3.1 Introduction

3.1.1. Introduction to Photochemistry

During the last 25 years there has been a remarkable growth in the use of fluorescence in biological sciences—biochemistry and biophysics. Fluorescence also finds application in environmental monitoring, clinical chemistry, DNA sequencing and genetic analysis by fluorescence in situ hybridization (FISH). In molecular biology, fluorescence is used for cell identification and sorting in flow cytometry, and in cellular imaging to reveal the localization and movement of intracellular substances by means of fluorescence microscopy. Because of the high sensitivity of fluorescence detection, expense and difficulties of handling radioactive substances, there is continuing development of medical tests based on the phenomenon of fluorescence. These tests include the widely used enzyme linked immunoassays (ELISA) and fluorescence polarization immunoassays.

As a result of light absorption, molecules attain higher singlet energy electronic levels, and in most cases this is accompanied by adjustment to a new equilibrium between the molecule and its environment. Deactivation of the excited molecule can take place either by the emission of fluorescence quanta (or, after inter-system crossing to triplet levels, phosphorescence) or by non-radiative energy dissipation to the solvent. Transitions between energy states occur in about $10^{15}$ s, a time too short for significant displacement of nuclei. This is the Franck-Condon principle.

The luminescent property of a molecule is mainly determined by the measurement of absorption wavelength ($\lambda_A$), emission wavelength ($\lambda_F$), quantum yield ($\phi_F$), fluorescent decay lifetime ($\tau_F$), radiative ($k_R$) and non-radiative decay ($k_{NR}$) constants. The fluorescence life time and quantum yields are the most important characteristics of a fluorophore. The quantum yield is the number of
emitted photons relative to the number of absorbed photons. The quantum yield is found by recording the total number of photons emitted by the fluorescing molecules throughout its fluorescence emission and comparing it with the number of photons absorbed. Thus if $I_f$ is the fluorescence intensity and $I_a$ is the intensity of the absorbed light, the quantum yield is then defined as

$$\phi_F = \frac{I_f}{I_a} \quad (3.1)$$

The maximum theoretical value of quantum efficiency $\phi$ is unity, since each quantum absorbed excites only one molecule, which can emit only one quantum of light on emission while decaying to the ground state. Since the quantum of light re-emitted is generally of longer wavelength than the one absorbed, the amount of energy re-emitted even at $\phi = 1$ is less than the amount absorbed, and the excess energy in transformed into heat. The quantum yield for most molecules decreases with increasing temperature.

The lifetime of the excited state is also an important photophysical parameter of any fluorophores as it determines the time available for the fluorophore to interact with or diffuse in its environment, and hence the information available from its emission. The lifetime of the fluorophore in the absence of non-radiative process is called the intrinsic or true life time, $\tau_F^0$. The intrinsic radiative life time $\tau_F^0$ of a state is the reciprocal of the rate constant for the disappearance of this state if emission were the only path of energy dissipation.

$$i.e., k_R = \frac{1}{\tau_F^0} \quad (3.2)$$

where $k_R$ is the radiative decay constant. However for most molecules measured fluorescence lifetime $\tau_F$, that is the mean lifetime is always less than $\tau_F^0$. The relationship between the two life times is given by
\[ \tau_F^0 = \frac{\tau_F}{\phi_f} \quad (3.3) \]

Where \( \phi_f \) is the fluorescence quantum yield.

Substituting in (3.2)

\[ k_R = \frac{\phi_f}{\tau_F} \quad (3.4) \]

\( k_{NR} \), the non radiative decay constant, can be related to the \( k_R \) and \( \tau_F \) by the equation;

\[ k_{NR} = k_{IC} + k_{ISC} + k_Q = \frac{1 - \phi_f}{\tau_F} = \tau_F^{-1} - k_R \quad (3.5) \]

Where \( k_{IC}, k_{ISC} \) and \( k_Q \) the decay constants due to internal conversion, intersystem crossing and quenching due to other mechanisms respectively.

The lifetime of the lowest singlet excited state, \( \tau_F \) is of importance in many applications. For strongly forbidden transitions, the lifetime is longer (10\(^{-3}\) s or more). Observed lifetime as noted before are generally less than the calculated values because of the other competing processes. Evidently, for molecules which do not fluoresce, the deactivation take place by other processes in a period less than 10\(^{-6}\) to 10\(^{-9}\) s.

### 3.1.2. Time-Correlated Single-Photon Counting (TCSPC)

At present almost all time-domain measurements are performed using TCSPC. Several comprehensive monographs dealing with TCSPC have appeared\(^{3-6}\). The insightful monograph of Ware\(^6\) clearly describes the concept of TCSPC, and anticipated many of its present applications. These instruments use high-repetition-rate picosecond or femtosecond laser light sources and high-speed MCP PMTs.
3.1.3. Principles of TCSPC

TCSPC is a digital technique for counting photons, which are time-correlated in relation to the excitation pulse (Figure 3.1). The heart of the method is a time-to-amplitude converter (TAC), which can be considered to be analogous to a fast stopwatch. The sample is repetitively excited using a pulse light source, often from a laser or flash lamp. Each pulse is optically monitored, by a high-speed photodiode or photo multiplier, to produce a start signal, which is used to trigger the voltage ramp of the TAC. The 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 a time channel using an analog-to-digital converter (ADC). Summing over many pulses, the MCA builds up a probability histogram of counts versus time channels. The experiment is continued until one has collected more than 10,000 counts in the peak channel. There can be no more than one photon detected per 100 laser pulses. Under these conditions, the histogram of photon arrival times represents the intensity decay of the sample.

Another important feature of TCSPC is the use of the rising edge of the photoelectron pulse for timing. This allows phototubes with nanosecond pulse widths to provide sub nanosecond resolution. This is possible because the rising edges of the single photon pulses are usually steeper than one would expect from the time response of the PMT. Also, the use of a constant fraction discriminator provides improved time resolution by removing the variability due to the amplitude of each pulse.
Figure 3.1. Schematic block diagram for TCSPC.
3. 2. Review of Literature

Coumarin derivatives are the subject of photophysical studies during the last few decades as they are highly fluorescing moieties. The nature and position of the substituents on coumarin ring has profound importance in deciding the photophysical behaviour of the substituted coumarin compounds.

Coumarins substituted at 7-position with an electron donating group are known to exhibit strong fluorescence. Since 7-aminocoumarins are highly fluorescent, they have been used as optical brighteners and fluorescent probes. Substituted 7-aminocoumarins also form an important class of laser dyes for the blue-green region. The photophysical properties of these compounds depend on the nature and position of a substituent group in the parent molecule and also change due to a change in the surrounding media. Coumarins are used as non-linear optical chromophores and as excellent probe to study solvation dynamics in the homogeneous solutions as well as organized media. In the recent past, numerous coumarin heterodimers have been synthesized and explored the possibility of their applications as laser dyes as organic scintillators and as triplet sensitizers. In a series of earlier works the effect of solvents, substituents and temperature on the various photophysical properties of coumarin compounds have been reported. It is found that the nature of solvents and substituents brings about a change in the values of fluorescence wavelength maxima, quantum yield, lifetime, polarization and excited state dipole moment of the coumarins. A systematic study of fluorescence quenching of 4- and 7-substituted coumarins by halide ions in aqueous media have also been studied in detail. A series of 2H-pyrano[3,2-c]chromen-5-one derivatives were synthesized, characterized and their photochromic and redox properties were investigated by the UV–Vis absorption spectroscopy recently.
The influence of the alkoxy substituent at position 7 and alkyl group at 4 of
the coumarins have been investigated by Diehl et al.\textsuperscript{24} and the spectroscopic
properties of 7-dialkylamino and 3-styryl substituted coumarins have been studied
by Raju et al.\textsuperscript{25} The solvent effect on the absorption and fluorescent spectra of
some 6-alkylamino-7-alkyl coumarin derivatives have been reported recently.\textsuperscript{26} The coumarins with bulky groups such as phenyl, phenylthio, benzylthio etc.
substituted at position 3 were spectroscopically analyzed in solvents of different
viscosity and polymer matrices.\textsuperscript{12a} The absorption-emission properties, fluorescent
decay, quantum yield and other photophysical parameters and their dependance
on concentration\textsuperscript{27}, polarity and viscosity of the solvents and effect of various
substituents on some biscoumarins\textsuperscript{28}, cyclopenta coumarins\textsuperscript{18} amino coumarins
\textsuperscript{29}, etc. have been reported in the preceding years.

3.3. Results and discussions

In the present work an array of coumarin derivatives have been subjected to
photophysical analysis. All these molecules possess the fluorescent property.
Hence our interest was to exploit this property of coumarins to find application in
industries and biological field. Nowadays coumarins are used in dye-laser
techniques, cell imaging, transcription assays, intrinsic probe for labeling peptides
etc. To know the applicability based on fluorescence of any molecule, one should
know the photophysical parameters and the factors governing it.

The aim of the present study is to determine the basic spectroscopic and
photophysical data for the compounds given in Figure 3.2. The photophysical
constants of the molecule $\phi_F$, $\tau_F$, $k_R$ and $k_{NR}$, and also the absorption spectrum (as
expressed by the molar absorption coefficient, $\varepsilon_A$) and the fluorescence spectral
distribution normalized to the quantum yield, form a basic set of data
characterizing a luminescent molecule. The determination of these parameters of
the newly synthesized coumarin derivatives and its variation in the presence and absence of some functional group is the aim of the present investigation. A comparison of these data of the new compounds with that of the known compounds (whose photo-physical studies are not extensively carried out) reveals some interesting results.

Figure 3.2. Substituted coumarin derivatives under study.

C 11  C 12  C 13

C 15  C 18  C 21  C 23

C 31  C 41  C 42  C 45
In the present study, the optical properties of newly synthesized coumarin derivatives have been investigated in detail by measuring the UV/Vis absorption, steady state and time resolved fluorescence. From these studies we have calculated the photophysical parameters like extinction coefficient ($\varepsilon_A$), quantum yield ($\phi_F$), lifetime values ($\tau_F$) radiative ($k_R$) and non-radiative decay constants ($k_{NR}$). An analysis on the correlation between the optical properties and structural characteristics of newly synthesized coumarin derivatives explores some interesting aspects.

3.3.1 Absorption and Emission Properties of Coumarin Derivatives

All the measurements have been made in the polar solvent, dimethyl sulfoxide, as it is the only solvent in which all the compounds are soluble. Absorption spectra are less sensitive to solvent polarity than emission spectra. Absorption of light occurs in about $10^{-15}$ s, a time that is too short for motion of the fluorophore or solvent. Absorption spectra are not affected by the decrease in the excited-state energy, which occurs after absorption has occurred. The absorption maxima ($\lambda_A$) and the molar extinction coefficient ($\varepsilon_A$) and absorption spectra are shown in Table 3.1 and Figure 3.3 respectively.

The effects of solvent and environment on fluorescence spectra are complex. Spectral shifts result from the general effect of solvent polarity whereby the energy of the excited state decreases with increasing solvent polarity. However, spectral shifts also occur due to specific fluorophore-solvent interactions and due to charge separation in the excited state. Emission from fluorophores generally occurs at wavelengths, which are longer than those at which absorption occurs. This loss of energy is due to a variety of dynamic processes, which occur following light absorption.
Table 3.1. Photophysical parameters of coumarin derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>$\lambda_A$ (nm)</th>
<th>$\varepsilon$ (M$^{-1}$cm$^{-1}$)</th>
<th>$\lambda_F$ (nm)</th>
<th>Stokes' Shift, $\mu_s$ (cm$^{-1}$)</th>
<th>$\phi_F$</th>
<th>$\tau_{av}$ (ns)</th>
<th>$K_R$ ($10^7$ s$^{-1}$)</th>
<th>$K_{NR}$ ($10^7$ s$^{-1}$)</th>
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<td>C11</td>
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<td>12431</td>
<td>421</td>
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<td></td>
</tr>
<tr>
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<tr>
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<td>0.8</td>
<td>3.00</td>
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<tr>
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<td>6800</td>
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<td></td>
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</tr>
<tr>
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<td>8600</td>
<td>466</td>
<td>11764</td>
<td>0.04</td>
<td>3.25</td>
<td>1.231</td>
<td>29.53</td>
</tr>
<tr>
<td></td>
<td>339</td>
<td>8400</td>
<td></td>
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</tr>
</tbody>
</table>
Figure 3.3a. Absorption spectra of coumarin derivatives.
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 $10^{-2}$ s due to internal conversion. Solvent effects shift the emission to still lower energy owing to stabilization of the excited state.
by the polar solvent molecules (Figure 3.4). 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.

**Figure 3.4. Jablonski diagram for fluorescence with solvent relaxation.**
The coumarins are itself polar in nature and hence display large sensitivity to the solvent polarity. Solvent polarity and the local environment have profound effects on the emission spectra of polar fluorophores. Literatures show that the substituted coumarin molecules exhibit solvatochromic effect in different solvent with varying polarity due to the intramolecular charge transfer (ICT). In the current investigation the solvatochromic effect of all the coumarin derivatives in different solvents could not be studied as the highly polar dimethyl sulfoxide (DMSO) is the only solvent in which all the compounds are soluble. The emission maxima ($\lambda_{em}$) and emission spectra are shown in Table 3.1 and Figure 3.5 respectively.

**Figure 3.5a. Emission spectra of coumarin derivatives.**
Figure 3.5b. Emission spectra of coumarin derivatives.
An interesting consequence of emission to higher vibrational ground states is that the emission spectrum is usually a mirror image of the absorption spectrum of the S\textsubscript{0} to S\textsubscript{1} transition. This similarity occurs because electronic excitation does not greatly alter the nuclear geometry. Hence the spacing of the vibrational energy levels of the excited state is similar to that of the ground state. As a result, the vibrational structures seen in the absorption and emission spectra are similar. Observations of the combined absorption-emission spectra of the coumarin derivatives (Figure 3.6) reveal that except few most of the molecules hold the mirror image rule. Although the mirror image rule is obeyed, an exact symmetry in spectra is not seen. It may be due to a faintly distorted geometry in the excited state. The generally symmetric nature of these spectra is a result of the same transition is being involved in both the absorption and emission and the similarities of the vibrational energy levels of S\textsubscript{0} and S\textsubscript{1}.

**Figure 3.6a. Combined absorption-emission spectra of coumarin derivatives.**
Figure 3.6b. Combined absorption-emission spectra of coumarin derivatives.
The deviation from the mirror image rule in the case of few molecules (C12, C45 etc.) indicates a different geometric arrangement of the nuclei in the excited state as compared to the ground state.

3.3.2 Stokes’ Shift

Energy losses between excitation and emission are observed generally for fluorescent molecules in solution. One cause of Stokes’ shift is the rapid decay to the lowest vibrational level of $S_1$. Furthermore, fluorophores generally decay to higher vibrational levels of $S_0$, resulting in further loss of excitation energy by thermalisation of the excess vibrational energy. In addition to these effects, fluorophores can display further Stokes’ shifts due to solvent effects, excited-state reactions, complex formation, and/or energy transfer. Some of the dynamic processes in solution involve fluorophore-solvent interactions and rotational diffusion. As was observed by Stokes, most fluorophores display emission at lower energies than their absorption. Rotational motions of small solvent molecules in fluid solution are rapid, typically occurring on a timescale of 40 ps or less. The relatively long timescale of fluorescence allows ample time for the solvent molecules to reorient around the excited-state dipole, which lowers its energy and shifts the emission to longer wavelengths. This process called solvent relaxation occurs in $10^{-10}$s in fluid solution. It is these differences between absorption and emission that result in the high sensitivity of emission spectra to solvent polarity, and the smaller spectral changes seen in absorption spectra. Solvent relaxation can result in substantial Stokes' shifts. Thus one of the reasons for high Stokes’ shift values observed for the coumarin derivatives are ascribed to the polarity of the solvent, DMSO. The Stokes’ shift values calculated for all the coumarin derivatives are represented in Table 3.2. The Stokes’ shift values are expressed in wavenumber and are calculated from the experimental parameters by
taking the difference between absorption and emission maxima expressed in wave
number using the equation,

\[
\text{Stokes’ Shift } \eta_S = (\bar{\nu}_A - \bar{\nu}_F) \times 10^7 \text{ cm}^{-1},
\]

Where, \( \bar{\nu}_A = 1/\lambda_A \text{ (nm)} \) and \( \bar{\nu}_F = 1/\lambda_F \text{ (nm)} \)

**Table 3.2. Stokes’ shift values of coumarin derivatives.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Stoke’s Shift; Wave number, cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>C11</td>
<td>6922</td>
</tr>
<tr>
<td>C12</td>
<td>4944</td>
</tr>
<tr>
<td>C13</td>
<td>5984</td>
</tr>
<tr>
<td>C15</td>
<td>6862</td>
</tr>
<tr>
<td>C18</td>
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</tr>
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<td>C41</td>
<td>7124</td>
</tr>
<tr>
<td>C42</td>
<td>9048</td>
</tr>
<tr>
<td>C45</td>
<td>11764</td>
</tr>
</tbody>
</table>

**3.3.3. Quantum Yield**

Quantum yield value of a molecule is a direct measure of its luminescent
property. If the absolute fluorescence efficiency of one substance (reference \( \phi_r \)) is
known, then that of the other (sample \( \phi_s \)) can simply be calculated. Quinine sulfate
in dilute \( \text{H}_2\text{SO}_4 \) (0.1 M) has been used as a standard substance for quantum yield
measurements. The quantum yield value of the solution of quinine sulfate in 0.1 M
\( \text{H}_2\text{SO}_4 \) is determined as 0.546 and hence the \( \phi \) values of the coumarin derivatives
are calculated knowing the other parameters. The values of \( \phi_s \) are determined by
the measurements of the area under the fluorescence curve (which have been suitably corrected for instrumental factors) using the equation;

\[ \phi_s = \phi_r \frac{A_r F_s}{A_s F_r} \left[ \frac{\eta_s^2}{\eta_r^2} \right] \]

Where \( \phi_r \) is the quantum yield of the reference (\( \phi_r = 0.546 \)). \( A_r \) and \( A_s \) are the absorbance of the ‘reference standard’ and ‘sample’ respectively at the excitation wavelength, \( F_r \) and \( F_s \) are the relative integrated fluorescent intensities (peak area) of the reference and samples respectively and \( \eta_r \) and \( \eta_s \) are respectively the refractive indices of the solvents in which the reference standard and samples are prepared. The measured values of the parameters and the calculated quantum yield value for all the coumarin derivatives have been tabulated in Table 3.3.

**Table 3.3. Quantum yield values of coumarin derivatives.**

\( \phi_r = 0.546; \quad \eta_s = 1.4785; \quad \eta_r = 1.33 \)

<table>
<thead>
<tr>
<th>Compound</th>
<th>( A_r )</th>
<th>( A_s )</th>
<th>( F_r )</th>
<th>( F_s )</th>
<th>( \phi_s )</th>
</tr>
</thead>
<tbody>
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<td>C11</td>
<td>0.108</td>
<td>0.104</td>
<td>( 4.63461 \times 10^8 )</td>
<td>( 3.67907 \times 10^7 )</td>
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</tr>
<tr>
<td>C12</td>
<td>0.109</td>
<td>0.116</td>
<td>( 5.09399 \times 10^8 )</td>
<td>( 7.58255 \times 10^6 )</td>
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</tr>
<tr>
<td>C13</td>
<td>0.108</td>
<td>0.123</td>
<td>( 3.01383 \times 10^8 )</td>
<td>( 7.78622 \times 10^7 )</td>
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</tr>
<tr>
<td>C15</td>
<td>0.111</td>
<td>0.116</td>
<td>( 3.97736 \times 10^8 )</td>
<td>( 1.92447 \times 10^6 )</td>
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<tr>
<td>C18</td>
<td>0.108</td>
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<td>( 4.23774 \times 10^8 )</td>
<td>( 1.62761 \times 10^6 )</td>
<td>0.002</td>
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<tr>
<td>C21</td>
<td>0.110</td>
<td>0.112</td>
<td>( 2.26758 \times 10^8 )</td>
<td>( 1.08984 \times 10^6 )</td>
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</tr>
<tr>
<td>C23</td>
<td>0.110</td>
<td>0.126</td>
<td>( 2.26758 \times 10^9 )</td>
<td>( 2.84662 \times 10^6 )</td>
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</tr>
<tr>
<td>C31</td>
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<td>( 2.26758 \times 10^8 )</td>
<td>( 2.78665 \times 10^6 )</td>
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<td>0.099</td>
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<td>( 2.96364 \times 10^8 )</td>
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<tr>
<td>C42</td>
<td>0.099</td>
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<td>( 2.42806 \times 10^6 )</td>
<td>0.04</td>
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</table>
A general trend of decrease in quantum yield value is observed for all these compounds in DMSO. This is supposed to be due to the solvent relaxation resulting from the excited state internal charge transfer (ICT) of fluorophores. The high Stokes’ shift values of these fluorophores also point towards an ICT emission in highly polar solvents. In addition to specific solvent-fluorophore interactions and an internal charge-transfer (ICT) state formation, the fluorophores can form a twisted internal charge-transfer (TICT) state.⁴

A keen examination of the quantum yield values also reveals some interesting results regarding the influence of substituents on the benzopyrone ring as well as the aromatic ring. The presence of electron withdrawing groups (CN, -COCH₃, p-NO₂-C₆H₄) at position 3 of the benzopyrone ring, found to decrease the fluorescent quantum efficiency and electron-donating functionalities (phenyl, p-Cl-phenyl) cause an increase in φ value of the molecules. Another observation that could be made from the study is that an increase in the fluorescent property of the molecule with the presence of an electron donating acetoxy group at position 7 of the coumarin ring. These results are in conjunction with the reported literatures.¹³,²⁴⁻²⁵

3.3.4. Abnormal Photophysical Observation in Presence of Methyl Group

On a perusal of the photophysical values (Table 3.1) of the coumarin derivatives under investigation, the noteworthy observations that can be made is that, when an alkyl group (methyl) is present at position 4 of the benzopyrone ring, the fluorescent property of the compound has diminished to a great extent, which is not in agreement with the early reports. This can be discussed in detail in view of the photophysical properties of the representative compounds C41 and C31, as given below (Figure 3.7).
Consider the compounds C41 and C31. The only difference between these two is the presence of one –CH$_3$ group at position 4. But the quantum yield value of C31 is found to be decreased 40 times to that of the compound C41. The methyl group, which is an electron donating group on the benzopyrane ring of the compound C31 can lead to an enhanced donor-acceptor capability on it compared to C41 and thus an improved charge transfer (by the formation of a better donor-accepter system). In this better donor-acceptor system C31, the excited state dipole moment of the molecule would be high compared to C41. This may also result to a higher stabilization of the excited state of C31 by solvent relaxation through charge transfer. From the Table 3.2 it is clear that compound C 31 is more red-shifted and has got high Stokes shift value. It is another evidence for the possible ICT due to the donor-acceptor pair formation in the presence of a methyl group. The $k_{NR}$ value for most of the compounds in Table 3.1 are found to have a considerable value, which may be ascribed due to the vibrational relaxation during the charge transfer.

**Figure 3.7**

\[
\begin{align*}
\text{C41} & : \phi_F = 0.25 \\
\text{C31} & : \phi_F = 0.006 \\
\text{Stokes Shift (cm}^{-1}) & : \mu_S = 7124 \\
& : \mu_S = 8344
\end{align*}
\]
Similarly a marginal decrease in the fluorescent property with the presence of a methyl group could be observed in the case of compounds C13 and C23 also.

**Figure 3.8**

![Chemical Structures](image)

C13

$\phi_F = 0.153$

Stokes’ Shift, $\mu_S = 5984$

C23

$\phi_F = 0.007$

$\mu_S = 6932$

Compounds C11 and C21 are considered as less emissive species in DMSO on the basis of the $\phi_F$ values obtained for them. Even in these low values a well-defined decrease in fluorescence in the presence of methyl group is apparent. The $\phi_F$ value of the C21, which is having a methyl group at position 4, is nearly 20 times less than that of the compound C11 that lacks it.

**Figure 3.9**

![Chemical Structures](image)

C11

$\phi_F = 0.06$

Stokes’ Shift, $\mu_S = 6922$

C21

$\phi_F = 0.003$

$\mu_S = 7115$

3.3.5. Fluorescent Lifetime

The fluorescent lifetime (decay time) of the excited state is defined by the average time the molecule spends in the excited state prior to return to the ground state. The fluorescent decay of all the substituted coumarin derivatives have been measured and plotted in Figure 3.10. From this figure, it is obvious that all the compounds follow a characteristic biexponential fluorescent decay, which reveal
the existence of two different emissive states for the molecule, which could be the locally excited state (LE, Franck-Condon state) and charge transfer state (CT). The $\chi^2$ value, which is known as the fitting parameter, determine fine fit for the biexponential decay and is in between 1.0 to 1.3. The average lifetime values are calculated using the equation:

$$\tau_{av} = \frac{\alpha_1 \tau_1^2 + \alpha_2 \tau_2^2}{\alpha_1 \tau_1 + \alpha_2 \tau_2}$$

Where $\tau_1$ and $\tau_2$ are the lifetime values of the two emissive states and $\alpha_1$ and $\alpha_2$ are called the pre-exponential factors, which give the abundance of each emissive states. The average lifetime values calculated for each compound are shown in Table 3.4.

**Figure 3.10a. Fluorescent lifetime decay plots of coumarin derivatives.**
Figure 3.10b. Fluorescent lifetime decay plots of coumarin derivatives.

\[ \chi^2 = 1.3 \]

\[ \lambda_m = 435 \text{ nm} \]
\[ \lambda_{ex} = 358 \text{ nm} \]

\[ \chi^2 = 1.25 \]

\[ \lambda_m = 435 \text{ nm} \]
\[ \lambda_{ex} = 335 \text{ nm} \]

\[ \chi^2 = 1.16 \]

\[ \lambda_m = 435 \text{ nm} \]
\[ \lambda_{ex} = 343 \text{ nm} \]

\[ \chi^2 = 1.24 \]

\[ \lambda_m = 406 \text{ nm} \]
\[ \lambda_{ex} = 315 \text{ nm} \]

\[ \chi^2 = 1.14 \]

\[ \lambda_m = 403 \text{ nm} \]
\[ \lambda_{ex} = 315 \text{ nm} \]
3.3.6. Radiative and Nonradiative Decay Constants

The fluorescence emission is a random process and emission occurs by a unimolecular process. The radiative lifetime of the excited state may be defined in terms of a first order decay process. The radiative decay constant ($k_R$) and non-radiative decay constant ($k_{NR}$) can be calculated by knowing the quantum yield ($\phi_F$) and lifetime values ($\tau$), which are related by the equation,

$$k_R = \frac{\phi_F}{\tau}$$

and

$$k_{NR} = \tau^{-1} - k_R$$
The calculated values of the $k_R$ and $k_{NR}$ are shown in Table 3.1. The high $k_{NR}$ values for few molecules (C13, C14, C42) may be due to the torsional vibrations of the substituents on the coumarin ring and vibrational relaxation during the charge transfer (CT).

Table 3.4. Average lifetime values for coumarin derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{ex}$(nm)</th>
<th>$\lambda_{em}$(nm)</th>
<th>$\tau_1$(ns)</th>
<th>$\alpha_1$ (%)</th>
<th>$\tau_2$(ns)</th>
<th>$\alpha_2$ (%)</th>
<th>$\tau_{av}$(ns)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C11</td>
<td>326</td>
<td>404</td>
<td>0.216</td>
<td>97</td>
<td>2.79</td>
<td>3</td>
<td>0.95</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>421</td>
<td></td>
<td>0.21</td>
<td>95</td>
<td>2.93</td>
<td>5</td>
<td>1.36</td>
<td>1.16</td>
</tr>
<tr>
<td>C12</td>
<td>358</td>
<td>435</td>
<td>0.75</td>
<td>48</td>
<td>4.23</td>
<td>52</td>
<td>3.74</td>
<td>1.3</td>
</tr>
<tr>
<td>C13</td>
<td>326</td>
<td>405</td>
<td>0.30</td>
<td>99</td>
<td>2.72</td>
<td>1</td>
<td>0.503</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>423</td>
<td></td>
<td>0.30</td>
<td>99</td>
<td>2.05</td>
<td>1</td>
<td>0.413</td>
<td>1.25</td>
</tr>
<tr>
<td>C15</td>
<td>335</td>
<td>435</td>
<td>0.99</td>
<td>57</td>
<td>4.45</td>
<td>43</td>
<td>3.66</td>
<td>1.25</td>
</tr>
<tr>
<td>C18</td>
<td>343</td>
<td>435</td>
<td>0.91</td>
<td>83</td>
<td>4.15</td>
<td>17</td>
<td>2.475</td>
<td>1.16</td>
</tr>
<tr>
<td>C21</td>
<td>315</td>
<td>406</td>
<td>0.83</td>
<td>66</td>
<td>4.0</td>
<td>34</td>
<td>3.09</td>
<td>1.24</td>
</tr>
<tr>
<td>C23</td>
<td>315</td>
<td>403</td>
<td>0.025</td>
<td>97</td>
<td>1.49</td>
<td>3</td>
<td>0.975</td>
<td>1.14</td>
</tr>
<tr>
<td>C31</td>
<td>315</td>
<td>431</td>
<td>0.77</td>
<td>76</td>
<td>3.73</td>
<td>24</td>
<td>2.56</td>
<td>1.2</td>
</tr>
<tr>
<td>C41</td>
<td>327</td>
<td>428</td>
<td>0.78</td>
<td>76</td>
<td>3.29</td>
<td>24</td>
<td>2.22</td>
<td>1.1</td>
</tr>
<tr>
<td>C45</td>
<td>335</td>
<td>466</td>
<td>1.01</td>
<td>36</td>
<td>3.6</td>
<td>64</td>
<td>3.25</td>
<td>1.25</td>
</tr>
</tbody>
</table>

3.4. Experimental

3.4.1. Electronic Spectral Measurements

Electronic absorption spectra were recorded on a Shimadzu UV-3101 PC UV-VIS-NIR Scanning Spectrophotometer and the emission spectra were recorded on a SPEX-Fluorolog F112x Spectrofluorimeter. The absorption measurements were carried out using 1 mm cuvette and fluorescence measurements were carried out using 1 x 1 cm cuvette. The fluorescence quantum
yields of all the coumarin derivatives in DMSO were estimated by comparison with Quinine sulfate in 0.1 M dilute sulfuric acid ($\Phi_F = 0.546$) as the standard reference.

3.4.2. Fluorescence Lifetime Measurements

Fluorescence lifetimes were measured using IBH (FluoroCube) Time-Correlated Picosecond Single Photon Counting (TCSPC) system. Solutions were excited with a pulsed diode laser ($<100$ ps pulse duration) at a wavelength of 375 nm (NanoLED-11) with a repetition rate of 1 MHz. The detection system consisted of a micro channel plate photomultiplier (5000U-09B, Hamamatsu) with a 38.6 ps response time coupled to a monochromator (5000M) and TCSPC electronics (Data station Hub including Hub-NL, NanoLED controller and preinstalled fluorescence Measurement and Analysis Studio (FMAS) Software). The fluorescence lifetime values were obtained using DAS6 decay analysis software.

3.5. Conclusions

In the present work newly synthesized coumarin derivatives have been subjected to the photophysical evaluation by studying the luminescence parameters. These data are compared with that of the values of the existing known molecules, the photophysical properties of which are not much investigated. Preliminary studies on the effect of substituents on the fluorescent properties of these coumarin derivatives have been carried out. Although the influence of the electron donating groups such as amino, substituted amino, hydroxy, alkoxy groups etc. at position 7 of the coumarin ring system have been extensively studied, the luminescent properties of the coumarin moieties with an acetoxy substituent have not been explored.
Most of the results obtained here are in close agreement with the early reports. However the interesting results regarding the substituent effect that emerged out from the present study are;

a) Presence of an electron donating substituent at position 3 of the coumarin ring increase the fluorescent properties of the molecules;

b) An electron withdrawing group at position 3 of the coumarin moiety found to decrease the fluorescence;

c) An electron donating acetoxy group at position 7 enhances the luminescent property to high extent and

d) Extraordinary declines in the fluorescence of coumarin derivatives are observed when a methyl group is present at position 4.

The first three results are in full concurrence with the literature, but last result is contradictory to the report of an enhanced quantum efficiency of the molecule with the presence of alkyl group at position 4. This discrepancy in fluorescence with the presence of a methyl group require an extensive experimental investigation with the support of a theoretical evaluation, which are beyond the scope of the present analysis. Thus it can be concluded that, depending upon the need for its application in industries, the photophysical properties can be varied suitably by incorporating electron donating or withdrawing substituents at different positions of coumarin scaffold. The compounds C13 and C41 have high Stokes’ shift value and reasonable quantum yield, which implies its possibility to use these compounds in cell imaging and dye-laser techniques.
3.6. References


