

# Fluorescence quenching study of fluorene by nitromethane and carbon tetrachloride in methanol and n-hexane solvents at room temperature

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## Abstract:

The steady-state quenching of fluorescence of fluorene was done by using carbon tetrachloride, and nitromethane as a quencher at room temperature, in methanol (polar solvent) and n-hexane (non-polar solvent). The present study was done by steady state and time resolved method to understand the quenching mechanism. The quenching rate constants and life time measurements were done in methanol and n-hexane with carbon tetrachloride, and nitromethane as quenchers. These parameters have shown that, the fluorescence quenching was generally governed by the well-known Stern–Volmer (S–V) plot. The positive deviation in the S–V plots has been observed for both the quenchers within the range of quencher concentration. The diffusion-limited rate constant,  $k_d$ , increases with the decrease in solvents viscosity, whereas, S–V quenching rate parameter,  $k_q$ , does not depend on the viscosity in any particular manner. The present work shows that nitromethane is an effective quencher than carbon tetrachloride for fluorene in both the solvents.

**Keywords:** Fluorescence quenching; Stern-Volmer plot and time resolved method; Polynuclear aromatic Hydrocarbons; Material diffusion.

## Introduction:

For the past 20 years, researchers have been studying the fluorescence quenching of organic molecules in solvents using a variety of quenchers, including carbon tetrachloride, aniline, oxygen, halide ions, and titanium dioxide nanoparticles [1-4]. The Stern–Volmer equation is a widely used for calculating the quenching rate parameters in the study of fluorescence quenching[5]. The process of fluorescence quenching involves transferring the electronic excitation energy of an excited fluorophore to a non-fluorescent quencher molecule through a variety of mechanisms, including diffusion, ground state complex formation, excited state reactions, energy transfer, charge transfer, and molecular reorganization [6]. Either static or dynamic (collisional) quenching mechanisms can account for this. Fluorescence quenching is influenced by a number of variables, including the quencher's nature and the surrounding medium's temperature, viscosity, solvent polarity, etc. [7-9]

Stern–Volmer (S-V) plots can be used to determine the quenching parameters, which can be used to investigate the fluorescence quenching mechanisms experimentally. The primary cause of the quenching mechanism is either collisional quenching or diffusion assisted dynamic quenching if S-V plots are linear [10, 11]. However, in addition to the dynamic process, processes like intersystem crossing, charge transfer complex formation, and static quenching may also be responsible for non-linear S-V plots with a positive deviation [12]. In order to comprehend the nature of molecular interactions, different quenchers such as carbon tetrachloride, bromobenzene, aromatic amines, halide ions, metal ions, etc. are used to quench the fluorescence of organic molecules [13]. Additionally, quenching studies can provide information on the location of fluorophores in proteins and membranes, as well as their accessibility to quenchers and diffusion rate of quenchers. In the fields of biology, chemistry, and medicine, quenching studies are also crucial. [14, 15]

The identification of polynuclear aromatic hydrocarbons has gained significant importance due to their perceived ability to seriously disrupt ecological balance in the environment and pose a risk to human health through inhalation, skin contact, and other means. Monitoring industrial and environmental pollution[16], diagnosing specific illnesses, ensuring the quality of food products, and other purposes all depend on detection. Aromatic amine vapours can enter the atmosphere through a many sources, including cigarette smoke, industrial emissions, garbage incineration, foul water, and automobile exhaust etc. IARC has classified polynuclear aromatic hydrocarbons and their derivatives as Group 3 root causes because they are extremely toxic to all living things, even at very low concentrations [17-19]. According to reports, lung cancer in humans can result from an acute inhalation exposure to high concentrations of aniline and its derivatives. Furthermore, since a lot of carcinogenic organic compounds, including o-toluidine, nitroaromatics, and aromatic amines, are found in tobacco smoke and are known to cause bladder cancer, it is crucial to detect them. Despite this, aromatic amines have found widespread application in the chemical, pharmaceutical, military, rubber, herbicides, pesticides, dye intermediates, and coal conversion industries. So, over the past several years, researchers from all over the world have focused a great deal of attention on creating highly sensitive, selective, and effective detection methods for aromatic amines [20-22].

Tricyclic aromatic hydrocarbon, fluorene, is a major component of fossil fuels and is commonly found in motor oils, crude oils, vehicle exhaust emissions, waste incineration, and products derived from the combustion of coal and oil. The European Environmental Agency (EEA) [23] and the United States Environmental Protection Agency (EPA) [24] have designated fluorene as a priority pollutant. Fluorene is a non-polar hydrophobic compound that is only weakly soluble in water. As a result, even though its concentration is lower than its solubility, fluorene cannot be accurately determined by fluorescence in an aqueous medium. In the present work, fluorescence quenching of the fluorene molecule is done by using the quenchers, carbon tetrachloride, and nitromethane, at room temperature, in both polar and non-polar solvents such as methanol and n-Hexane respectively.

## **Experimental:**

### **Instruments and Methods:**

The absorption spectra of solutions were recorded at room temperature, on UV-Visible spectrophotometer (UV-3600 SHIMADZU) using 1.00 cm quartz cell. The Steady-State fluorescence spectra of the solutions were recorded on PC based Spectrofluorophotometer (JASCO Model-FP-750, Japan). The fluorescence spectrometer has a 150 W xenon lamp used as a light source. In all the spectroscopic measurements quartz cuvettes having 1 cm width and 5 cm height are used. Fluorescence lifetime measurements were carried out using an ISS Chronos-BH (ISS-90021) time correlated single photon counting spectrometer (USA). The pulsed light emitting diode with excitation wavelength 340 nm operating at 8 MHz frequency is used as an excitation source. The fluorescence decay curves were recorded by keeping the emission wavelength at 330 nm and to measure the fluorescence lifetime decay curves were fitted biexponentially with a  $\chi^2$  value close to unity.

### **Materials:**

Fluorene (Aldrich) of 98% purity was tested for its fluorescence purity by comparing with reported fluorescence spectrum (to ensure that there is no contamination) and used as such. Carbon tetrachloride and Nitromethane used AR grade parents from Avra Synthesis Pvt. Ltd., Bangalore, India. Solvents like methanol and n-hexane have purified by usual method. Before being utilized in the current investigation, both the quencher is doubly distilled. A semi-microbalance with an accuracy of 0.01 mg was used to weigh the solutes. Fluorene concentration is fixed at  $3.0 \times 10^{-6}$  M in order to prevent the self-quenching process. While the quencher concentration was adjusted between 0.00-0.06 M in the solutions. All measurements were carried out at room temperature.

## **Results and Discussion:**

### **Steady-state absorption and fluorescence Studies:**

Fluorene absorption spectra were measured in methanol and n-hexane. In both the solvents, the wavelengths that correspond to the absorption maxima are 260.8 nm and 261.6 nm, respectively.

Fig.1 displays the fluorene fluorescence spectra with and without quenchers (0.00-0.06 M), nitromethane and carbon tetrachloride. From the fluorescence data, the characteristic intense peaks of fluorene are found at 302 nm and 302.5 nm in methanol and n-hexane respectively. Fluorescence intensity is shown to be significantly quenched as quencher concentration rises, with no change in the location or form of the fluorescence maxima. This implies that the probe and quenchers have substantial molecular interactions[25, 26].

The quenching efficiency of the fluorene displayed in Table 1, has been estimated by the equation (1), [27]. In both the solvents, nitromethane is a more efficient quencher for fluorene than carbon tetrachloride. The high value of quenching efficiency (91.34%) was obtained in methanol solvent by nitromethane while around 82% quenching by carbon tetrachloride in both the solvents.

$$\eta = \frac{I_0 - I}{I_0} \times 100 \quad (1)$$

The Stern–Volmer [S–V] equation is used in fluorescent quenching to calculate the quenching rate parameters. The equation is,

$$I_0/I = 1 + K_{S-V}[Q] \quad (2)$$

where  $I_0$  and  $I$  are the fluorescence intensities in the presence and absence of the quencher,  $[Q]$  is the quencher concentration, and  $K_{S-V}$  is the S-V constant. The slope of plot of  $I_0/I$  versus  $[Q]$  is used to estimate the S–V constant ( $K_{S-V}$ ), which is equal to  $k_q \tau_0$ , where  $\tau_0$  is the solute fluorescence lifetime without quencher and  $k_q$  is the S–V quenching rate parameter.

For fluorene molecule,  $I_0$  and  $I$  were measured at various quencher concentrations. When quenchers carbon tetrachloride and nitromethane are added, the intensity of the fluorescence decreases. Figure 2 shows the S-V plots with intercepts close to unity. It is evident from this that the S–V relation is followed by fluorescence quenching. The least square fitting technique was used to calculate the slope of the S–V plots, which provide the dynamic quenching constant ( $K_{S-V}$ ). Using the experimental values of  $K_{S-V}$  and  $\tau_0$ , the fluorescence quenching rate parameter ( $k_q$ ) is computed using the equation (3), [28]

$$k_q = K_{S-V} / \tau_0 \quad (3)$$

Table 2, displayed the values of  $K_{S-V}$  and  $k_q$  for the fluorene molecule in studied solvents. The effective quenching of fluorescence is suggested by the higher values of  $k_q$ . The positive deviations (non-linearity) is due to the small static quenching component in the overall dynamic quenching. Fig. 3 reveals the simultaneous occurrence of dynamic and static quenching when the fluorophore, fluorene, is quenched both by excited state collisions and by ground state complex formation with the quencher. [29]

The presence or absence of a transient quenching component is verified by using a published method[29]. Accordingly, a plot of  $[Q] / [(I_0/I - 1)]$  against  $[Q]^{1/3}$ . These plots are non linear with a negative slope (Fig.4). This prompted us to suggest that the positive deviation in the

Stern-Volmer plot in the studied solvents is due to the presence of a small static quenching component in the overall dynamic quenching.[29]

Standard Gibbs free energy change was calculated by equation (4) and the values are listed in Table 2.[30]

$$\Delta G^0 = -RT \ln K_{S-v} \quad (4)$$

As solvent viscosity drops, it is expected that the diffusion-limited quenching will increase. The Stokes-Einstein equation is used to compute the diffusion coefficients for solute,  $D_s$  and quencher,  $D_Q$ , in order to comprehend the impact of solvent viscosity ( $\eta$ ). The Stoke's Einstein equation (5) is used to estimate these coefficients [28, 31].

$$D = \frac{kT}{a \pi \eta R} \quad (5)$$

In this equation 'a' is the Stoke's Einstein number, which is equal to 6 for the solute and 3 for the quencher and  $K$ ,  $T$ , and  $\eta$  stands for the Boltzmann constant, temperature, and solvent viscosity, respectively. The sum of the radii of the quencher and solute is  $R = R_S + R_Q$ , and these values were calculated using Edward's recommended methodology [28, 31]. The values for the solute radius, quencher's radii, the sum of the solute and quencher's radii, and the solute life time without a quencher for the fluorene molecule is put under the Table 3. Calculated values of  $D_s$  and  $D_Q$  for the fluorene molecule and the quencher molecule are displayed in Table 3.

The equation (6) is used to estimate the values of  $k_d$  for fluorene based on the values of  $R$  and  $D$ ; these values are also displayed in Tables 3 [7, 8, 28].

$$k_d = 4 \pi N D R [1 + R / (2D \tau_0)]^{1/2} \quad (6)$$

Table 3 reveals the values of viscosity of the solvents ( $\eta$ ), diffusion coefficient of solute ( $D_s$ ), quencher ( $D_Q$ ) and sum of the diffusion coefficient of solute and quencher ( $D$ ), diffusion rate parameter ( $k_d$ ) for the fluorene molecule. In the studied solvents, the  $k_d$  values increases with the decrease in solvent viscosity.

### Time Resolved Fluorescence Studies:

Time-resolved fluorescence quenching experiments using different concentrations of quenchers have been performed. The decay profiles are shown in Fig. 5 [32, 33] along with the fluorescence lifetime of fluorene at different quencher concentrations. The fluorescence decay profile was fitted bi-exponentially, with a residual  $\chi^2$  value that is almost equal to one. Table 4 represents the values of the fluorescence lifetime.

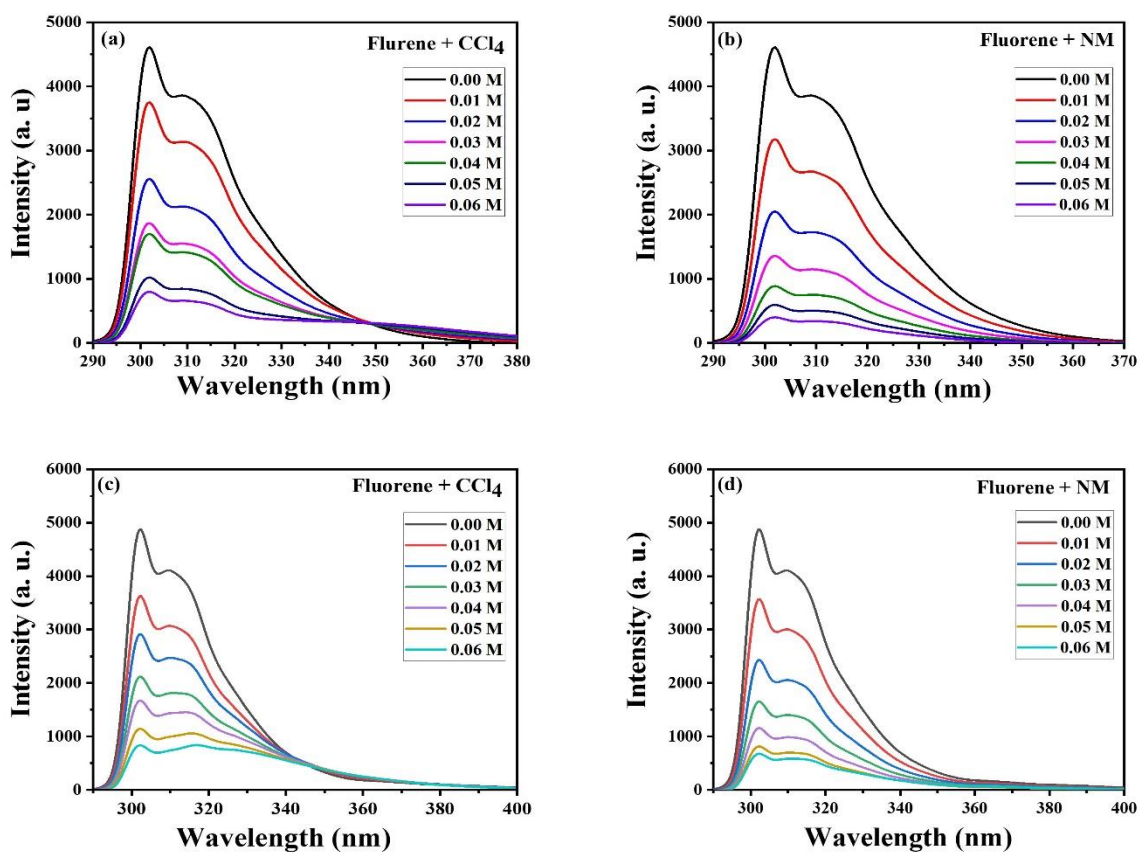
Time-resolved measurements allow for the distinction between the fluorescence quenching mechanisms, which are static and dynamic. As the concentration of the quencher is increased during the static quenching process, the fluorescence decay lifetime of the fluorophore will not change. On the other hand, the average fluorescence lifetime of the excited molecule will be seen to decrease in collisional quenching. It can be seen from Fig. 3 that as the quencher concentration increases, the fluorescence lifetime decreases. This implies that collisional interactions between the fluorophore and the quencher [12, 15, 34-37].

**Conclusion:**

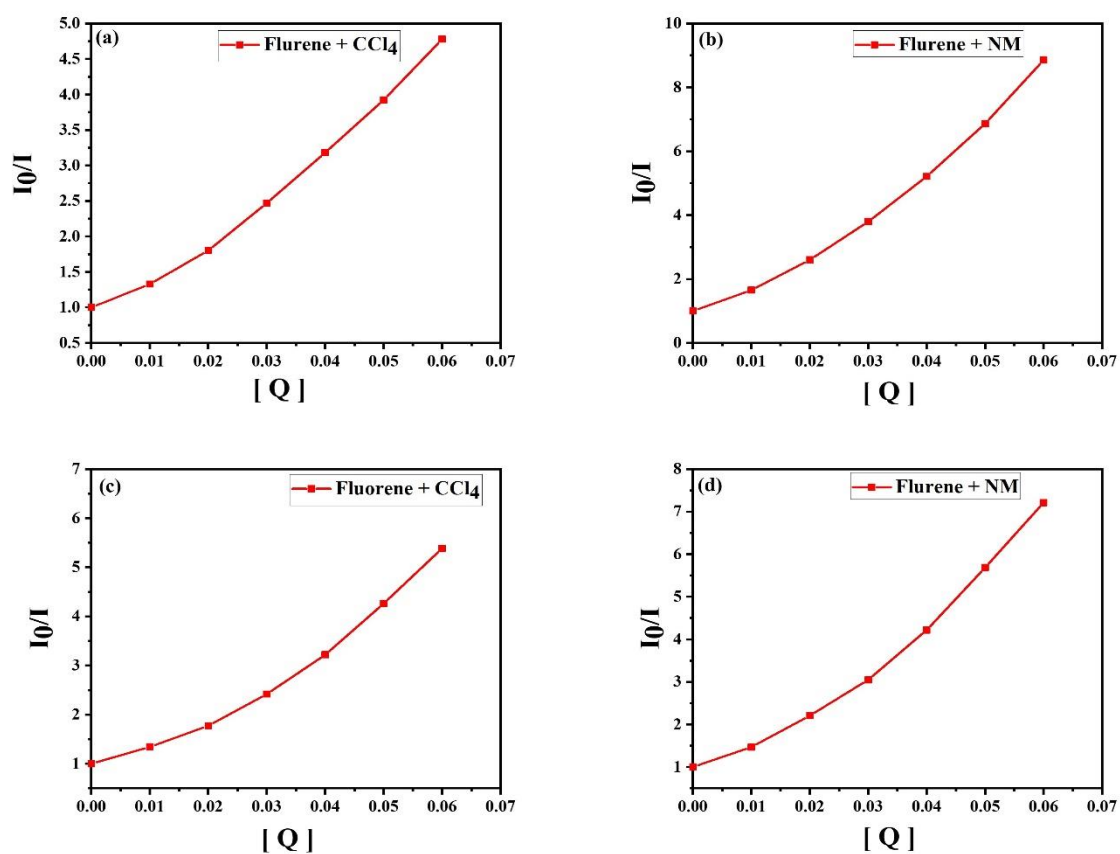
The quenching study of fluorophore, fluorene, is carried out using carbon tetrachloride and nitromethane in polar and non-polar solvents like methanol and n-Hexane, respectively. From the fluorescence data, the characteristic intense peaks for fluorene are found at 302 nm and 302.5 nm in methanol and n-hexane respectively. Efficient fluorescence quenching is explained by using the Stern-Volmer constant to calculate some of the quenching parameters. The data suggests that the dynamic quenching mechanism is followed in the studied system in presence of small static quenching. A comparison between the quenching rate parameter ( $k_q$ ) and the diffusion limited rate constant ( $k_d$ ) indicates that material diffusion is not the only cause of the quenching mechanism. As solvent viscosity rises, the values of  $k_q$  and  $k_d$  fall. A notable impact of solvent polarity on the quenching rate parameter  $k_q$  has been observed. The present study of fluorescence quenching of fluorene can be used in its detection as it is a pollutant as well as the environmental contaminant. This type of study is useful in understanding the mechanism of bimolecular fluorescence quenching and the different ways of interaction of fluorophore, fluorene, with the quenchers.

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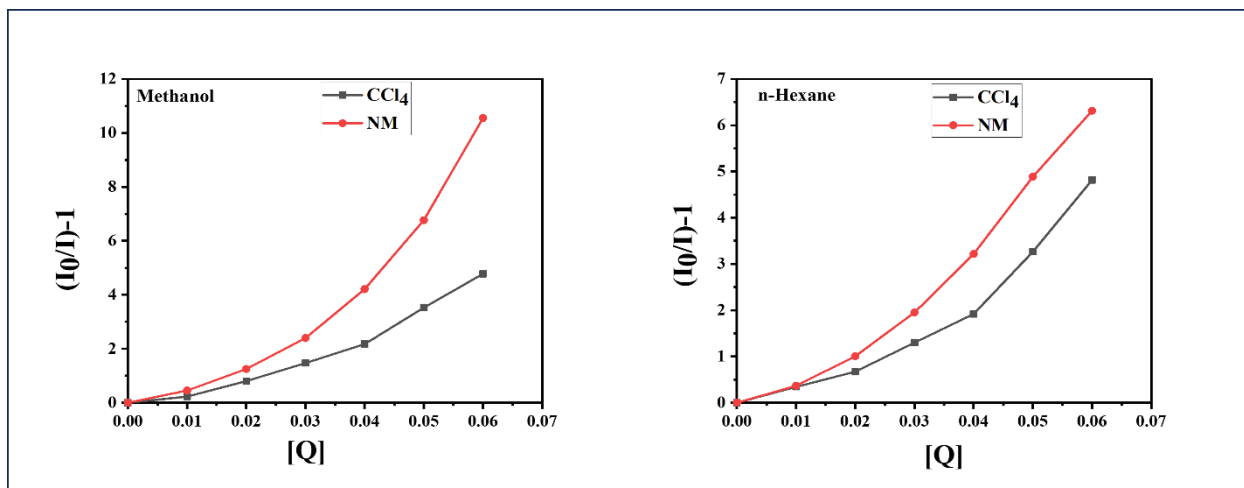


**Fig.1** Fluorescence spectra, (a) and (b) are Fluorene + carbon tetrachloride and Fluorene + nitromethane respectively in methanol solvent, and (c) and (d) are Fluorene + carbon tetrachloride and Fluorene + Nitromethane respectively in n-hexane solvent.

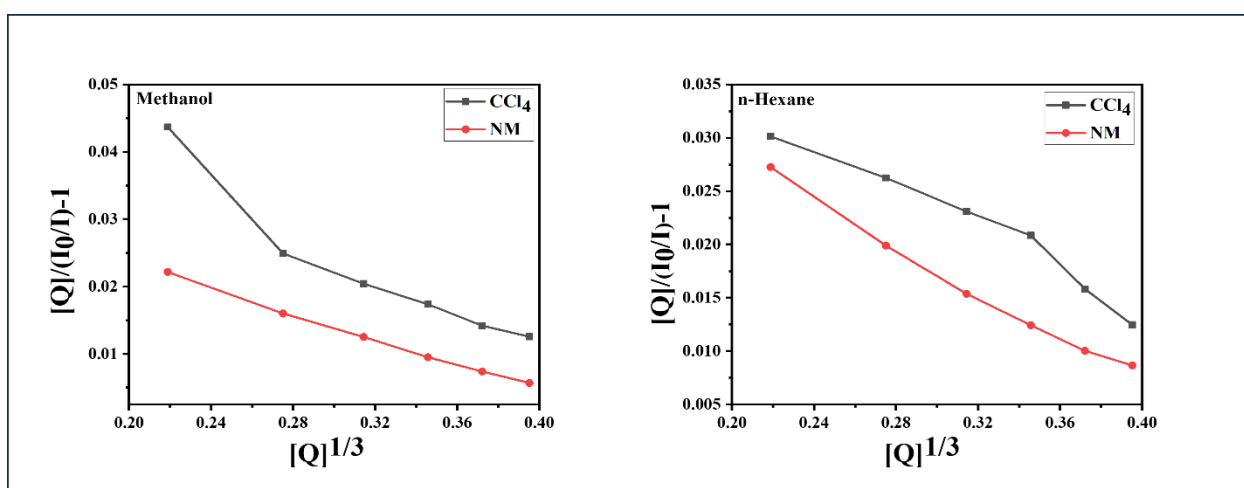


**Fig.2. Steady-state Stern–Volmer plot, (a) and (b) are Fluorene + carbon tetrachloride and Fluorene + Nitromethane respectively in methanol solvent, and (c) and (d) are Fluorene + carbon tetrachloride and Fluorene + Nitromethane respectively in n-hexane solvent.**

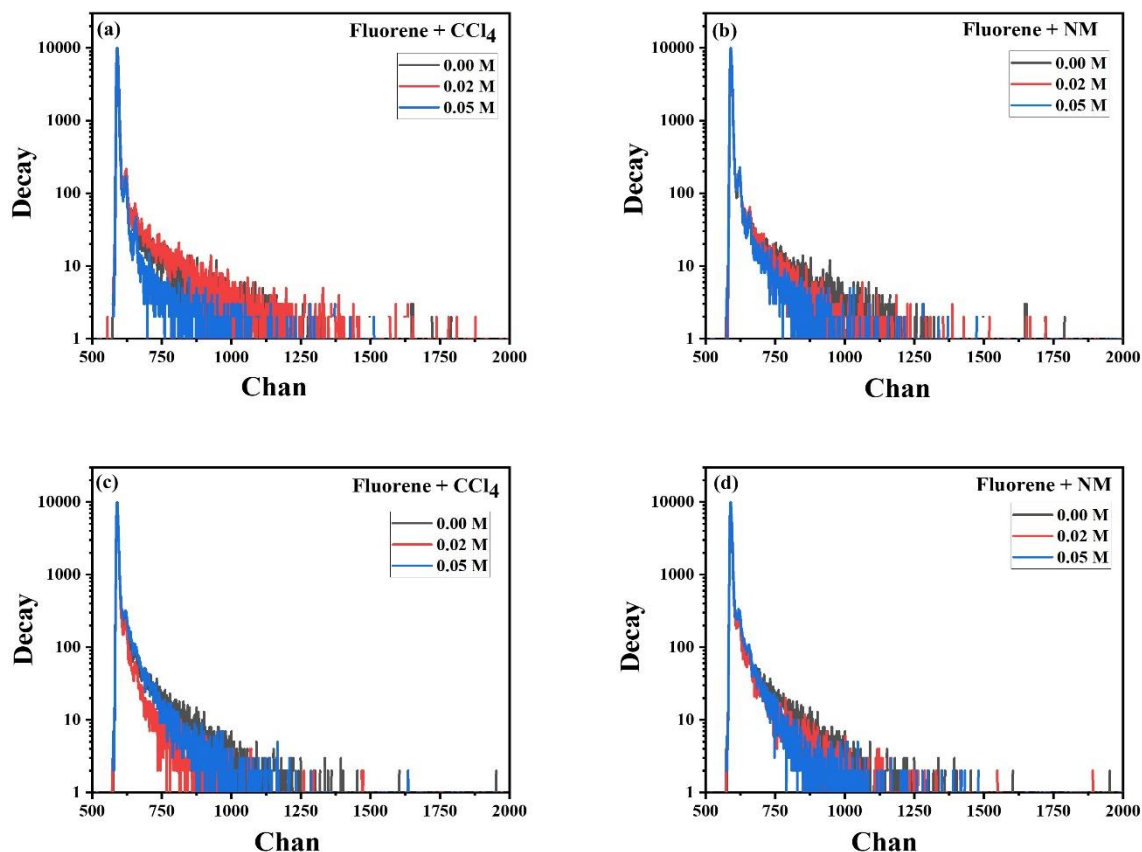




**Fig.3 Non-linear plot of  $[(I_0/I) - 1]$  against  $[Q]$  for fluorene fluorescence quenching by  $\text{CCl}_4$  and nitromethane, in methanol and n-hexane.**



**Fig.4 The plot of  $[Q]/[(I_0/I) - 1]$  vs.  $[Q]^{1/3}$  for fluorene fluorescence quenching by  $\text{CCl}_4$  and nitromethane, in methanol and n-hexane.**



**Fig.5. Fluorescence decay profile, (a) and (b) are Fluorene + carbon tetrachloride and Fluorene + Nitromethane respectively in methanol solvent, and (c) and (d) are Fluorene + carbon tetrachloride and Fluorene + Nitromethane respectively in n-hexane solvent.**

**Table 1. Quenching efficiency of the fluorene**

Quencher	Solvent	Conc. of Quencher	Efficiency in %
Carbon tetrachloride	Methanol	0.01	18.62515
		0.02	44.53002
		0.03	59.51035
		0.04	63.12800
		0.05	77.89604
		0.06	82.69472
	n-Hexane	0.01	25.54983
		0.02	40.20456
		0.03	56.50635
		0.04	65.73497
		0.05	76.54465
		0.06	82.80100

Nitromethane	Methanol	0.01	31.09683
		0.02	55.50889
		0.03	70.56298
		0.04	80.80940
		0.05	87.12805
		0.06	91.34471
	n-Hexane	0.01	26.83229
		0.02	50.13203
		0.03	66.11401
		0.04	76.28959
		0.05	83.29837
		0.06	86.13680

**Table 2. The values of S–V constant ( $K_{S-v}$ ), quenching rate parameter ( $k_q$ ), lifetime of the solute in the absence of quencher ( $\tau_0$ ), and Gibbs free energy for fluorene molecule in different solvents.**

Quencher	Solvent	$K_{S-v}$ ( $\text{mol}^{-1}$ )	$k_q \times 10^9$ ( $\text{mol}^{-1} \text{s}^{-1}$ )	$\tau_0$ (ns)	$\Delta G^0$ (kJ/mol)
Carbon tetrachloride	Methanol	63.9480	16.3192	3.91856	-10.48
	n-Hexane	72.9978	14.9179	4.89328	-10.80
Nitromethane	Methanol	130.9394	33.3641	3.91856	-12.28
	n-Hexane	103.9061	21.2344	4.89328	-11.70

**Table 3. The values of viscosity of the solvents ( $\eta$ ), diffusion coefficient of solute ( $D_s$ ), quencher ( $D_Q$ ) and sum of the diffusion coefficient of solute and quencher ( $D$ ), diffusion rate parameter ( $k_d$ ) for the fluorene molecule.**

Quencher	Solvent	$\eta$ m. Pa. s.	$D_s \times 10^{-5}$ ( $\text{cm}^2 \cdot \text{s}^{-1}$ )	$D_q \times 10^{-5}$ ( $\text{cm}^2 \cdot \text{s}^{-1}$ )	$D \times 10^{-5}$ ( $\text{cm}^2 \cdot \text{s}^{-1}$ )	$k_d \times 10^9$ ( $\text{mol}^{-1} \text{s}^{-1}$ )
Carbon tetrachloride	Methanol	0.507	0.6703	1.3407	2.0110	11.5664
	n-Hexane	0.28	1.2138	2.4276	3.6414	19.9526
Nitromethane	Methanol	0.507	0.6839	1.3679	2.0519	11.5179
	n-Hexane	0.28	1.2384	2.4769	3.7154	19.8942

For the Fluorene molecule:  $R_S = 3.8 \text{ A}^0$  [38],  $R_Q(\text{CCl}_4) = 2.73 \text{ A}^0$  [39],  $R_Q(\text{NM}) = 2.6 \text{ A}^0$  [40]

**Table 4. Lifetime of the solute in the absence and presence of quencher( $\tau_0$ ).**

Quencher	Solvent	Conc. of Quencher	Life-time
Carbon tetrachloride	Methanol	0.00 M	3.91856
		0.02 M	3.44696
		0.05 M	3.31085
	n-Hexane	0.00 M	4.89328
		0.02 M	4.19172
		0.05 M	3.97942
Nitromethane	Methanol	0.00 M	3.91856
		0.02 M	3.80343
		0.05 M	3.60718
	n-Hexane	0.00 M	4.89328
		0.02 M	4.63145
		0.05 M	3.99650

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