CHAPTER VI

FLUOROMETRIC INVESTIGATION

OF

INTERACTION BETWEEN NITROPHENOLS

AND

7- HYDROXY-4-AZIDOMETHYLCOUMARIN
INTRODUCTION

The phenomenon of quenching of fluorophores by small molecules like carbon tetrachloride, aniline, bromobenzene, halides and metal ions has been an area of intense investigation in physical, chemical and biological sciences [1,2]. The property of coumarin derivatives to exhibit fluorescence has been studied thoroughly leading to their application in a variety of fields. Tricyclic coumarins with emission bands in the visible region have been employed as tunable dye lasers [3,4]. Aromatic hydroxyl and alkoxy coumarins have been used as fluorescent probes in the study of biochemical mechanism [5] and detection of DNA [6]. 3-Heterocyclic coumarins with 7-dialkylamino groups have been synthesized as models to study the intermolecular energy transfer processes [7]. Photochemical reactions of 6-azido and 7-azido coumarins have been studied to explore their potential as photo affinity labels [8]. 3-azido coumarins have been employed to generate strongly fluorescent 1, 2, 3-triazolyl coumarins 1 [9] and recently this reaction has been used in non-destructive verification of PNA immobilization [10].

Azidowarfarins have been found to be useful photo affinity probes for rat liver cytochrome P450 [11]. Huisgen 1, 3-dipolar cycloadditions of 4-azidomethyl coumarins have lead to angularly oriented triazoles [12]. Recently, a series of sulphonamides containing 4-azido methyl coumarin moiety have been reported as potential anti-microbial agents [13].
Bulut et al. [14] reported the synthesis, photophysical, photochemical and electrochemical properties of crown ether bearing coumarin substituted phthalocyanines 2.

\[
\begin{align*}
R & \quad R \\
M & = \text{Zn (5), Cu (6), Co (7)}
\end{align*}
\]

Kadadevarmath et al. [15] studied the role of solvent polarity on the fluorescence quenching of newly synthesized 7,8-benzo-4-azidomethyl coumarin 3 by aniline in benzene–acetonitrile mixtures. Fluorescence quenching of these dipolarophilic 4-azidomethyl coumarins has been studied using aniline as the neutral quencher in various solvents and benzene–acetonitrile binary mixtures. It was found that quenching is due to diffusion limited processes, which have been explained in terms of sphere of action and finite sink approximation models [16-18]. It is of interest to note that the high energy absorption band (\(\lambda_{\text{max}}\)) of the quencher is significantly different from the low energy emission band (\(\lambda_{\text{max}}\)) of the azides.

\[
\begin{align*}
N_3 & \\
3
\end{align*}
\]

Hanagodimath et al. [19-20] carried out fluorescence quenching of newly synthesized biologically active coumarin derivative 4 by aniline in binary solvent
mixtures. In this case also there was considerable difference between the emission band of compound 4 and the absorption band of the quencher viz., aniline.

Khan et al. [21] studied the interaction between Coumarin C440 5 with Fullerene C60 by fluorescence and time resolved spectroscopic techniques.

Sharma et al. [22] reported the fluorescence quenching of 3-methyl 7-hydroxyl coumarin 6 using acetone as quencher.

Sarkar et al. [23] studied photo induced intermolecular electron transfer from dimethyl aniline to 7-amino coumarin dyes 7-11 on the surface of β-cyclodextrin.

Zeng et al. [25] carried out binding studies of 1-phenyl-3-(coumarin-6-yl)sulfonylurea 14 to bovine serum albumin by fluorescence spectroscopy.

Hrdlovic et al. [26] reported characteristics of the excited states of 3-substituted coumarin derivatives 15 and transfer of electronic energy to N-oxyl radicals (quencher).

Chattopadhyay et al. [27] reported super quenching of the laser dye, coumarin 153 (C153) 16 by 16-59 nm gold nanoparticles (AuNPs) from the steady state and time-resolved fluorometric investigations.
Onganer et al. [28] carried out the photophysical features of 7-amino-4-trifluoromethylcoumarin (C151) 17 in colloidal suspension that contains CdS semiconductor nanoparticles by using molecular UV–Vis absorption, steady-state and time-resolved fluorescence, and electron spin resonance (ESR) spectroscopy techniques.

Bulut et al. [29] carried out synthesis and investigation of photophysical and photochemical properties of highly soluble 7-oxy-3-(4-methoxyphenyl)coumarin bearing zinc phthalocyanines 18.

Hillebrand et al. [30] studied quenching of the fluorescence of 3-carboxy-5,6-benzocoumarin 19 by aromatic amines.
Pal et al. [31] reported the electron transfer (ET) from diphenylamine (DPA) and triphenylamine (TPA) to a series of excited coumarin dyes 20-22 having differently substituted 7-amino groups in acetonitrile solution.

Qian et al. [32] studied the intermolecular energy transfer from coumarin-120 23 to rare earth ions (Eu$^{3+}$, Tb$^{3+}$) in silica xerogels.

Zappia et al. [33] reported the new coumarin-urea based receptor for a selective off-on fluorescence response to fluoride.
Electron transfer (ET) reactions between excited coumarin dyes 25-31 with different aliphatic/aromatic amine donors have been investigated by Pal et al. [34] in acetonitrile solution using steady-state and time-resolved fluorescence quenching measurements.

![Chemical structures](image)

Chen et al. [35] reported the creation of 3,4-bis-triazolocoumarine sugar conjugates 32 via fluorogenic dual click chemistry and their quenching specificity of silver(I) in aqueous media.

![Chemical structure](image)

Ghosh et al. [36] reported the colorimetric and fluorescence sensing of fluoride ion using thiourea based coumarin receptors 33-34.
Hillebrand et al. [37] reported experimental study of the interaction of coumarin 3-carboxylic acid derivatives 35-36 with aniline in triton-X-100 micelles.

In the present work, we have employed the industrially important [38] and mutagenic [39] nitrophenols as quenchers and 7-hydroxy-4-azidomethyl coumarin as the fluorophore which has been newly synthesised since their emission bands [380-450 nm] are closer to the absorption band of the azide [350-420 nm], which facilitates the so called FRET [40,41]. The results have been obtained under the steady state experimental setup using a variety of solvents like ethanol, methanol, butanol, DMSO, THF and acetonitrile, with a view to understand the quenching mechanism and to correlate the dielectric constant (polarity) of a solvent with Stern-Volmer quenching constant $K_{sv}$, binding constant ‘K’ and number of binding sites ‘n’.
PRESENT WORK:

The synthetic work carried out during the present investigation has been described in Scheme I. Ethyl 4-bromoacetoacetate obtained from the bromination of ethylacetoacetate, was treated with resorcinol under Pechmann cyclisation conditions using neat sulphuric acid as the condensing agent. The reaction resulted in the formation of 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one 1. The reactivity of 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one has been explored by azidation. The required 4-(azidomethyl)-7-hydroxy-2H-chromen-2-one 2 was synthesized by the reaction of sodium azide with 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one 1 in aqueous acetone at room temperature. After completion of the reaction, reaction mixture was quenched in ice water, obtained solid was filtered washed with water, dried and recrystallised by using ethanol.


RESULTS AND DISCUSSION

In the IR spectrum, presence of intense band at 2119 cm$^{-1}$ along with bands at 1692 and 3407 cm$^{-1}$ indicated the formation of 4-(azidomethyl)-7-hydroxy-2H-chromen-2-one and it was further supported by $^1$H-NMR spectral data. Singlet at 4.78 ppm integrating for two protons indicated the presence of two methylene protons. Singlet at 6.23 ppm integrating for one proton attributed to C3-proton. Doublets at 6.82 and 7.55 ppm integrating for one proton each corresponding to C5 and C3 protons.
respectively. Singlets at 6.75 and 10.66 ppm integrating for one proton each attributed to C₈ and -OH protons respectively Figure 1.

$^1$H NMR (CDCl₃, 300 MHz, TMS): ppm

\[ \text{CH}_2-\text{N}_3 - 4.78 \text{ (s, 2H)} \]
\[ \text{H}_9 - 6.75 \text{ (s, 1H)} \]
\[ \text{H}_3 - 6.27 \text{ (s, 1H)} \]
\[ \text{H}_5 - 7.55 \text{ (d, } J = 8.9 \text{)} \]
\[ \text{H}_6 - 6.82 \text{ (d, } J = 8.9, \text{1 H)} \]
\[ -\text{OH} - 10.66 \text{ (s, 1H).} \]

IR (KBr) cm$^{-1}$: 1692 (lactone C=O), 2119 (N₃), 3407 (-OH)

Figure 1. Spectral data of 4-(azidomethyl)-7-hydroxy-2H-chromen-2-one 2.
SPECTRAL DATA:

Spectrum No. 1. IR (KBr) of compound 2.
Spectrum No. 2. 1H-NMR (DMSO, d6) of compound 2.

- 7.55 (d, 1H, J = 8.4 Hz) 7.35 (d, 1H, J = 8.4 Hz)
- 6.82 (d, 1H, J = 8.4 Hz)
- 6.75 (s, 1H, C=H)
- 6.27 (s, 1H, C=H)
- 4.78 (s, 2H, CH₂-N₂)
- 10.66 (s, 1H, -OH)
- 7.55 (d, 1H, J = 8.4 Hz)

Structure:

![Structure Image]
Fluorescence quenching

Energy transfer between 7-hydroxy-4-azidomethylcoumarin and nitrophenols

7-hydroxy-4-azidomethylcoumarin shows emission bands in the region 380-450 nm and nitrophenols show absorption bands in the region 350-420 nm. Fluorescence resonance energy transfer occurs whenever the emission spectrum of fluorophore overlaps with the absorption spectrum of quencher. Thus during the present study it was attempted to employ 7-hydroxy-4-azidomethylcoumarin as fluorophore and nitrophenols as quencher.

Fluorescence resonance energy transfer depends on the extent of overlap between absorption spectrum of quencher and emission spectrum of fluorophore. In case of o-nitrophenol the space for electron delocalization is less than that of p-nitrophenol which in turn is less than picric acid. In quencher molecule Figure 2, as the space for electron delocalization (resonance) increases absorption tail shifts towards visible region, consequently extent of overlap of absorption band of quencher with the emission band of fluorophore increases. Thus the extent of overlap between absorption spectra of nitrophenols with emission spectrum of 7-hydroxy-4-azidomethylcoumarin follows the order o-nitrophenol < p-nitrophenol < picric acid.

Fluorescence intensity of a compound can be decreased by a variety of molecular interactions, viz., excited state reactions, molecular rearrangements, fluorescence resonance energy transfer (FRET), ground state complex formation and collisional quenching. In present study decrease in the fluorescent intensity of fluorophore is mainly due to FRET.
Figure 2. Overlap of emission spectrum E of 7-hydroxy-4-azidomethylcoumarin with absorption spectra A of i) o-nitrophenol

ii) p-nitrophenol

iii) picric acid
UV-Visible absorption Spectroscopy

UV-visible absorption measurement is an accurate method applicable for exploring the structural change [42] and to detect the complex formation [43]. In the present study, we have observed a red shift in UV absorption spectra of 7-hydroxy-4-azidomethylcoumarin, nitrophenols and 7-hydroxy-4-azidomethylcoumarin-nitrophenol system as shown in Figure 3. It is evident that the UV absorption intensity of azide increased regularly with the variation of nitrophenol concentration (0–25.17mM). The change in the λ_{max} indicates the change in polarity around the azide moiety. This indicates that the binding between azide and nitrophenols leads to a change in azide conformation. These observations clearly suggest that there is an initial complex formation between the azide and nitrophenols.

Figure 3. Absorption spectra of azide – nitrophenol system. Azide concentration was kept fixed at 4.6μM and nitro phenol concentration for 1) 0.0mM 2) 3.597mM 3) 10.791mM 4) 17.985mM 5) 25.179mM
Nuclear Magnetic Resonance Spectroscopy

This technique has been employed in order to ascertain the complex formation between 7-hydroxy-4-azidomethylcoumarin and nitrophenol Figure 4. Initially the $^1$H NMR spectrum of p-nitrophenol (0.23M) was recorded in which the -OH proton was observed at 10.92 ppm. Addition of 1.16M of 7-hydroxy-4-azidomethylcoumarin did not show any change in the integration of the peak at 10.92 ppm. However addition of 2.3M of azide reduces the integration of the peak at 10.88 ppm, further on addition of 4.7M of azide resulted in complete disappearance of the peak at 10.88 ppm and appearance of a peak at 3.71 ppm which further shifted up to 4.08 ppm upon addition of 9.4M of azide.

Figure 4. $^1$H-NMR spectra of p-nitro phenol-azide system. p-Nitro phenol concentration was kept fixed at 0.23M and azide concentration for i) 0.0M ii) 2.3M iii) 4.7M.
Figure 5. Interaction between 7-hydroxy-4-azidomethylcoumarin and nitrophenols.

Conversely an initial spectrum of the azide was recorded which was at 0.24 M concentration exhibited a low intensity peak at 10.46 ppm. Upon addition of 11.5 M of p-nitrophenol the integration of the peak was reduced to 50% of the original value with significant line broadening.

These two experiments support the complex formation between the phenolic – OH of the nitrophenol and the anionic nitrogen of the azide Figure 5. The other mode of association i.e., through the 7-hydroxy group of coumarin and the nitro group of the phenol also occurs but its expression is less significant.

Quenching Mechanism

The Fluorescence quenching data was analyzed by the Stern-Volmer equation $I_0/I = 1 + K_{sv}[Q]$ where $I_0$ and $I$ are fluorescence intensities of 7-hydroxy 4-azido methyl coumarin in the absence and presence of quenchers viz. o-nitrophenol, p-nitrophenol and picric acid respectively. $K_{sv}$ is the Stern-Volmer quenching constant and $[Q]$ is the concentration of the quencher.

S-V plots obtained by using fluorescence emission intensity measurements for 7-hydroxy-4-azidomethylcoumarin and o-nitrophenol system are found to be linear in all the solvents which are shown in the Figure 6. The linearity of the $I_0/I$ versus $[Q]$ revealed the quenching type, as static or dynamic, since the characteristic Stern-Volmer plot of combined quenching(both static and dynamic) exhibits positive deviation.
Figure 6. Stern-Volmer (S-V) plots from fluorescence emission intensity measurements for 7-hydroxy-4-azidomethylcoumarin and o-nitrophenol system in different solvents: 1) butanol 2) methanol 3) acetonitrile 4) DMSO 5) THF 6) ethanol

To investigate the quenching mechanism, fluorescence was recorded at three different temperatures i.e. 293 K, 303 K, 308 K. The fluorescence quenching data at different temperatures were analyzed by the Stern-Volmer equation. The results are shown in Figure 7. The $K_{sv}$ and Pearson's correlation coefficient values, $R^2$, (the linear correlation between $I_0/I$ and $[Q]$) obtained at different temperatures are shown in Table 1. From these results, we can see that the $K_{sv}$ values decrease with increasing temperature, which is consistent with the static type of quenching. In view of this the interaction between 7-hydroxy-4-azidomethyl-coumarin and o-nitrophenol can be suggested as static quenching but not dynamic quenching. That is, 7-hydroxy-4-azidomethylcoumarin bound to o-nitrophenol and azide-o-nitrophenol complex was formed, which resulted in quenching of the fluorophore.
Figure 7. Stern-Volmer (S-V) plots from fluorescence emission intensity measurements for 7-hydroxy-4-azidomethylcoumarin and o-nitrophenol system at different temperatures 1) 293 K 2) 303 K 3) 308 K.

Table 1. Stern-Volmer constant at different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>20 °C (293 K)</th>
<th>30 °C (303 K)</th>
<th>35 °C (308 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{sv}$ Lmol$^{-1}$</td>
<td>37.121</td>
<td>33.974</td>
<td>31.825</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9066</td>
<td>0.9054</td>
<td>0.9269</td>
</tr>
</tbody>
</table>

But in case of $p$-nitrophenol and picric acid the S-V plots are found to be nonlinear in all the solvents, showing positive deviation, are shown in the Figure 8. Similar experimental results have been reported in the literature [45-49]. Thus positive deviation from linearity suggests that quenching is not purely collisional and reveals the role of the static quenching process (concave towards the Y-axis) indicating the presence of both static and dynamic quenching mechanism.
Analysis of binding equilibria

When small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules [42, 50] is given by

$$\text{log}(I_0-I)/I = \log K + n\log[Q]$$

where \('K' and \('n'\ are the binding constant and the number of binding sites respectively. Thus a plot of log \((I_0-I)/I\) versus log \([Q]\) is found to linear as shown in the Figure 9 and can be used to determine \('K' as well as \('n'. The value of \('K' indicated that, from the Table 2, there is a strong interaction and the formation of a complex between 7-hydroxy-4-azidomethylcoumarin and quenchers o-nitrophenol, p-
nitrophenol and picric acid. As the space for electron delocalization in quencher molecule increases the extent of overlap between absorption spectrum of quencher and emission spectrum of donor increases which has direct impact on binding constant. Hence the binding constant for picric acid is higher than that of p-nitrophenol which in turn is greater than o-nitrophenol. From the Figure 10 it is clear that the number of binding sites, \( n \), varies with change in dielectric constant, which clearly indicates that since there is possibility of formation of H-bonding between fluorophore and quencher, as the polarity of the solvent changes the extent of interaction between the fluorophore and quencher varies because of H-bonding with solvents.

Figure 9. Plots of Log \((I_0-I)/I\) versus Log [Q] for 7-hydroxy-4-azidomethylcoumarin with i) o-nitrophenol ii) p-nitrophenol iii) picric acid in different solvents 1) acetonitrile 2) DMSO 3) THF 4) methanol 5) butanol 6) ethanol
Table 2. Values of binding constant and number of binding sites in different solvents

<table>
<thead>
<tr>
<th>Solvents</th>
<th>( o\text{-nitrophenol} )</th>
<th>( p\text{-nitrophenol} )</th>
<th>Picric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BS</td>
<td>BC</td>
<td>BS</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1.5554</td>
<td>1.9266( \times 10^2 )</td>
<td>1.7126</td>
</tr>
<tr>
<td>Butanol</td>
<td>1.0386</td>
<td>0.4237( \times 10^2 )</td>
<td>1.3513</td>
</tr>
<tr>
<td>DMSO</td>
<td>2.0247</td>
<td>6.5614( \times 10^2 )</td>
<td>1.5834</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.9752</td>
<td>0.1264( \times 10^2 )</td>
<td>1.3319</td>
</tr>
<tr>
<td>THF</td>
<td>0.9157</td>
<td>0.1171( \times 10^2 )</td>
<td>1.7171</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.0284</td>
<td>13.1673( \times 10^2 )</td>
<td>1.2916</td>
</tr>
</tbody>
</table>

BS - Binding sites, BC - Binding constant

Figure 10. Variation of binding sites of i) \( o\text{-nitrophenol} \)

ii) \( o\text{-nitrophenol} \)

iii) Picric acid

as a function of dielectric constant \( D \). BS - Binding sites.
Determination of acting force between 7-hydroxy-4-azidomethylcoumarin and nitrophenols

Considering the dependence of binding constant on temperature, a thermodynamic process was considered to be responsible for the formation of a complex. The binding forces between a fluorophore and quencher include hydrogen bond, van der Waals force, electrostatic force, hydrophobic force, and so on. The thermodynamic parameters were determined using van't Hoff equation:

$$\log K = \frac{-\Delta H^0}{2.303RT} + \frac{\Delta S^0}{2.303R}$$

The logK versus 1/T plot enabled the determination of $\Delta H^0$ and $\Delta S^0$ for the binding process. The value of $\Delta G^0$ was calculated from the relation:

$$\Delta G^0 = \Delta H^0 - T \Delta S^0$$

Where $\Delta H^0$, $\Delta G^0$ and $\Delta S^0$ are, respectively, standard enthalpy change, standard free energy change and standard entropy change. The binding studies were carried out at 293 K, 298 K, and 308 K and the values are given in Table. 3. At these temperatures the 7-hydroxy-4-azidomethylcoumarin does not undergo structural degradation. Ross and Subramanian [51] have characterized the sign and magnitude of the thermodynamic parameter associated with various individual kinds of interaction. The negative value of $\Delta G^0$ reveals that the interaction process is spontaneous. The negative $\Delta H^0$ and $\Delta S^0$ values indicate that the binding is mainly enthalpy driven and entropy is unfavorable for it, and that the hydrogen bonding and weak van der Waals forces played major role in the interaction [42, 51, 52]. The positive values of both $\Delta H^0$ and $\Delta S^0$ imply typical hydrophobic interaction.
Table 3. The thermodynamic parameters of Azide-nitro phenol system at 293 K, 298 K, 308 K

<table>
<thead>
<tr>
<th>Quencher</th>
<th>$\Delta H^\circ$ kJmol$^{-1}$</th>
<th>$\Delta S^\circ$ Jmol$^{-1}$K$^{-1}$</th>
<th>$\Delta G^\circ$ kJmol$^{-1}$</th>
<th>T (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-nitrophenol</td>
<td>-69.3576</td>
<td>-0.1293</td>
<td>-69.3197</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-69.3190</td>
<td>298</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-69.3177</td>
<td>308</td>
</tr>
<tr>
<td>p-nitrophenol</td>
<td>137.6700</td>
<td>0.5802</td>
<td>137.5199</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>137.4971</td>
<td>298</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>137.4913</td>
<td>308</td>
</tr>
<tr>
<td>Picric acid</td>
<td>20.7393</td>
<td>0.2538</td>
<td>20.6649</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.6636</td>
<td>298</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.6611</td>
<td>308</td>
</tr>
</tbody>
</table>
EXPERIMENTAL

Synthesis of 7-Hydroxy-4-azidomethylcoumarin 2.

7-Hydroxy-4-bromomethylcoumarin 1 was synthesized according to the reported procedure from resorcinol and bromoethyl acetoacetate [53]. 7-Hydroxy-4-azidomethylcoumarin was prepared by dissolving 7-hydroxy-4-bromomethylcoumarin (0.01 mol, 2.17 grams) in 25 mL of acetone and stirred the reaction mixture for 5 minutes at room temperature. Then NaN$_3$ (0.012 mol, 0.78 grams) dissolved in 3.8 mL of water was added drop wisely to the 7-hydroxy-4-bromomethylcoumarin in acetone. Further the reaction mixture was stirred at room temperature for 6h and reaction is monitored by TLC. After completion of the reaction, reaction mixture was quenched in ice water (20 mL) so as to get yellow coloured solid which was filtered and washed with 15 mL water and recrystallised from ethanol. mp-164-166°C. IR(KBr) cm$^{-1}$: 1624 (-C=C), 1692 (lactone C=O), 2119 (N$_3$), 3407 (-OH); $^1$H NMR (300MHz, DMSO, 8ppm), 4.78 (s, 2H, CH$_2$-N$_3$), 6.27 (s, 1H, C$_3$-H), 6.82 (d, $J$ = 8.9Hz, 1H, C$_6$-H), 6.75 (s, 1H, C$_g$-H), 7.55 (d, $J$ = 8.9Hz, 1H, C$_5$-H), 10.66 (s, 1H,-OH).

Procedures of fluorescence measurement

Fluorescence measurements were carried out by taking fresh solution each time in a rectangular quartz cell having an airtight stopper. The solutions were prepared by keeping the concentration of 7-hydroxy-4-azidomethylcoumarin fixed (4.6$\mu$M) and varying the quencher concentration (0.0-50mM). First the fluorescence intensity $I_0$ of 7-hydroxy-4-azidomethylcoumarin was measured without the quenchers o-nitrophenol, p-nitrophenol and picric acid and then the fluorescence intensity $I$ was measured at different quencher concentrations. Titrations were
Temperature dependent fluorescence measurements were carried out at 293 K, 298 K, 303 K, 308 K and 318 K.

**Analysis of fluorescence quenching**

Fluorescence quenching of organic molecules in solution by various quenchers like aniline, bromobenzene, carbon tetrachloride, ethyltrithiocarbonate, halide ions, metal ions, etc. has been studied by several investigators by steady state [54-56, 45-49] and transient methods [42-44, 53]. In almost all the cases, the experimental results follow the linear Stern-Volmer (S-V) relation and is given by

\[
\frac{I_0}{I} = 1 + K_{sv} [Q]
\]

where \(I_0\) and \(I\) are fluorescence intensities of 7-hydroxy-4-azidomethylcoumarin in the absence and presence of quenchers nitrophenol, p-nitrophenol and picric acid respectively. \(K_{sv}\) is the S-V constant and \([Q]\) is the quencher concentration. But in some cases, it has been observed that the experimental results show positive deviation from a linear S-V relation [45, 53-56]. This positive deviation is attributed to various processes like intersystem crossing, formation of charge transfer complexes both at ground and excited states, static and dynamic quenching, etc. Apart from this, the polarity of the solvents and the range of quencher concentration are expected to play a role in these mechanisms [57].

**CONCLUSIONS**

7-hydroxy-4-azidomethylcoumarin was quenched by o-nitrophenol through static quenching mechanism. Quenching by p-nitrophenol and picric acid show positive deviation from linearity indicating the presence of both static and dynamic quenching. Quenching mechanism is independent of solvent polarity.
constant increases with increase in the space for electron delocalization in quencher molecule. The number of binding sites varies with the change in polarity of the solvent. Azide interacts with $o$-nitrophenol through H-bonding and weak van der Waals forces. Azide has hydrophobic interaction with $p$-nitrophenol and picric acid. Since the absorption bands of the quenchers (nitrophenols) are close to the emission band of the fluorophore (azide) all the processes occur via the fluorescence resonance energy transfer (FRET).
References


