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Fluorescence Study Of 4-(4-Nitro-Phenoxymethyl)-Benzo[H]Chromen-2-One (NM2BC)

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Abstract

Fluorescence quenching analysis reveals that the process is governed by a synergistic interplay between diffusion-controlled and activation-controlled mechanisms. In the diffusion-controlled pathway, quenching efficiency is primarily dictated by the rate at which quencher molecules encounter the excited fluorophore, highlighting the influence of solvent viscosity and molecular mobility. In contrast, the activation-controlled component indicates the presence of an energetic barrier that must be surmounted before non-radiative deactivation can occur, demonstrating that simple collisional encounters are insufficient for effective quenching. The coexistence of these processes' points to contributions from both dynamic and static quenching mechanisms: dynamic quenching arises from excited-state interactions, while static quenching involves the formation of weakly bound ground-state complexes that diminish fluorescence intensity. Overall, the combined effects of diffusion, activation barriers, and complex formation offer a comprehensive framework for understanding the intricate quenching dynamics observed in the system.

Keywords: *Fluorescence Quenching, Activation Energy, Diffusion Energy.*

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I. Introduction

Fluorescence spectroscopy is one of the most powerful and sensitive techniques used to investigate molecular interactions, excited-state dynamics, and solute–solvent effects in chemical and biological systems. The fluorescence properties of organic chromophores are highly sensitive to the microenvironment, allowing researchers to probe subtle changes in polarity, hydrogen bonding, and solvation dynamics at the molecular level (Lakowicz, 2006; Valeur & Berberan-Santos, 2012) [1-2]. Among various fluorescence-based phenomena, fluorescence quenching serves as an essential tool to study the nonradiative deactivation processes and intermolecular interactions between fluorophores and quenchers.

Fluorescence quenching can occur through several mechanisms, including dynamic (collisional) quenching, static quenching, energy transfer, or charge transfer processes (Turro et al., 2010) [3]. In dynamic quenching, the excited fluorophore is deactivated via collision with a quencher molecule during its excited-state lifetime, whereas in static quenching, a non-fluorescent ground-state complex is formed between the fluorophore and quencher before excitation (Lakowicz, 2006). The Stern–Volmer relationship is often employed to distinguish between these quenching mechanisms and to evaluate the strength and nature of molecular interactions in different solvent environments (Lakowicz, 2006; Ware, 1962).

In mixed solvent systems, the preferential solvation of a solute plays a crucial role in modulating its spectroscopic and photophysical properties. Preferential solvation occurs when a solute molecule interacts more strongly with one component of a solvent mixture than the other, leading to local compositional inhomogeneities around the solute (Marcus, 1993; Reichardt & Welton, 2011) [4-6]. This microscopic environment often differs from the bulk composition of the solvent mixture, resulting in notable shifts in absorption or emission maxima, changes in fluorescence intensity, or variations in quenching efficiency (Ferreira et al., 2009) [7]. The extent of preferential solvation depends on parameters such as solvent polarity, hydrogen-bond donating and accepting abilities, and specific solute–solvent interactions, which can be quantitatively interpreted using solvatochromic or Kamlet–Taft correlations (Kamlet & Taft, 1976; Catalán, 2009) [8-9].

Understanding the interplay between fluorescence quenching and preferential solvation provides valuable insights into solute–solvent interactions, excited-state stabilization, and the photophysical behavior of chromophores in complex solvent environments. Such studies are particularly relevant for designing efficient fluorescent probes, optimizing solvent systems for photochemical reactions, and elucidating the molecular basis of solvent-induced spectral shifts in organic and biomolecular systems (Valeur, 2001; Demchenko, 2009) [10].

II. Materials And Methods

The process described in the literature was used to produce the coumarin derivative of 4-(4-Nitro-phenoxymethyl)-benzo[h]chromen-2-one (NM2BC) [11]. Each solvent utilized is of spectroscopic grade and is purchased from S.D. Fine-Chem. Ltd. To investigate the derivative's photophysical properties, absorption and emission spectra measurements have been made in binary mixture, including, dioxane, acetone, diethyl ether and methanol. At room temperature, absorption and fluorescence spectra were evaluated utilizing "UV-VIS spectrophotometer (Model: T-90+) and a fluorescence spectrophotometer (Model: Hitachi F-2700)." Low concentrations, around 1×10^{-5} M, have been used in the measurements for reducing self-absorption impact and formation of aggregation. The measurement of fluorescence lifetime (τ) was conducted by applying a Time-Correlated Single Photon Counting (TCSPC) spectrometer (Model: F-7000) on a binary mixture. The diode laser source is employed with 330 nm as an excitation wavelength, and the instrument specifications are tuned to the lifetime range of 10^{-12} - 10^{-2}s.

Evaluation of Fluorescence Quenching

Fluorescence quenching of the NM2BC coumarin derivative was examined using aniline as the quencher, chosen for its electron-rich nitrogen and aromatic ring, which enable photoinduced electron transfer (PET) and π–π or charge-transfer interactions with the excited coumarin. No significant quenching was observed with carbon tetrachloride or bromobenzene. The quenching mechanism was analyzed using steady-state fluorescence measurements and Stern–Volmer (S–V) plots, described by:

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \qquad (1)$$

where "I_0" and "I" are the fluorescence intensities in the absence and presence of quencher, [Q] is the quencher concentration, and $K_{SV} = K_q\tau_0$, with "K_q" representing the bimolecular quenching rate constant. The steady-state quenching rate is expressed as:

$$K_q = K_d p. \qquad (2)$$

where "p" is the probability of quenching per encounter and ''K_d'' is the encounter frequency. Fully diffusion-controlled quenching corresponds to (p =1), but the solute primarily determines "p", indicating that solvent alone does not govern the process. The encounter frequency "K_d" is given by:

$$K_d = 4\pi N'DR\left[1 + \frac{R}{(2D\tau_0)^{1/2}}\right], \qquad (3)$$

and the mutual diffusion coefficient "D" is obtained from the Stokes–Einstein relation:

$$D = \frac{KT}{a\pi\eta R}, \qquad (4)$$

where "K" is the Boltzmann constant, "T" the absolute temperature, "η" the medium viscosity, "a" the Stokes–Einstein factor, and "R" the encounter distance. The ratio $\frac{K_q}{K_d}<1$, indicates that diffusion alone does not fully control quenching. The activation energy of the process is described by:

$$E_a = E_d + RT\ln\left[\frac{1}{P} - 1\right], \qquad (5)$$

where (E_d) is the diffusion activation energy. When $E_d > E_a$, diffusion significantly contributes to quenching; otherwise, other activation processes also play a role. Using these analyses, Stern–Volmer constants, quenching and diffusion activation energies, and the probability of quenching per encounter were systematically determined, providing a comprehensive understanding of the quenching dynamics in NM2BC [17].

III. Results And Discussion

Analysis of Fluorescence Quenching of the NM2BC Molecule

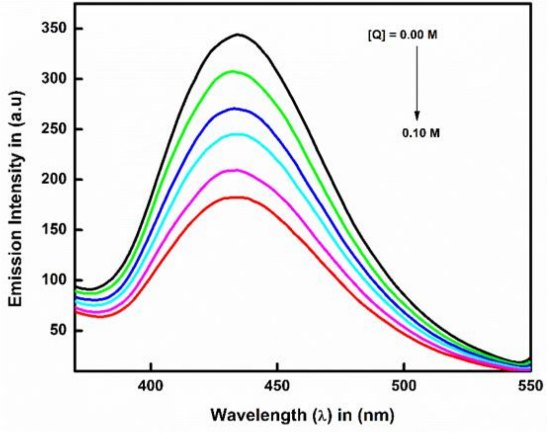

Fig 1. Shows the Fluorescence quenching of the molecule.

Fig. 1 presents the fluorescence spectra of NM2BC in various solvents, demonstrating a significant decrease in fluorescence intensity with increasing concentrations of the quencher (aniline). Notably, the spectral band maxima and shape remain largely unchanged, suggesting the absence of exciplex formation in the system. To investigate the quenching mechanism, Stern–Volmer (S–V) plots were constructed under steady-state conditions for NM2BC.

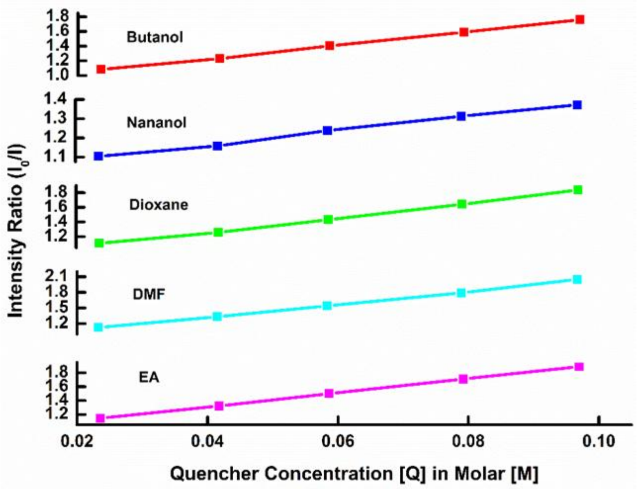

Fig 2. Plots of Stern – Volmer in different Solvents.

These plots exhibit linearity in all solvents, indicating the presence of a single quenching mechanism. The steady-state Stern–Volmer behavior is illustrated in Fig. 2. Table 1 summarizes the fluorescence intensities of NM2BC as a function of quencher concentration across different solvents. The corresponding Stern–Volmer constants (K_{SV}), calculated using Eq. (1), are presented in Table 3. Further analysis of the quenching kinetics, specifically, the quenching rate constant (K_q), the frequency of encounters (K_d), and the probability of quenching per encounter (p) was performed using Eq. (2), with results listed in Table 3. The ratio $\frac{K_q}{K_d}$, also derived from Eq. (2), is found to be less than unity in all solvents.

Table 1 Fluorescence intensity (**I**) of NM2BC as a function of quencher (aniline) concentration [**Q**], fixed solute concentration ($1\times 10^{-6}M$) in different solvent media for a steady state at room temperature.

[Q] (M)	Butanol		Nonanal		Dioxane		DMF		EA	
	I_o	I_o/I	I_o	I_o/I	I_o	I_o/I	I_o	I_o/I	I_o	I_o/I
0.00	1978	-	2064	-	344	-	496	-	747	-
0.02	1795	1.10	1845	1.11	306	1.12	451	1.09	618	1.20
0.04	1615	1.22	1788	1.15	269	1.27	362	1.37	585	1.27
0.06	1420	1.39	1654	1.24	244	1.40	322	1.54	537	1.39
0.08	1232	1.60	1559	1.32	201	1.64	277	1.79	434	1.72
0.10	1120	1.76	1501	1.37	182	1.89	241	2.05	386	1.93

DMF- Dimethylformamide
EA- Ethyl acetate

Table 2 Steady-state values of fluorescence lifetime **(τ_o)**, and diffusion coefficient **(D)** for NM2BC compound in different solvent media.

Solvent	τ_o (ns)	Dielectric Constant (ε)	Viscosity H (cP)	K_{SV} M^{-1}	$D_y\, cm^2s^{-1}$ (10^{-5})	$D_Q\, cm^2s^{-1}$ (10^{-5})	D cm^2s^{-1} (10^{-5})
Butanol	5.6	17	4.2	8.5	1.66	2.36	4.01
Nonanal	2.9	8.8	0.8	3.5	0.14	0.58	1.00
Dioxane	4.0	2.3	1.2	9.5	0.59	0.85	1.45
DMF	6.3	37	0.8	11.7	6.22	8.83	15.0
EA	8.0	6	0.4	9.6	9.38	13.3	22.7

$R(NM2BC) = R_S + R_Q = 6.86$ Å

Table 3 Quenching rate parameter (**k_q**), frequency of encounter (**k_d**), probability (**p**), activation energy for diffusion (**E_d**), and activation energy (**E_a**) for NM2BC compound in different solvents.

Solvents	k_q $(M^{-1}s^{-1})10^9$	k_d $(M^{-1}s^{-1})10^9$	p	E_d kcal mole^{-1}	E_a kcal mole^{-1}
Butanol	1.52	22.9	0.06	5.3	6.0

Nonanal	1.19	6.68	0.17	2.6	3.5
Dioxane	2.39	9.03	0.26	3.1	2.3
DMF	1.86	81.9	0.02	5.3	8.5
EA	1.20	12.6	0.09	8.4	1.1

This suggests that the quenching process is not purely diffusion-controlled and does not involve long-range energy transfer, and the diffusion-controlled rate constant (K_d) was determined using Eq. (3). Table 2 compiles additional physical parameters, including the steady-state diffusion coefficient (D), solvent viscosity (η), and the fluorescence lifetime (τ_0) of NM2BC in the absence of quencher. Also provided are the molecular radii of the quencher (R_q) and solute (R_s). The calculated kinetic distance (r) exceeds the encounter distance (R), indicating that quenching occurs within the reactive encounter sphere. Lastly, the values of activation energy (E_a) and diffusion energy (E_d) for the quenching process, presented in Table 3, reveal that $E_d < E_a$ in all case of butanol, nonanal, DMF, $E_d > E_a$ in Dioxan and EA. This confirms that the activation and diffusion energy dominates, suggesting that the quenching mechanism is governed by an activation-controlled process as well as by diffusion.

IV. Conclusions

The present study demonstrates that the fluorescence of the NM2BC molecule is effectively quenched by aniline, where both activation energy and diffusion processes play vital roles in governing the quenching efficiency. The fluorescence quenching behavior can be well interpreted by an activation-controlled mechanism coupled with a diffusion-driven process, indicating that both energetic and molecular motion factors influence the quenching dynamics.

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Data Availability: During the current investigation, no datasets were created; only examined the occurred data.

Declarations
Competing Interests: The authors declare no competing interests.

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