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Analysis of Fluorescence Quenching of Indole Derivative by Stern-Volmer Plots

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Abstract. The fluorescence quenching of the newly synthesized indole derivative Ethyl 5-Methyl-3-phenyl-1H-indole 2-Carboxylate [5-MPIC], has been examined in various solvents, including Benzene, Toluene, 1,4 Dioxane, Acetonitrile. Aniline was used as a quencher. The molecular behavior in various media has been explored by using solvents with different refractive index and dielectric constants. Spectroscopic techniques like UV/Vis spectroscopy and time-resolved single-photon counting were utilized to characterize the molecules at room temperature. The study of fluorescence quenching demonstrated a linear dependence in Stern-Volmer (SV) plots across solvents with varying dielectric constants. This indicates that the quenching reactions are dynamic. Additionally, key quenching parameters were determined, and the specific type of quenching reaction involved was identified. at room temperature, and the study took into account the role of diffusion in the quenching process. The probability of quenching per encounter (p) was calculated for all solvents. Furthermore, the values of p were used to estimate both the activation energy for quenching and the activation energy for diffusion.

KEY WORDS: Indole derivative, quenching, aniline, Stern-Volmer.

1 Introduction

Among the many photophysical properties of organic molecules, fluorescence quenching is a well-known spectroscopic property that provides valuable information about membranes, proteins, and macromolecular assemblies. Quenching reactions are typically non-destructive, require only small samples, and can be applied to almost all systems containing fluorescent probes. Research has shown that indole possess remarkable therapeutic and medicinal properties, leading to their use in various therapeutic and pharmaceutical applications, including as antioxidants, anticancer agents, antineoplastic agents, antimicrobials, antineuro degenerative agents, chemosensors, and anticoagulants. Furthermore, indole derivatives exhibit excellent charge transfer characteristics, making them suitable for molecular electronics applications [1–4]. Recently, we reported on the spectroscopic properties of indole derivatives, specifically focusing on ground and excited-state dipole moments and fluorescence quantum yield. Indole 2-carboxylic esters exhibit significant biological activity and are widely utilized as fluorescent brighteners and probes for monitoring various physiological disorders. Additionally, they are used in photochemotherapy and electroluminescent devices. 5-MPIC has unique applications in DNA binding studies, making it valuable for various biological and pharmaceutical research. Recent studies provide crucial insights into how the fluorescence intensity of the 5-MPIC molecule is influenced by different quenchers, depending on their concentrations in various solvent media. In-depth analysis of fluorescence quenching in indole composites has been instrumental in understanding complex biological systems [5–7].

Fluorescence quenching, which occurs due to molecular interactions such as molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching, is highly sensitive to the polarity of the solvent and the type of quencher used. The efficiency of these quenching reactions is typically measured using Stern-Volmer kinetics. Depending on the quenching mechanism, the resulting graphs may show straight lines, or positive or negative deviations [8]. Fluorescence quenching of organic heterocyclic molecules, including those like indole derivatives, plays a critical role in understanding complex biophysical and biochemical systems. This process involves the reduction in fluorescence intensity of a fluorophore, which occurs due to various molecular interactions in both ground and excited states. These interactions can include molecular rearrangements, energy transfer mechanisms such as Förster Resonance Energy Transfer (FRET), and complex formation. Common quenchers used in solution-based studies include bromobenzene, aniline, carbon tetrachloride (CCl4), and metal ions. Each of these quenchers can influence the quenching efficiency and help scientists analyze how different molecular mechanisms operate under various conditions [9–11]. This knowledge is crucial in areas like biochemical research, where fluorescence quenching serves as a powerful spectroscopic tool. Considering interaction between fluorophore and quencher molecules basically there are two types of quenching mechanisms:

(i) Static quenching and
(ii) Dynamic quenching

Static Quenching

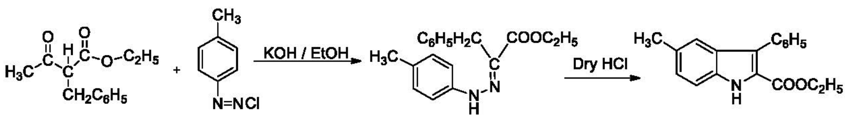
Occurs when the fluorophore forms a non-fluorescent complex with the quencher in the ground state, before any excitation happens. This complex leads to a decrease in fluorescence intensity without altering the fluorophore's excited-state properties. Importantly, the fluorescence lifetime remains unchanged because the quenching happens before the fluorophore can emit any fluorescence [12].

Dynamic Quenching

Also known as collisional quenching, it occurs when the fluorophore and quencher interact after excitation. The quencher collides with the excited fluorophore, returning it to the ground state without emitting light, leading to a reduction in fluorescence intensity. Unlike static quenching, dynamic quenching impacts the fluorescence lifetime because the interaction occurs during the excited state. The Stern-Volmer equation is commonly used to evaluate the quenching process and differentiate between static and dynamic quenching based on their distinct effects on fluorescence intensity and lifetime [13].

2 Materials and Method

The indole derivative Ethyl 5-Methyl-3-penyl-1H-indole-2-Corboxylate [5-MPIC], was synthesized following established literature methods [14].



Scheme: Synthesis of ethyl 5-methyl-3-penyl-1H-indole-2-corboxylate.

The structure of the dye is depicted in Figure 1. All solvents used for the experiments were of spectroscopic grade and procured from S. D. Fine Chemicals Ltd., India. Aniline, used as the quencher, was purified by distillation to ensure its quality. For the fluorescence quenching studies, solutions were prepared by

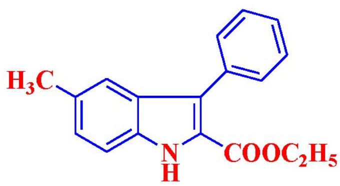


Figure 1. Structure of ethyl 5-methyl-3-phenyl-1H-indole-2-carboxylate (5-MPIC).

maintaining a constant dye concentration of 1×10^{-5} M/L, while the quencher concentration was varied between 0.00 to 0.10 M/L across all solvent systems. This setup allows for the investigation of the interaction between the dye and the quencher, providing insights into the quenching mechanism through variations in fluorescence intensity with changing quencher concentration.

The fluorescence emission spectra were obtained both with and without the quencher using a Fluorolog-3 spectrofluorometer from Horiba Jobin Yvon. For measuring fluorescence lifetimes without the quencher, the Time-Correlated Single Photon Counting (TCSPC) technique was employed, specifically using the ISS model 90021. All these measurements were conducted at room temperature, which is 300 K. The reproducibility of the experimental values is within a 5% margin of error, ensuring consistency and reliability in the data.

3 Result and Discussion

One of the most popular equations used to study the fluorescence quenching by experimentally is Stern-Volmer equation [15].

$$\frac{I_0}{I} = 1 + K_{S-V}[Q]\,. \tag{1}$$

The fluorescence intensity measurements for the dye 5-MPIC were conducted both in the presence and absence of the quencher (aniline). As the concentration of aniline increased, the fluorescence intensity of the dye decreased. This reduction in fluorescence is likely due to electron transfer from aniline to the dye. The fluorescence emission spectra were recorded in different solvent, with aniline concentrations ranging from 0.00 to 0.10 M/L. The Stern-Volmer plots, which describe the relationship between fluorescence quenching and quencher concentration, were found to be linear, indicating that the quenching mechanism follows the Stern-Volmer equation [16]. The intercepts of the plots were close to unity, and the slope provided the dynamic quenching constant (K_{S-V}). The fluorescence quenching rate constant (k_q) was calculated using the values of K_{S-V} and the lifetime of the unquenched fluorescence (τ_0), as given by the appropriate equation

$$k_q = \frac{K_{S-V}}{\tau_0}\,. \tag{2}$$

The values of K_{S-V} and k_q for 5-MPIC dye in different solvents are tabulated in Table 1. The diffusion coefficient for the dye (D_D) and for quencher (D_Q) can be estimated using the Stokes-Einstein equation [17]

$$D = \frac{kT}{a\pi\eta R}\,, \tag{3}$$

where k, T and η are the Boltzmann constant, temperature and viscosity of the solvent respectively and 'a' is the Stokes-Einstein number $a = 6$ for dye and

Table 1. The value of viscosity of solvents (η), slope K_{S-V} ($= k_q\tau_0$), quenching rate parameter (k_q) and quenching probability per encounter (p), for 5-MPIC

Solvent	τ_0 ns	$\eta \times 10{-}4$ (P)	K_{S-V} (mol^{-1})	$k_q \times 10^{10}$	p
Benzene	8.75	4.62	5.25	5.91	0.42
Toluene	5.60	3.33	11.91	16.35	0.12
1,4 Dioxane	8.45	1.20	6.79	12.31	0.29
Acetonitrile	6.12	3.88	7.60	11.80	0.36

$a = 3$ for quencher, since the radius of the dye is larger than the quencher. $R = R_D + R_Q$ is the sum of the radii of dye and quencher and these values were estimated using the method as suggested by Edward [18]. The values of radius of the dye, radius of quencher, sum of the radii of molecule and quencher and the lifetime of the molecule without quencher for 5-MPIC are shown at the bottom of Table 2. Using the values of R and D, the values of k_d for 5-MPIC molecule were estimated using Eq. 4 and these values are tabulated in Table 1

$$k_d = 4\pi NDR\left\{1 + \frac{R}{(2D\tau_0)^{1/2}}\right\}, \tag{4}$$

where N is Avogadro's number per millimole. Further, the value of probability of quenching per encounter (p) was estimated using Eq. 5:

$$k_q = k_d p\,. \tag{5}$$

The data for 5-MPIC in different solvents indicates that the probability of quenching per encounter (p) is less than unity. This means that not every encounter between the molecule and the quencher results in quenching, which aligns with findings from other studies. To analyze the role of activation in quenching, it is essential to determine both the activation energy for diffusion (E_d) and the activation energy for the quencher (E_a). The relationship between the probability of quenching (p) and these activation energies is used to estimate the values

Table 2. The value of diffusion coefficients D, D_s and D_Q, diffusion rate parameter (k_d) activation energy for diffusion (E_d) and activation energy for quenching (E_a) for 5-MPIC. $R = R_S + R_Q = 6.54$ Å ($R_S = 3.73$ Å and $R_Q = 2.81$ Å)

Solvents	D_S^{-5} ($\mathrm{cm^2s^{-1}}$)	$D_Q \times 10^{-5}$ ($\mathrm{cm^2s^{-1}}$)	$D \times 10^{-5}$	$k_d \times 10^{-9}$	E_d	E_a
Benzene	9.30	18.60	3.25	1.80	7.89	9.87
Toluene	7.23	14.46	2.45	2.19	6.80	10.34
1,4 Dioxane	7.89	15.78	1.90	1.35	5.46	6.70
Acetonitrile	13.2	26.4	4.78	2.35	7.90	9.10

of E_a. These values were calculated using a specific equation (Eq. 6) and are summarized in Table 2. The lower probability of quenching per encounter

$$E_a = E_d + RT \ln[(1/p) - 1] \,, \tag{6}$$

where R is the gas constant and T is the temperature in Kelvin. It is clear from Table 2 that values of E_a are less than those of E_d in all the solvents studied. It indicates that in the bimolecular quenching reaction the influence of diffusion process is greater than that of quenching process. The fluorescence spectra of 5-MPIC were recorded for various concentrations of the quencher aniline in solvents such as benzene, toluene, 1,4 dioxane and acetonitrile. The study found no significant shift in the emission wavelengths across different quencher concentrations, as shown in Figures 2. Fluorescence intensities, both without quencher (I_0) and with different quencher concentrations (I), were measured in all solvents at fixed solute concentrations. The fluorescence intensity decreases as the quencher concentration increases and the values are given in Table 3. The Stern-Volmer plots of $[I_0/I]$ against $[Q]$ are plotted in all the solvents and are shown in Figure 3. From figures it is observed that, the S-V plots are linear, without any deviation, throughout the quenching process, with intercept nearly equal to unity. This clearly shows that, the dynamic quenching process follows Stern-Volmer relation [19].

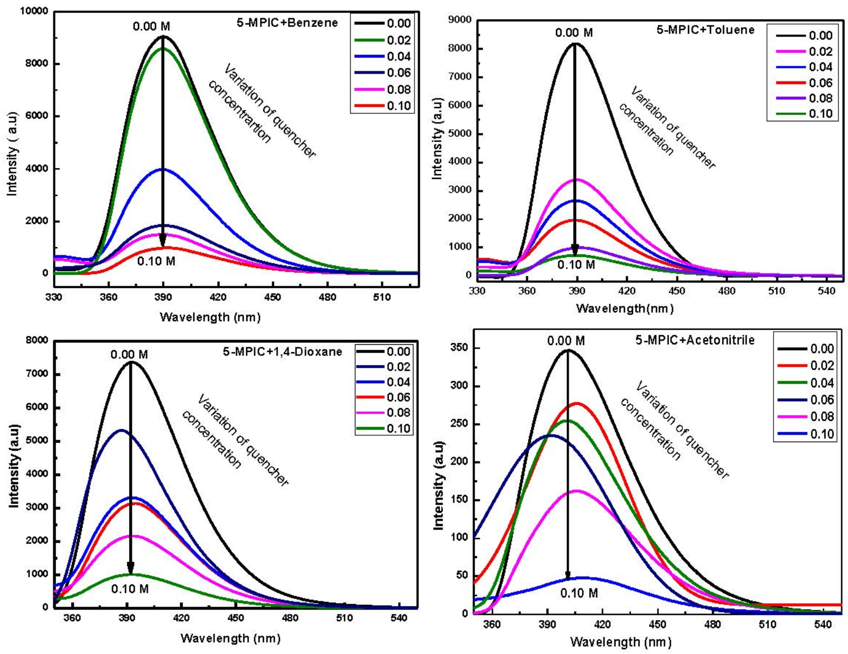


Figure 2. Fluorescence spectra of 5-MPIC in: (a) Benzene; (b) Toluene; (c) 1,4-Dioxane; (d) Acetonitrile for different quencher concentration.

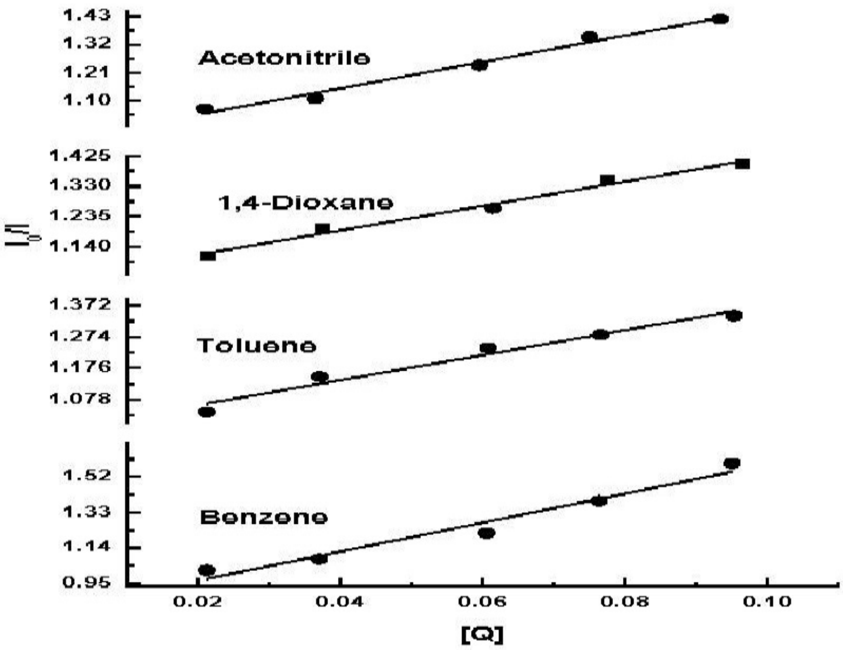


Figure 3. Stern-Volmer plot of $[I_0/I]$ against $[Q]$ in different solvents for 5-MPIC with aniline.

The fluorescence decay profiles for 5-MPIC show Figure4 a significant decrease in fluorescence lifetime as the quencher concentration increases. This observation indicates dynamic quenching, where the quencher interacts with the excited state fluorophores, leading to a reduction in their emission lifetimes [20].

Table 3. Fluorescence intensity in four different solvents for different quencher concentration of 5-MPIC

Concentration (Q) M	Benzene		Toluene		1,4 Dioxane		Acetonitrile	
	I_0	I_0/I	I_0	I_0/I	I_0	I_0/I	I_0	I_0/I
0.00	11.20	—	45.80	—	14.40	—	18.42	—
0.02	10.36	1.08	42.20	1.08	13.60	1.05	17.43	1.05
0.04	09.30	1.20	42.00	1.09	12.25	1.17	16.67	1.10
0.06	08.65	1.29	38.02	1.20	10.00	1.44	14.80	1.24
0.08	08.07	1.38	35.33	1.30	09.20	1.56	14.23	1.29
0.10	07.35	1.52	25.80	1.77	8.44	1.70	12.13	1.51

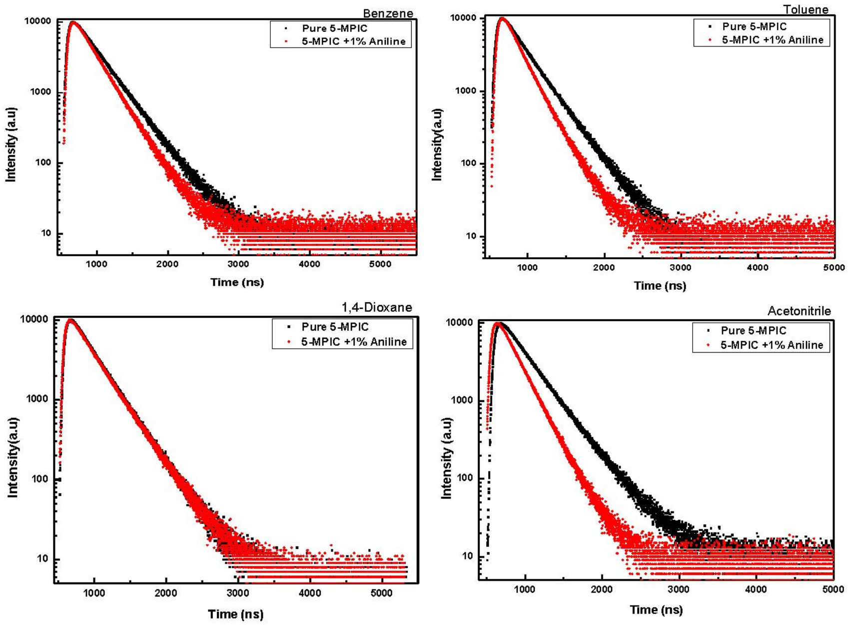


Figure 4. Fluorescence decay curve of 5-MPIC in different solvents with aniline.

4 Conclusion

Based on the above results and discussion, it was observed that the 5-MPIC coumarin derivative dye undergoes fluorescence quenching in the presence of aniline. The fluorescence intensity of the dye decreases with the addition of aniline, likely due to electron transfer from aniline to the dye. The Stern–Volmer plots were linear across all the solvents studied. In each solvent, the probability of quenching per encounter was found to be less than unity. Therefore, the fluorescence quenching of -5-MPIC by aniline in various solvents appears to involve not only molecular diffusion but also energy transfer from the quencher**.**

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