Chapter II

EXPERIMENTAL TECHNIQUES

2.1 UV / Vis spectrophotometer
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As described in the earlier chapter, the present investigation deals with the study of solute-solvent interactions based on the steady state absorption and fluorescence emission measurements and the measurements of fluorescence anisotropy and life times of a selected series of exalite dyes. The various instruments employed for the purpose are uv/vis spectrophotometer, fluorescence spectrophotometer (with polarizer accessories and thermostatic cell holder), picosecond time-correlated single photon counting spectrometer, LCR data bridge and dielectric cell. A brief description of these is presented in this chapter.

2.1 Absorption spectrophotometer

Absorption spectra of dilute solutions at concentrations in the range of $10^{-4}$ - $10^{-5}$ M/L of the exalite dyes in various solvents were recorded using uv/visible double beam ratio recording absorption spectrophotometer (Hitachi, Model 150-20). It is a sensitive instrument for the study of absorption spectrum in the uv and visible region. Its light source compartment contains a tungsten lamp for the visible region and a deuterium lamp for the uv region. The instrument has automatic corrections for the light source as well as the detector.

The optical layout of the spectrophotometer is shown in Fig.2.1. The light emitted from the source passes through the stray light cut filter $F$ and slit $S_1$ and illuminates a concave diffraction grating (600 l/mm, blaze wavelength 250 nm) in the Seya Namioka mount monochromator.
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W: Tungsten lamp (10 V, 4 A)
D2: Deuterium discharge lamp
F: Filter (FI: 900 ~ 600, F2: 390 ~ 340, U: 360)
G: Grating (600 l/mm, blazed at 250 nm)
PMT: Photomultiplier (R928)
S1: Entrance slit (H: 6 mm, W: 0.1 mm)
S2: Exit slit (H: 6 mm, W: 0.1 mm)

Fig. 2.1: Optical ray diagram of UV-Visible Absorption spectrophotometer (Hitachi Model: 150-20)
The monochromatic light output of the monochromator falls on rotating mirror $M_3$ where it is divided into two light beams. One beam is used as a reference beam and the other passes through the sample. These two light beams, the reference and sample beams, after passing through the sample compartment alternately illuminate the R928 ultra sensitive detector where they are converted into current signals.

### 2.2 Fluorescence spectrophotometer

A fluorescence spectrophotometer (Hitachi, Model F2000) was used for recording the fluorescence spectra of the dyes chosen. Fig. 2.2, depicts the optical arrangement of the instrument in which two separate monochromators are used—one for the excitation beam and the other for the emission beam. The monochromatic excitation beam at a desired wavelength illuminates the sample cell and the fluorescence collected at $90^\circ$ is focussed onto the entrance slit of emission monochromator. The output of the emission monochromator is detected by a PMT. 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. A thermostatic cell holder (Hitachi, Model 251-9061) with stirrer is used for measuring the effect of temperature on fluorescence and anisotropy. The stirrer speed of 500 to 1200 rotations per minute and temperature range from 5 to 75 °C are available.
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Fig. 2.2: Optical system configuration of the fluorescence spectrophotometer of the model F2000 Hitachi.
2.3 Measurement of Fluorescence Decays

The fluorescence decays of the exalite dyes in alkanes and alcohols were measured using a picosecond laser as an excitation source and a time correlated single photon counting technique (TCSPC) with micro channel plate photomultiplier tube (MCP-PMT) as a detector. The fluorescence lifetime spectrometer employed for this purpose is described in the following pages:

a. Time Correlated Single Photon Counting Technique (TCSPC)

Fluorescence lifetimes of the fluorophores can be measured employing various methods among which, TCSPC is the best technique due to many advantages. TCSPC is a digital technique and counts the photons which are time correlated with the excitation pulse. The sample is repetitively excited using a pulsed light source. Each pulse from the source is optically monitored by a high-speed photodiode, which produces a start signal that triggers a voltage ramp in the time to amplitude converter (TAC). This voltage ramp is stopped when the first fluorescence photon from the sample is detected. The TAC provides an output pulse whose voltage is proportional to the time between the start and stop signals. A multichannel analyzer (MCA) converts this voltage to time channel using an analog-to-digital converter (ADC). The MCA thus builds up a probability histogram of counts versus channels by summing over a large number of pulses.

A schematic representation of fluorescence lifetime spectrometer (IBH, U.K., Model 5000U) is shown in Fig. 2.3. The second harmonic output from the Tsunami mode locked picosecond laser was used as the excitation source. The mode locked
Fig. 2.3 Picosecond Laser and TCSPC Setup
375 nm laser pulses are focussed in the sample and the fluorescence photons from the sample were collected at right angles to the excitation beam. These emitted photons were detected by a 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 photodetector (DET 210, Thor Labs Inc). The fundamental output (750 nm) from the Tsunami mode locked 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 the MCA Card (Oxford Corporation, UK) and the data collection was carried out by a software (Datastation 2000) provided by IBH. Repetitive laser pulsing and emitted photon collection produces 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.

b. Picosecond Laser source

The diode laser pumped cw Nd-YVO₄ laser (Millennia 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
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having 19 diode elements. These diodes have an output at 809 nm with a power of 13 Watts, which was routed through the fibre optical bundles to the Millennia.

The 809 nm output pumped the Nd-YVO₄ rod which in turn produced an output at 1064 nm. This is the fundamental output and is converted to 532 nm by a phase-matched doubler crystal (Lithium triborate). The 532 nm output is taken out through a dichroic output coupler of the Millenna V laser.

The 532 nm laser beam (5W) pumps the mode locked Ti: sapphire laser which is capable of generating pico/femto second pulses with appropriate optics. The pump beam is incident on the Ti: sapphire rod at Brewster's angle. The output of the Ti: sapphire laser is tunable from 690 to 1080 nm. In the present investigation standard optics is used to get an output between 720 and 850 nm and the wavelength selection is achieved by the birefringent filters. 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 train of pulses of 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 in time and space in BBO crystal (beta barium borate). 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 (R 3809U MCP-PMT, Hamamatsu).

c. Fluorescence decay analyses

The measured fluorescence decay is the convolution of true fluorescence decay, excitation function and the instrument response function. The fluorescence kinetic parameters (lifetime, amplitude, etc.) are obtained by deconvoluting the excitation and the instrument response function from the measured fluorescence decay. The data analysis was accomplished by a software (IBH, DAS-6) based on reconvolution technique using iterative nonlinear least square methods. The reconvolution is preceded by a series of iteration until a Chi-Square value is reduced. The quality of fit is normally identified by the reduced $\chi^2$, weighted residual and the autocorrelation function of the residuals.

2.4 LCR bridge and Dielectric cell

The determination of dipole moment of a molecule in a given solvent mainly requires the measurement of the dielectric constants of the solvent and
solutions when the samples are 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 small 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 the several methods reported in literature for the measurement of very small changes in capacitance a method suggested by Ferre et al. [1-4] is widely used. In the present investigation, a digital LCR data bridge of the type 6421 supplied by Forbes Tinsley Co. Pvt. Ltd., Aurangabad, India, having a resolution of 0.001pf and basic accuracy of ± 0.01 % with four terminal integral test jig connections, is used.

Dielectric Cell

The dielectric cell (Fig. 2.4) used to measure static dielectric constant $\varepsilon_{12}$, was fabricated in the laboratory [4]. It comprises two concentric metallic cylinders kept in position with a small glass strip between the cylinders and their leads are coated with gold. The entire cell is placed in a glass tube with a ground glass stopper for closing after filling with the solution.
Fig. 2.4: Schematic representation of the cell used for the determination of static dielectric constant.
2.5 References


