Preliminary Time-Resolved Fluorescence Studies of Some Olefin appended Dibenzobarrelenes and Bisdibenzobarrelenes Employing TCSPC Technique

4.1. Abstract

To understand the primary and secondary physicochemical processes in a photochemical reaction it is necessary to characterise the excited states and the transient intermediates during their short lifetime. A number of methods developed on the basis of the physical properties of the transient species are available for their detection. Time-correlated single-photon counting technique has been utilised in the present study of the excited states of olefin appended dibenzobarrelenes and bisdibenzobarrelenes.

4.2. Introduction

Fluorescence spectroscopy\(^1\) is a powerful technique to investigate the structural and dynamic properties of molecules. The excited states of fluorophores are very sensitive to the changes in their environment, which can result in a shift of the absorption or emission wavelength, or an intensity variation. Alternatively, when time-resolved measurements are performed, the different decay rates of fluorescence intensity or the polarization anisotropy can be recorded. The former stems from the fact that many dynamic events can deactivate the excited state\(^2\) and hence influence the lifetime.

Time-resolved measurements are widely used in fluorescence spectroscopy, particularly for studies of biological macromolecules and for cellular imaging.\(^3\) Time-resolved measurements contain more information
than is available from the steady-state data. One can distinguish static and
dynamic quenching using lifetime measurements. The time-resolved donor
decays are highly informative about the purity of the sample as well as the
donor-to-acceptor distance.

The studies presented in this chapter focuses on the aspect of time-
resolved fluorescence. In this method, the fluorophores are excited by a
sudden pulse of light, which results in population of excited states. Then this
population decays through two channels: 1) random deactivation through
fluorescence emission and nonradiative processes, 2) quenching due to
energy transfer. In the case of the novel bisdibenzobarrelenes, there might be
chances of excimer formation.

Fox et al. as a case study for the design of donor-acceptor
compounds with tailor-made properties, has conducted an INDO/S level
molecular orbital investigation of organic molecules where aromatic donors
are rigidly linked to a maleic ester group via a barrelene-type bridge. The
study indicates that dibenzobarrelenes with dicarbomethoxy ester groups
resemble a bridged donor-acceptor molecule, where the aromatic residues act
as electron donors and the dicarbomethoxy ester groups act as electron
acceptors. The INDO/S calculations yielded a low-lying state with definite
charge-transfer characteristics.

The fluorescence quantum yields ($\phi_f^s$) were determined by comparing
the integrated fluorescence spectra of the sample with that of the reference, while keeping the excitation wavelength equal. Equation 1 was used for the
determination of fluorescence quantum yield ($\phi_f^s$).

$$\phi_f^s = \phi_f^r \frac{A_f^s}{A_f^r} \frac{OD_r^x}{OD_s^x} \frac{(\eta^s)^2}{(\eta^r)^2}$$

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where $A_j$ and $A'_j$ are the area under the fluorescence spectra of the sample and the reference respectively and $OD$ and $OD'$ are the respective optical densities of the sample and the reference solutions at the wavelength of excitation. A correction was applied for the variation of the refractive indices of the solvents. $n_j$ and $n'_j$ are the respective refractive indices of the sample solution and reference solution. These measurements were carried out to characterise the excited singlet ($S_1$) state.

Time dependent fluorescence measurement is an important method to investigate the dynamics of the singlet ($S_1$) state of the molecules. If the $S_1$ state is involved in some complex photochemical processes, such behaviour will be reflected in the fluorescence decay and the analysis of decay curves reveals the mechanistic details of the processes undergone by the $S_1$ state. In majority of the cases it is seen that the fluorescence decay can be expressed as a sum of exponentials as,

$$F(t) = \sum F_i(0) \exp \left(-t/\tau_i\right)$$

where, $F_i(0)$ is the fluorescence intensity of the $i^{th}$ component at time zero. The time dependent fluorescence of a molecule can be measured by a number of methods. Time-correlated single-photon counting spectrometer has been used in the present work and is described here in great detail.

4.2.1. Basic Principles of the Fluorescence Life Time Measurements by Time-Correlated Single-Photon Counting (TCSPC) Technique

Fluorescence lifetimes were measured using a time domain fluorescence spectrometer Model EL-199 (Edinburgh Instrument, U.K.). The schematic diagram of the set up is shown in Figure 1. The instrument operates
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on the principle of time-correlated single-photon counting (TCSPC). This technique relies on the concept that the probability distribution of a single photon emission after an excitation event represents the fluorescence intensity variation with time. An optical excitation pulse from a flash lamp is split into two parts, one part is used to excite the sample which is kept in the sample chamber and the other part is used to generate a start pulse in a start PMT. The optical signal at the start PMT generates an electrical START pulse which is routed through a Constant Fraction Discriminator (CFD) to the START input of the Time to Amplitude Converter (TAC) to initialise its charging operation. The part of the optical pulse which excites the sample effectively gives emission photons, which are then detected by a stop PMT (at the right angle to the direction of excitation) to generate a STOP pulse. The STOP pulse is also routed to TAC through another CFD and variable delay. On receiving the STOP signal, TAC stops its charging operation and generates an electrical output (TAC-output) having amplitude proportional to the time differences (Δt) between the START and STOP pulses reaching the TAC. The TAC output pulse is then fed to the input of a Multichannel Analyzer (MCA) through an analog to digital converter (ADC). The ADC generates a numerical value corresponding to the TAC output pulse and thus selects an appropriate address (channel) of the MCA and a count is added to this address. The above cycle (from excitation to data storage) is repeated large number of times and as a result a histogram of counts against the channel number of the MCA is generated. It represents exactly the fluorescence decay curve only when the collection rate of emission of the photon by the stop PMT is very low usually <0.02 photon/excitation pulse, and is illustrated by statistical treatment.13 Fluorescence lifetime of the samples are calculated from such curves after proper time calibration of the MCA channels.
4.2.2. Detailed Description of the Time Domain Fluorimeter (EI-199):

(a) Coaxial Flash Lamp: Low pressure, gated, thyratron-triggered coaxial flash lamp of high repetition rate is used for excitation. The lamp compartment and the thyratron are housed together in a large metal box, where the thyratron is coaxial with thoriated tungsten electrodes. The pulse repetition rate of the lamp can be adjusted by means of an oscillator, driving the thyratron pulser.

Figure 1. Schematic diagram of the Time-Correlated Single-Photon Counting Fluorescence Spectrometer, EI-199. 199F, the Flash lamp with control units; 199s, the spectrometer part; 199D, the SPC detection system and 199M, the data analyser.

Hydrogen gas is used at ~0.5 atm. Pressure in the lamp chamber and
the gap between the two electrodes is kept ~0.7 mm. The typical discharge voltage is applied about 6 to 8 kV and repetition rate for the lamp pulsing is kept ~30 kHz. Using the above parameters, ~1 ns (FWHM) pulses from D₂ lamp can be obtained.

The most important development for TCSPC since 2000 is the introduction of pulsed-laser diodes (LDs) and pulsed light-emitting diodes (LEDs) as simple solid-state sources. These devices consume little power, are easy to operate and require almost no maintenance.

(b) Discriminator: The signals from the PMT are routed through the discriminator in order to improve the signal to noise ratio (S/N) and to get correct timing information. As simple leading edge discriminator is always associated with timing errors, the Constant Fraction Discriminators (CFD) are used in SPC instruments. A threshold level is set on the front panel of the CFD to filter out low amplitude noises from the input pulses.¹³

(c) Variable Delay Lines: A variable delay line is incorporated in the path of the STOP signal between the CFD and the TAC to trigger the TAC-MCA combination in such a way that an optimum placement of the decay curve can be obtained in the MCA channels.

(d) Time to Amplitude Converter (TAC): The time correlation between excitation and emission events is carried out by the TAC unit and is called the heart of the SPC instrument. After receiving the START pulse and after a fixed delay, a timing capacitor is charged linearly from a constant current source. The charging is discontinued on the arrival of a STOP pulse and as a result an output pulse is generated equivalent to the amplitude of the final charge in the capacitor. Since charging ramp of the capacitor is linear with time, the TAC output signal height is proportional to the time differences (Δt) between the START and the STOP pulses.
(e) Multichannel Analyzer (MCA): The MCA used in the EI-199 is equipped with an AD-converter, a memory consisting of 1024 channels or multiple of this to store the time correlated data and a CRT screen. MCA can be operated either in Pulse Height Analysis (PHA) mode or in Multichannel Scaling (MCS) mode. For fluorescence lifetime measurements MCA is operated in PHA mode. The data stored in MCA channels are transferred to the computer for analysis.

(f) The Start PMT: As the light intensity detected by the Start PMT is quite high, an ordinary PMT with medium gain and reasonably low transit time can be used. IP-28 PMT (Hamamatsu) is used in EI-199 spectrometer.

(g) The Stop PMT: The spread in transit time (the time difference between the emission of a photoelectron and its arrival to the anode) has a pronounced effect on the time resolution of an SPC instrument. As the transit time becomes shorter the spread also becomes shorter and thus the time resolution increases. Thus a fast PMT is suitable for TCSPC measurements. As the detected light level is very low the gain of the Stop PMT should also be very high. To get better gain one has to increase the number of dynodes, as a result

Figure 2. Functioning of TAC
transit time increases in the process. For this purpose specially designed 12-stage Philips XP-2020Q end-on PMT is used as Stop PMT, which is having a high gain ($3 \times 10^7$ at 2.2 kV) and reasonably lower transit time (~28 ns). The cathode material of XP-2020 Q is bi-alkali KCsSb with spectral response from 200-650nm. The dark current is so low that one can work at room temperature.

4.2.3. Time Calibration in EI-199 Spectrometer:

Time Calibration of the MCA channels of EI-199 is done using a number of accurately calibrated delay lines. For this purpose the stop signal is split into two parts, one is fed to the START input of the TAC and the other is routed through the precisely calibrated delay lines and then fed to the STOP input of the TAC. As the same STOP signal is used for both the START and STOP input of the TAC, counts will be collected at a single channel of the MCA depending on the TAC range used and delay introduced in the path of the STOP pulse. For different delays, counts are collected at different channels of the MCA. MCA data are then processed in a computer and the time calibration is done using suitable program.

4.2.4. Data Analysis:

Since the excitation pulses used for practical purposes have a finite time width and the detection system has also a finite response time, the observed decay curve $I(t)$ is a convolution of the true decay curve $G(t)$ and the effective time profile of the excitation pulse, $P(t)$. Observing $I(t)$ and $P(t)$ experimentally and assuming a proper decay function $G(t)$, a convoluted function $Y(t)$ can be calculated and compared with the experimentally observed decay curve $I(t)$. Hence, $Y(t)$ can be expressed as

$$Y(t) = \int_0^t P(t') G(t - t') dt'$$

(3)
For mathematical analysis, the function $G(t)$ is assumed to be a sum of exponentials, such that

$$G(t) = \sum B_i \exp\left(\frac{t}{\tau_i}\right) + A$$  \hspace{1cm} (4)

Where, $B_i$ is the pre-exponential factor for the $i$th component, $\tau_i$ is the corresponding fluorescence lifetime and $A$ is a correction term for constant background. The mathematical procedure is used to calculate the function $Y(t)$ and then $G(t)$ is extracted out from $Y(t)$ by the nonlinear least square iterative reconvolution method and is assumed to be either a monoexponential or biexponential or multiexponential function and the best $G(t)$ is selected from the reduced chi-square ($\chi^2_r$) value and distribution of weighted residuals.

(a) Reduced Chi-square value: The reduced chi-square ($\chi^2_r$) value is defined as

$$\chi^2_r = \frac{\sum_{i=n_1}^{n_2} W_i \left\{Y(i) - I(i)\right\}^2}{(n_2 - n_1 + 1 - p)}$$  \hspace{1cm} (5)

Where, $W_i \{= 1/I(i)\}$, is the weighting factor of the counts in the $i$th channel, $n_1$ and $n_2$ are the first and last channels of the section of the analysed decay curve, and $p$ is number of degrees of freedom for the particular fitting (namely, three for single exponential fitting, i.e. $B$, $\tau$ and $A$ and so on). In the actual analysis the function $G(t)$ is assumed to be either a monoexponential or a biexponential or a triexponential function and for each of these cases the parameter $B_i$, $\tau_i$ and $A$ are varied as long as a minimum ($\chi^2_r$) value is obtained.

When the decay curve with its peak channel having 5-10 thousand counts, is considered to be having a suitable precision, the number of counts in
each channel $I(i)$, follows a Poission distribution with a standard deviation $\sigma_i$, given by

$$\sigma_i = \sqrt{I(i)}$$  \hspace{1cm} (6)

Thus, the best selection of $G(t)$ will be the one for which the ($\chi^2$) value is very close to unity.

(b) Distribution of Weighted Residuals: The weighted residual for the $i$th channel is defined as

$$r_i = W_i \{Y(i) - I(i)\}$$ \hspace{1cm} (7)

For a good fit the weighted residuals among the data channels should be randomly distributed about zero and should follow a Gaussian distribution, i.e. 68%, 95%, 99.7% and 100% of the weighted residuals should be within 1, 2, 3 and 4 respectively.

4.2.5. Overview of Fluorescence Spectroscopy

Fluorescence spectroscopy can be applied to a wide range of problems in the chemical and biological sciences. The measurements can provide information on a wide range of molecular processes, including the interactions of solvent molecules with fluorophores, rotational diffusion of biomolecules, distances between sites in biomolecules, conformational changes and binding interactions. The usefulness of fluorescence is being expanded by advances in technology for cellular imaging and single-molecule detection. Fluorescence spectroscopy will continue to contribute to rapid advances in biology, biotechnology and nanotechnology.
4.3. Results and Discussion

We chose the dibenzobarrelenes 1a-d and the bisdibenzobarrelenes 1e-h (Chart 1) for the time-resolved studies employing TCSPC (Time-Correlated Single-Photon Counting) Technique. The absorption spectra of 1a-h was recorded in dichloromethane at room temperature, is depicted in Figure 3. Dibenzobarrelenes 1a was chosen as the reference for our study.
Figure 3. Absorption spectra of 1a-h at room temperature in $10^{-5}$ M dichloromethane

In the case of dibenzobarrelene type molecular systems, the absorption band with peak intensity at 280 nm, is attributed to the O-O transition of $S_0 \rightarrow S_1$.

The fluorescence spectrum of the selected molecules taken in acetonitrile (ACN) and dichloromethane (DCM) is shown in Figures 4 to 10.
Figure 4. $\lambda_{\text{max}} = 450$ nm in DCM; $\lambda_{\text{max}} = 465$ nm in ACN

Figure 5. $\lambda_{\text{max}} = 470$ nm in ACN
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Figure 6. $\lambda_{\text{max}} = 425$ nm in DCM

Figure 7. $\lambda_{\text{max}} = 473$ nm in DCM; $\lambda_{\text{max}} = 445$ nm in ACN
**Figure 8.** $\lambda_{\text{max}} = 435$ nm in DCM

**Figure 9.** $\lambda_{\text{max}} = 415$ nm in DCM; $\lambda_{\text{max}} = 425$ nm in ACN
Of the selected molecules, 1c and 1f were non-fluorescent. It might be due to large rate of internal conversion or a slow rate of emission.\textsuperscript{1}

The fluorescence $\lambda_{\text{max}}$ of 1a-h showed marked sensitivity to solvent polarity. This behaviour can be explained through the Jablonski diagram shown in Figure 11. The processes that occur between the absorption and emission of light are usually illustrated by the Jablonski\textsuperscript{16} diagram. These diagrams are used in a variety of forms, to illustrate various molecular processes that occur in excited states.
Emission from fluorophores generally occurs at wavelengths that are longer than those at which absorption occurs. This loss of energy is due to a variety of dynamic processes that occur following light absorption as shown in Figure 11. The fluorophore is typically excited to the first singlet state ($S_1$), usually to an excited vibrational level within $S_1$. The excess vibrational energy is rapidly lost to the solvent. If the fluorophore is excited to the second singlet state ($S_2$), it rapidly decays to the $S_1$ state in $10^{-12}$ s due to internal conversion. Solvent effects shift the emission to still lower energy due to stabilisation of the excited state by the polar solvent molecules. Typically, the fluorophore has a larger dipole moment in the excited state ($\mu_E$) than in the ground state ($\mu_G$). Following excitation, the solvent dipoles can reorient or relax around $\mu_E$, which lowers the energy of the excited state. As the solvent polarity is increased, this effect becomes larger, resulting in emission at lower
energies or longer wavelengths. In general, only fluorophores that are themselves polar display a large sensitivity to solvent polarity. Nonpolar molecules, such as unsubstituted aromatic hydrocarbons, are much less sensitive to solvent polarity.

The marked difference in the fluorescence absorption maximum of the molecules in acetonitrile and dichloromethane, point towards the formation of an internal charge transfer state (ICT).\textsuperscript{17} Cortes \textit{et al.}\textsuperscript{10} has shown that dibenzobarrelene with dicarbomethoxy ester groups resemble a bridged donor-acceptor molecule, where the aromatic residues act as electron donors and the dicarboxy ester groups act as electron acceptors. Fluorophores possessing electron-donating and electron-accepting groups, upon excitation undergoes an increase in charge separation within the molecule.

The fluorescence emission spectrum of 1e in Figure 8, shows a broad, structureless emission band, characteristic of an excimer emission. But the non-gaussian nature of the curve and the curve being interrupted due to the second harmonic function of the flash lamp, does not confirm an excimer formation. The fluorescence emission spectra of 1g and 1h shows no excimer formation.

4.3.1. Fluorescence Lifetimes

The fluorescence lifetime is one of the most important characteristics of a fluorophore. The lifetime of a fluorophore is the average time between its excitation and return to the ground state and moreover it determines the time available for the fluorophore to interact with or diffuse in its environment, and hence the information available from its emission.

In the excited singlet states, the electron in the excited orbital is paired to the second electron in the ground-state orbital. Consequently, return to the ground state is spin allowed and occurs rapidly by emission of a photon. The
emission rates of fluorescence are typically $10^8 \text{ s}^{-1}$, so that a typical fluorescence lifetime is near 10 ns ($10 \times 10^9 \text{ s}$). Due to the short timescale of fluorescence, measurement of the time-resolved emission requires sophisticated optics and electronics. Despite the added complexity, time-resolved fluorescence is widely used because of the increased information available from the data, as compared with steady-state measurements.

The intensity decay for samples 1a-h, is shown in Figures 12 to 22. The curve L in the figures is the instrument response function, i.e. the response of the instrument to a zero lifetime sample. This curve is typically collected using a dilute scattering solution such as colloidal silica (Ludox) and no emission filter. This decay represents the shortest time profile that can be measured by the instrument.

The measured intensity decay of the various samples is shown as a histogram of dots. The height of the dots on the y-axis represents the number of photons that were detected within the time interval $t_k$ to $t_k+\Delta t$, where $\Delta t$ is the width of the timing channel. In the case of 1a in ACN (Figure 12), the largest number of counts has recorded approximately 3000 photons.
Figure 12

Counts vs Time (ns)

Figure 13

Counts vs Time (ns)
Counts < 3

Counts

Figure 14

Time (ns)

In ACN cozcv-1, '4 Wat

Figure 15

Time (ns)

In DCM cozma
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Figure 16

Figure 17
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Figure 18

Figure 19
Figure 20

Figure 21
A general property of fluorescence is that the same fluorescence emission spectrum is generally observed irrespective of the excitation wavelength. This is known as Kasha's rule.\textsuperscript{18} In this study, hydrogen flash lamp emitting 290 nm radiation and diode lasers emitting 408 nm radiation were utilised.

An important concept is that the lifetime is a statistical average and fluorophores emit randomly through out the decay. The fluorophores do not all emit at a time delay equal to the lifetime. For a large number of fluorophores some will emit quickly following the excitation, and some will emit at times longer than the lifetime. This time distribution of emitted photons is the intensity decay.
Table 1

Table 1 depicts the lifetimes of the excited species of 1a-h. The biexponential and triexponential decay of the excited species of these molecules, suggest complex processes involved in their deactivation, which could be explained only through further mechanistic investigations.

4.3.2. Fluorescence Quantum Yield

Quantum yield, one of the most important characteristics of a fluorophore, is defined as the number of emitted photons relative to the
number of absorbed photons. Substances with the largest quantum yields, approaching unity, such as rhodamines, display the brightest emissions.

The quantum yield exhibited by the selected molecules 1a-h is illustrated in Table 2. The measurements were carried out using dimethyl aminobenzonitrile (DMABN) in acetonitrile as standard, with its quantum yield as 0.03. It was used because the excitation wavelength of DMABN matched with those of the samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fluorescence Quantum Yield ($\phi_f$)</th>
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<tbody>
<tr>
<td></td>
<td>In CH$_2$CN</td>
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<tr>
<td>1a</td>
<td>0.015</td>
</tr>
<tr>
<td>1b</td>
<td>0.001</td>
</tr>
<tr>
<td>1c</td>
<td>0.0004</td>
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<tr>
<td>1d</td>
<td>0.002</td>
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<tr>
<td>1e</td>
<td>0.002</td>
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<tr>
<td>1f</td>
<td>0.001</td>
</tr>
<tr>
<td>1g</td>
<td>0.0006</td>
</tr>
<tr>
<td>1h</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 2

The dibenzobarrelene moiety without an olefin appendage at the bridgehead position, exhibited the largest quantum yield. The rest of the samples, having olefin moieties at the bridgehead position, showed very low
quantum yields, which might be due to the interactions between the olefin moieties and the dibenzobarrelene. Studies have shown that low values of $\Phi_f$ suggest a "flexible" molecule for which a rapid radiationless deactivation may occur via a twisting motion about a carbon-carbon double bond.\(^2\) It might also be due to quenching processes or might be the result of competing $S_1 \rightarrow T_1$ intersystem crossing.

4.4. Conclusion

The preliminary photophysical study of the dibenzobarrelenes 1a-d and the bisdibenzobarrelenes 1e-h were conducted utilising Time-Correlated Single-Photon Counting (TCSPC) Technique. The molecules 1a, 1b, 1d, 1e, 1g and 1h exhibited the formation of an internal charge transfer state upon excitation. 1c and 1f were non-fluorescent, which might be due to a large rate of internal conversion or a slow rate of emission. The biexponential and triexponential fluorescence decay, suggests complex processes involved in their deactivation. The excited state interaction between the bridgehead olefin appendages and the dibenzobarrelene moiety is confirmed through the very low fluorescence quantum yield.
References


15. Ware, W. R. Creation and Detection of the Excited State, Marcel Dekker, New York, 1971.