier and HV power supply. Electronic control unit connects a computer with PMT and step motor of the optical delay. The unit is connected to a computer via serial port using a software Lumex version 3.1.

A schematic layout of the FOG100 optical unit in fluorescence transmission configuration is shown in Fig.2.8. The input beam can be directed from one of the two sides of the unit: input IN1 or IN2 (optional), depending on
Figure 2.7: Schematic layout (top view) of the FOG100 system (with single monochromator)
Figure 2.8: Schematic layout of the FOG100 optical unit in fluorescence transmission configuration.
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complete laser system configuration. Mirror M1 (Fig.2.8) is removed in case of IN1 input, and fundamental frequency radiation goes together with the second harmonic from external CDP2015 frequency conversion unit through IN1. Lenses L1, L2 and NC1 nonlinear crystal serves as second or third harmonic generator (SHG or THG). It is SHG when the input radiation is fundamental frequency only, and THG when the input radiation is fundamental frequency together with second harmonic radiation. NC1 has about 15%-20% efficiency for the second harmonic generation and 3%-4% efficiency for the third harmonic generation together with the second harmonic generation inside the CDP2015 frequency conversion unit. Beamsplitter BS1 is used to split the input beam to excitation and gate beams. For the second harmonic or third harmonic excitation, corresponding beamsplitters BS1 are used. They transmit >95% of fundamental radiation, and reflect >99% of second or third harmonic radiation, respectively. Note that BS1 dichroic beamsplitter, separating second (third) harmonic fundamental radiation, reflects several percent of fundamental (gate) radiation, which can be successfully removed using additional BS2 beamsplitter (identical to BS1). The gate beam is directed by mirrors M2 and M3 to gold-coated reflector mirror R, connected to the optical delay line (DL). These mirrors are used for precision beam alignment parallel to DL travel. R reflects an input beam exactly back. Mirrors M4 and M5 direct the gate beam into the nonlinear crystal NC2.

The excitation beam (second harmonic) is directed to a sample S with the help of BS1 and BS2. Typically the sample is kept in a specially designed rotating cell. Lens L3 (f = +40 mm) focuses the excitation radiation into the sample, and a filter F2 can be used for the excitation attenuation. The Berek variable waveplate gives appropriate polarization of the excitation light.
Sample fluorescence is collected with an achromatic lens AC (f = +40 mm) and directed to the nonlinear crystal NC2, where sum frequency mixing with gate radiation takes place. The filter F3 eliminates residual excitation light and transmits fluorescence. Fluorescence and gate radiation are focusing to NC2 with lens L4 (f = +80 mm). Sum frequency radiation, generated in the NC2, is focused with L5 (f = +60 mm) to an input slit of the monochromator (together with fluorescence and gate radiation). Iris aperture A3 and filter F4 select (in addition to monochromator) sum frequency radiation from other radiation.

II.5.2.3. **Rotating sample cell:** A rotating sample cell is usually used for liquid samples to minimize photo degradation during long excitation with femtosecond pulses. The cell contains two silica windows separated by either 1 mm or 0.4 mm Teflon ring spacer. The cell has 27 mm clear aperture, but it can be filled partially in case of smaller amount of sample. Narrow ring of sample is formed in this case during rotation. The cell is driven with DC motor giving variable moderate (1-5 Hz) rotation velocity. A special cell holder minimizes any cell deviations during rotation.
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II.6. REFERENCES


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