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EFFECT OF TEMPERATURE ON THE FLUORESCENCE QUENCHING OF BIOLOGICALLY ACTIVE CARBOXAMIDE

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ABSTRACT

Fluorescence quenching of biologically active carboxamide namely (E)-2-(4-Chlorobenzylideneamino)-N (2-chlorophenyl) 4,5 6,7-tetrahydrobenzo[b]thiophene-3- carboxamide [ECNCTTC] by aniline and carbon tetrachloride has been carried out at room temperature (298 K) to understand the role of quenching mechanisms. The study has been carried out by both steady state in different solvents and by time resolved method in n-heptane and cyclohexane. The Stern–Volmer plot has been found to be linear for all the solvents studied. The probability of quenching per encounter ‘p’ is determined in all the solvents and is found to be less than unity. It is found that, the activation energy E_a (E'_a) is greater than the activation energy of diffusion, E_d. The results obtained by the transient method infer that the thermally assisted intersystem crossing, a non-radiative deactivation process from S_1 to T_2 is responsible for observed decrease in quantum yield and lifetime. Hence, from both the methods it can be concluded that quenching mechanism is not solely due to the material diffusion, but there is also contribution from the activation energy.

Keywords: ECNCTTC, Steady state and time resolved method, Fluorescence quenching, S–V plot, Activation energy, Material diffusion, Intersystem crossing.

1. INTRODUCTION

Quenching mechanisms of organic molecules in solution by various quenchers like carbon tetrachloride [2–5], aniline [6, 7], bromobenzene [8], metal ions [9] and halide ions [10], etc. has been studied by several investigators. Our study is to understand the nature of bimolecular reactions taking place under both steady state and transient conditions. The study has been of importance in physical, chemical, biological and medical sciences [12, 13]. The process of quenching competes with the spontaneous emission and causes reduction in the fluorescence intensity and lifetime of the molecule. Basically it is a process in which the electronic excitation energy of an excited molecule is transferred to a quencher molecule via several mechanisms such as diffusion, charge transfer, etc., leading to non-fluorescent emission of quencher molecule.

The phenomenon of quenching is generally governed by the well-known linear Stern-Volmer (S–V) equation given by [14]

$$\frac{I_0}{I} = 1 + k_q\tau_0[Q] \qquad (1)$$

$$\frac{\tau_0}{\tau} = 1 + k'_q\tau_0[Q] \qquad (2)$$

where I_o and I are the fluorescence intensity, τ_0 and τ are the fluorescence lifetime, in the absence and presence of the quencher, respectively. [Q] is the quencher concentration and k_q (k'_q) is the quenching rate parameter [15]. The term $k_q\tau_0 = K_{sv}$ ($k'_q\tau_0$ = K'sv) is called the S–V constant. The phenomenon of quenching is supposed to occur due to the short-range collision between the excited molecules and the quencher molecules. If the close collision between the reactants should occur then the reactants should occupy the contagious position in the solution and separate due to diffusion after the collisional encounter.

The probability of quenching per encounter 'p' and frequency of encounter 'k_d' are given by

$$k_q = k_d p \qquad (3)$$

$$k'_q = k_d p' \qquad (4)$$

The value of k_d can be calculated from the theoretical equation giving the bimolecular reactions controlled by material diffusion given by [16]

$$k_d = 4\pi NDR10^{-3}\left\{1 + R/(2D\tau_0)^{1/2}\right\} \qquad (5)$$

where N is the Avogadro's number, D (= $D_Y + D_Q$) is the sum of the diffusion coefficients of solute (D_Y) and quencher (D_Q) and R (= $R_Y + R_Q$) is the sum of the molecular radii of solute (R_Y) and quencher (R_Q), respectively. The degree to which the material diffusion controls the quenching and also the efficiency of quenching can be determined by comparing the values of k_d and k_q. In the present work, we have used both steady state and transient state experimental setup to investigate the fluorescence quenching of [ECNCTTC] with aniline and CCl_4 as quenchers in order to understand the role of diffusion in the quenching mechanism. We have also studied the effect of temperature on non-radiative deactivation process of [ECNCTTC].

2. EXPERIMENTAL

2.1 Materials

The structure of the solute [ECNCTTC] is shown in Fig. 1. The solvents cyclohexane, n-decane, n-heptane, n-hexane, n-pentane and toluene were of HPLC grade (S.D.Fine Chemicals Ltd., Mumbai, India) and were used without any further purification. The quenchers aniline and CCl_4 were used after double distillation. The concentrations of the solute molecule in all the solvents were kept low (=$1X10^{-5}$M) to minimize the inner effect for which the reliable and stable recording of the fluorescence spectra was ensured and varying the concentration of the quencher (0.02 to 0.1M) in all solvents.

2.2 Steady state measurements

The absorption spectra were recorded using (ELICO Model SL-160) UV/VIS Spectrophotometer. The fluorescence intensity measurements were recorded using HORIBA-FLUOROLOG JOBIN YVON (FL-3) Fluorescence Spectrophotometer. For temperature-dependent studies, we have used a hollow cell holder, through which water from a constant temperature bath was circulated. The temperature in the cuvette could be controlled within ± 0.2°C between 30^0C and 60°C and was monitored directly by thermocouple immediately after the fluorescence measurement. The excitation wavelength chosen was the absorption maximum 395 nm. The concentrations of the solute molecule in all the solvents were kept low (=1X10^{-5}M) to minimize the inner effect for which the reliable and stable recording of the fluorescence spectra was ensured. The fluorescence intensity was usually determined by the relative measurement of relative intensity at the emission maxima, by varying the temperature from 30 to 60°C. The shape and position of the emission spectra were not noticeably altered by changing the temperature.

2.3 Lifetime Measurement

The fluorescence lifetimes of [ECNCTTC] were measured using computer controlled Time Correlated Single Photon Counting (TCSPC) pico seconds spectrometer HORIBA-FLUOROLOG JOBIN YVON (Model 5000U, IBH, UK) available at National Centre for Ultrafast Processes – NCUFP, University of Madras Taramani Campus Chennai-600 113, India. Short pulsed diode light source Nano-LED at 395nm excitation wavelength was used. The fluorescence lifetime measurements were performed in different solvents at temperature (30°C). The analysis of fluorescence lifetime data were carried out by using the software provided by IBH (DAS-6) which is based on reconvolution technique using iterative non–linear least square methods. The reconvolution is preceded by the series of iterations until the Chi–Square is reduced. The quality of the fit is normally identified by the reduced χ^2, weighted residual and the autocorrelation function of the residuals. In our measurements, the χ^2values are less then unity. The experimental values are reproducible within 5% of the experimental error.

3. RESULTS AND DISCUSSION

The S–V plots, for each solvent, are obtained by using experimentally determined values of I_o and I. The plots are shown in Figs. 2 and 3. These plots are linear with intercept nearly equal to unity. This clearly indicates that the steady state quenching phenomenon in all solvents follow the S–V relation.

Fluorescence emission spectra of [ECNCTTC] in the presence of aniline in n-heptane at 30^{o}C and CCl_4 in cyclohexane at 40^{o}C are shown in Figs 4 and 5. Further, the fluorescence lifetimes τ_o and τ measured for the solute in n-heptane and cyclohexane, without and with quenchers aniline and CCl_4 are given in Table 1. In order to check whether these reactions are diffusion limited, the temperature dependence of rate constant k_q is examined. The fluorescence quenching was carried out in the temperature range of 30–60^{0}C by both steady state and transient methods. The fluorescence lifetime τ of [ECNCTTC] in n-heptane and cyclohexane at different temperatures is measured by using TCSPC. The fluorescence lifetime decreases slightly with increase in temperature. The decay curve for [ECNCTTC] in n-heptane and cyclohexane in the temperature range 30–60^{0}C are shown in Figs. 6 and 7. By varying the temperature of the medium, an insight in to the thermally activated process of deactivation from the excited singlet state is obtained. When thermally activated mechanism is possible, the fluorescence decay rate constant k_f (= 1/τ) is given by

$$k_f = k^0 + k' exp\left(\frac{\Delta E}{RT}\right) \qquad (6)$$

where k^0 is temperature independent rate constant, k^0 is frequency factor for thermally assisted deactivation process and involves the entropy component [18] and ΔE is the activation energy for deactivation process. The values of k_f are given in values of ΔE (=20.316 kJ mol^{-1}and 23.078 kJ mol^{-1}) separation between S_1 and T_2 states and k' (=1.858 X10^{10} s^{-1}and 2.066X10^{10} s^{-1}) can be

Table 2. The plot of lnk_f versus 10^3/T according to Eq. (6) should be linear with its slope equal to -ΔE /R and intercept equal to lnk^0. Fig. 8 shows plot of lnk_f versus 10^3/T for [ECNCTTC]. The

determined by Eq. (6). In order to see whether the mechanism of intersystem crossing plays an important role, the singlet state energy (S_1=

22935cm^{-1}) was determined from the excitation and emission spectra [18]. From the experimentally determined value of ΔE and S_1, the values of T_2 are determined and are equal to 24632 cm^{-1} for n-heptane and 24913 cm^{-1} for cyclohexane. From these values, it is clear that T_2 lies above S_1, indicating that the deactivation of the excited molecule proceed via the triplet state [19–21]. Hence in case of [ECNCTTC], there is a possibility of intersystem crossing $S_1 \rightarrow T_2$ with increase in temperature. From steady state measurements in the temperature range 30–60^{0}C, S–V plots show linear variation with intercept nearly equal to unity, as shown in Figs. 9 and 10. The values of K_{sv} and k_q were determined for temperatures (30–60^{0}C), and are listed in Table 2. From this table, it is clear that the values K_{sv} and k_q increase with temperature. It has been assumed that k_q is proportional to exp (-E_q/RT) [8], where E_q is the activation energy for collisional process and determined from the least square fit value of the slope of the plot of lnk_q versus inverse of absolute temperature T and R is the gas constant. Fig. 11 shows the plot of lnk_q versus 10^3/T for [ECNCTTC], which is linear. The value of E_q given in Table 2 is nearly equal to the values obtained by others [17, 22] and this suggests that the process of quenching in this system is controlled by the material diffusion. The S–V plot τ_0/τ versus [Q] using Eq. (2) in n-heptane with aniline and cyclohexane with CCl_4 is shown in Fig. 12, which is found to be linear with the intercept nearly equal to unity. This clearly indicates that the phenomenon of quenching by transient method follows the S–V relation. We present the values of slopes K_{sv} (= $k_q\tau_0$) for each solvent medium, the quenching rate parameter k_q obtained using the experimentally determined value of τ_0, K'_{SV}, p' and k'_q in Table 3. In order to calculate the rate parameter k_d according to Eq. (5), the sum of the diffusion coefficients D of solute and quencher were calculated using Stokes–Einstein's relation [23]

$$D = \frac{kT}{a\pi\eta r} \qquad (7)$$

where k is the Boltzmann's constant, T the absolute temperature, Z the viscosity of the medium, r the radius of the molecule (calculated according to Edward [24]) and 'a' is the Stokes– Einstein number. For self-diffusion a=3 [25] and for diffusion of larger molecules in a liquid of smaller molecules a= 6 [26]. The term in the larger bracket of Eq. (5) is neglected, as its contribution is negligible. The values of k_d for all the solvents are given in Table 4. Further using the experimentally determined values of k_q and the calculated values of k_d, the probability of quenching per encounter p was determined according to Eq. (3) for all the solvents and the values are given in Table 3. The values of p are less than unity for all the solvents, indicating reaction of quenching that is not solely controlled by material diffusion and may depend on other processes [16]. This fact is also observed by others [5, 11, 15]. From Fig. 13, we see that although the frequency of collisional encounter k_d increases as the viscosity decreases, the rate constant k_q does not depend on the viscosity of the solvent. Hence, we may infer that the phenomenon of quenching is not solely controlled by material diffusion. Therefore, in addition to diffusion, it may also depend on the activation process. In order to see whether the activation process is playing a role, we have calculated the activation energy (E_a) for the quenching reaction given by

$$E_a = E_d + RT\ln\left[\frac{1}{p} - 1\right] \qquad (8)$$

where E_d is the activation energy for diffusion. Using the literature values of E_d and the experimentally determined values of p, the values of E_a are calculated and are given in Table 4. From this, it is clear that $E_a > E_d$ ($E'_a > E_d$ in cyclohexane) in all the solvents, this clearly proves the fact that the quenching reaction is not controlled by the material diffusion alone. Further for pure dynamic quenching I_0/I should be equal to τ_0/τ [27]. This means that the values of quenching rate parameters k_q and k'_q determined from Eqs. (1) and (2), respectively, should be same. But for [ECNCTTC], we observe that in case of n-heptane and cyclohexane $k'_q < kq$. This indicates that fluorescence quenching of [ECNCTTC] by aniline in n-heptane and CCl_4 in cyclohexane are not purely dynamic. This supports our experimental result that quenching reaction is not solely controlled by material diffusion, but there is also contribution due to some activation processes.

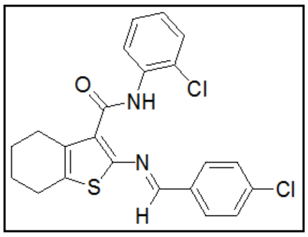

Figure 1
Molecular Structure of [ECNCTTC]

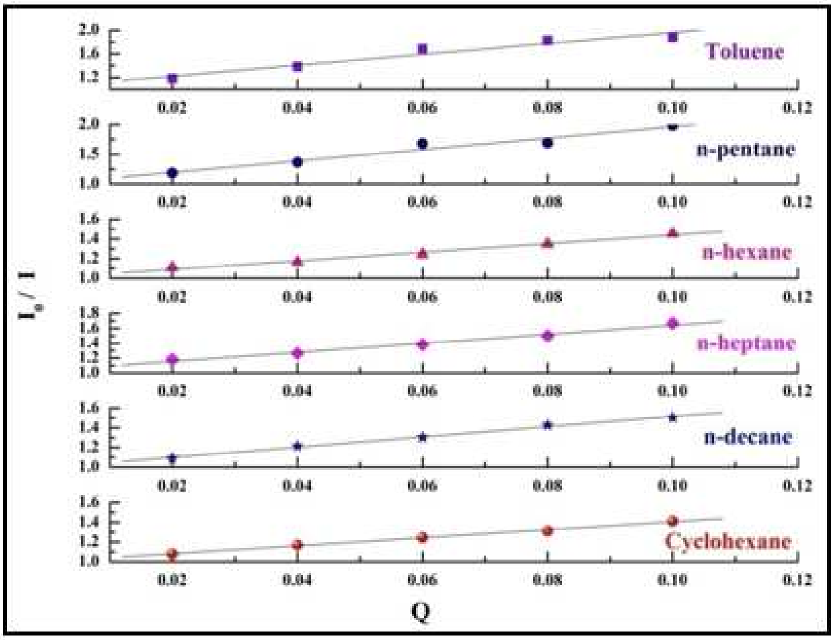

Figure 2
S-V plots of [ECNCTTC] from steady state fluorescence emission intensity measurements in different solvents with aniline.

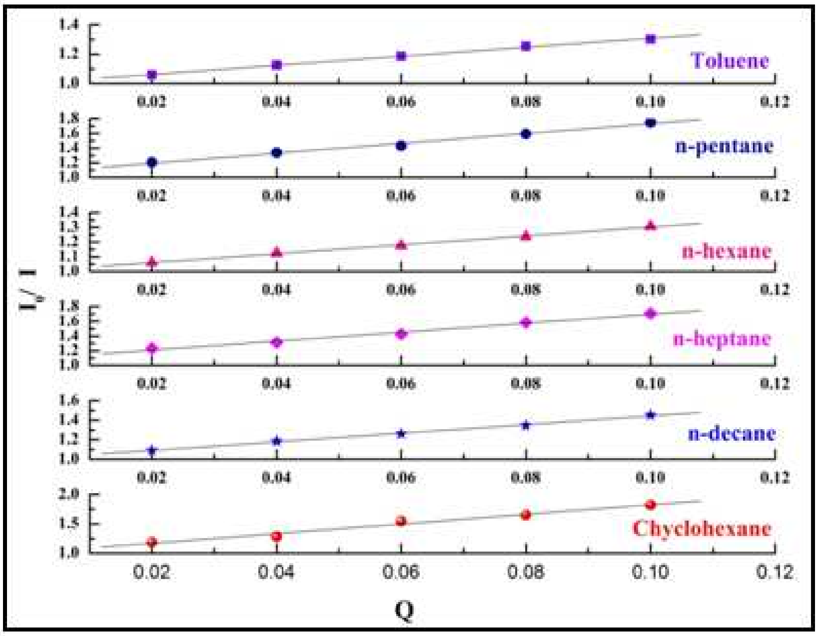

Figure 3
S-V plots of [ECNCTTC] from steady state fluorescence emission intensity Measurements in different solvents with CCl_4

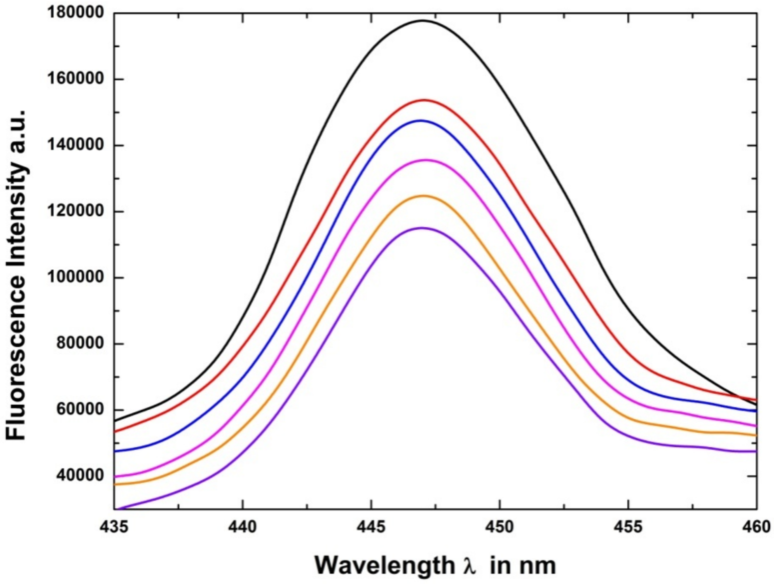

Figure 4
Fluorescence emission spectra of [ECNCTTC] ($C=1x10^{-5}M$, λ_{exc}=340 nm) in the presence of aniline in n-heptane at 30^oC. Concentrations of aniline (in M/L (1) 0.00 (2) 0.02 (3) 0.04 (4) 0.06 (5) 0.08 (6) 0.10

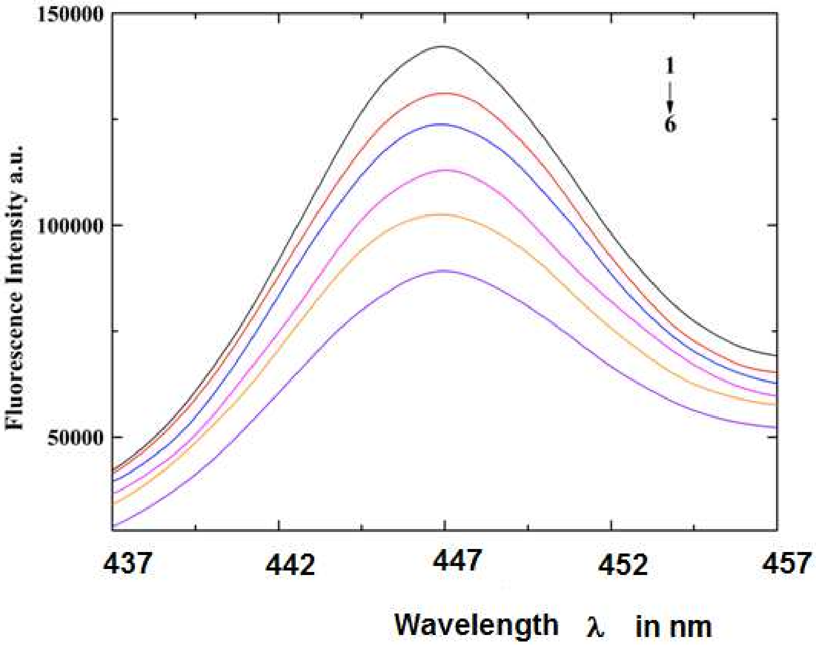

Figure 5

Fluorescence emission spectra of [ECNCTTC] ($C=x10^{-5}M$, $\lambda_{exc}=340$ nm) in the presence of CCl_4 in cyclohexane at 40^oC. Concentrations of aniline (in M/L) (1) 0.00 (2) 0.02 (3) 0.04 (4) 0.06 (5)0.08 (6) 0.10

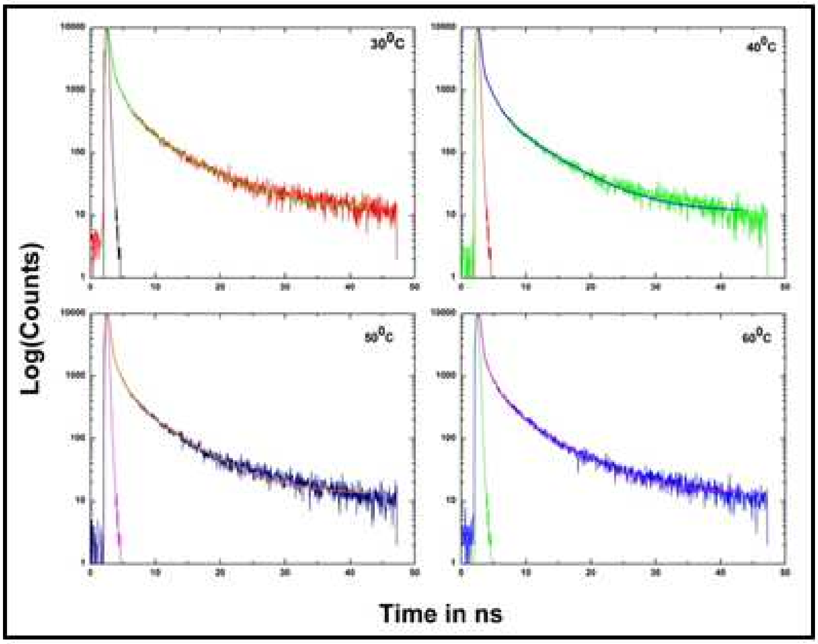

Figure 6

Fluorescence decay curves of [ECNCTTC] in n-heptane at different temperatures 30^0C to 60^0C

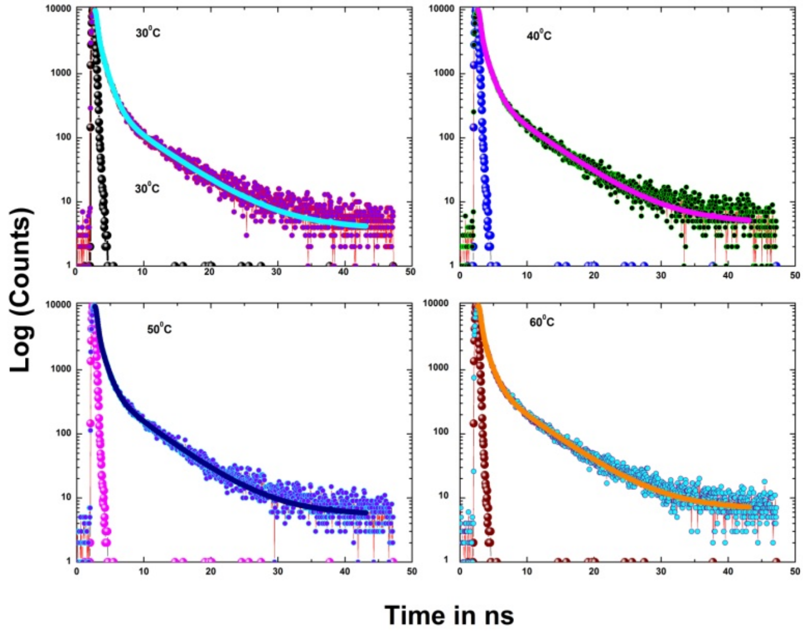

Figure 7
Fluorescence decay curves of [ECNCTTC] in cyclohexane at different temperatures 30^0C to 60^0C

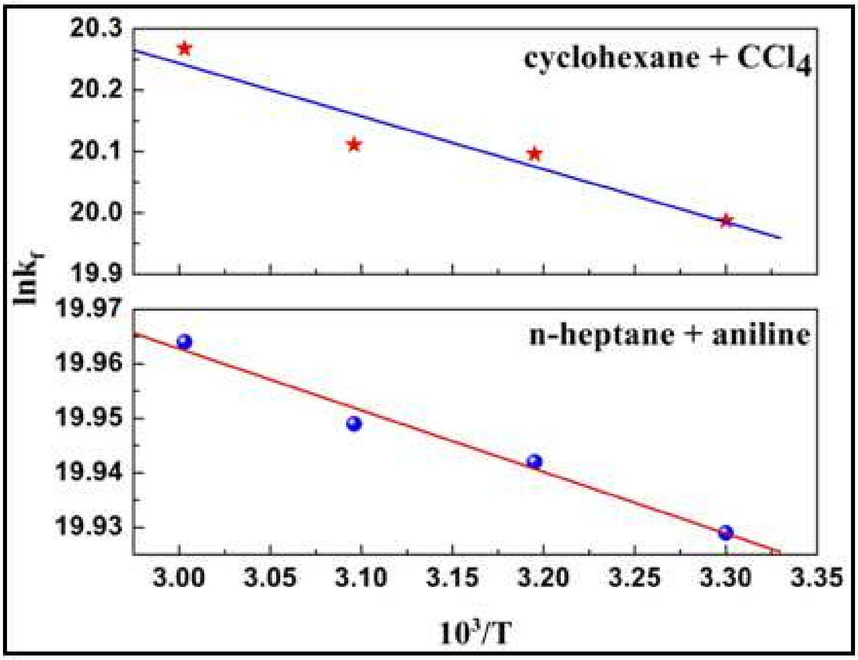

Figure 8
Plot of $\ln k_f$ versus $10^3/T$ for [ECNCTTC]

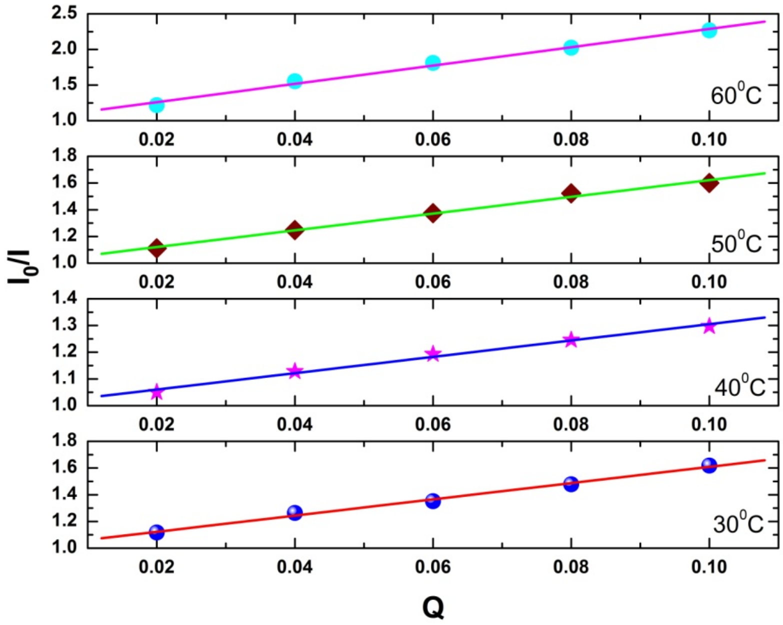

Figure 9
Stern-Volmer (S-V) plots from steady state fluorescence emission intensity measurements for [ECNCTTC] + aniline system in heptane at different temperatures.

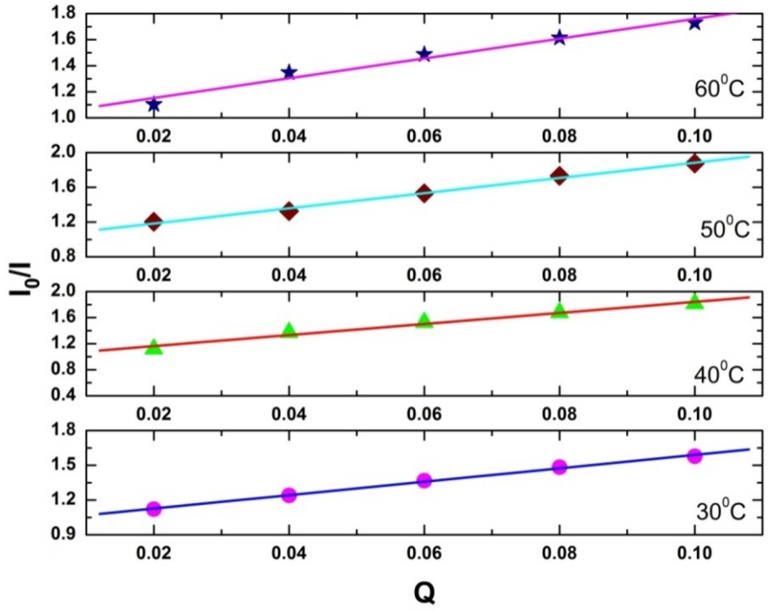

Figure 10
Stern-Volmer (S-V) plots from steady state fluorescence emission intensity measurements for [ECNCTTC] + CCl_4 system in cyclohexane at different temperatures.

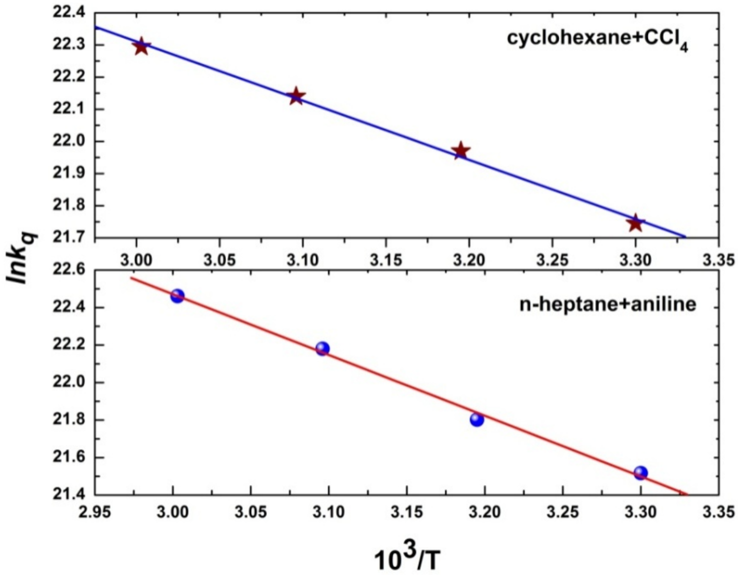

Figure 11
Plot of lnk$_q$ versus $10^3/T$ for [ECNCTTC] with n-heptane+ aniline and cyclohexane+CCl_4systems

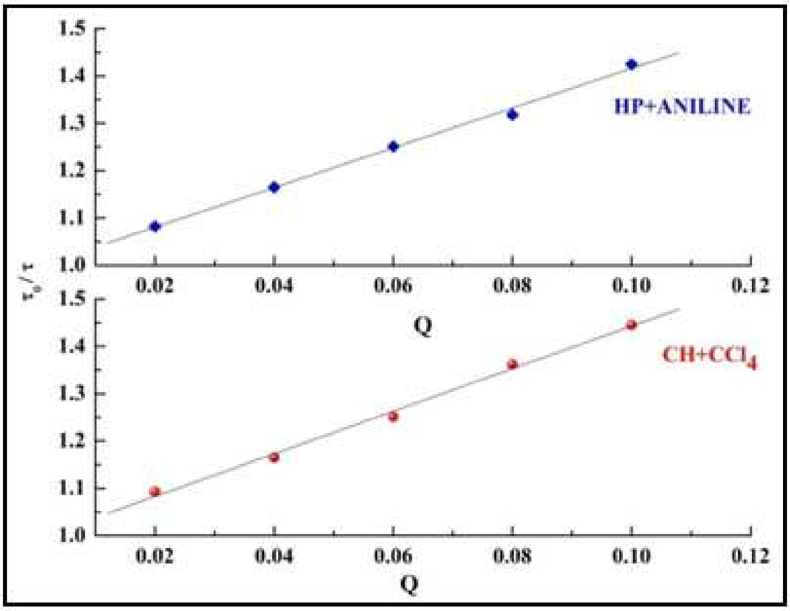

Figure 12
S-V plots of τ_0/τ versus [Q] in n-heptane+aninline (HP+ANILINE) and cychlohexane+CCl_4 (CH+CCl_4)

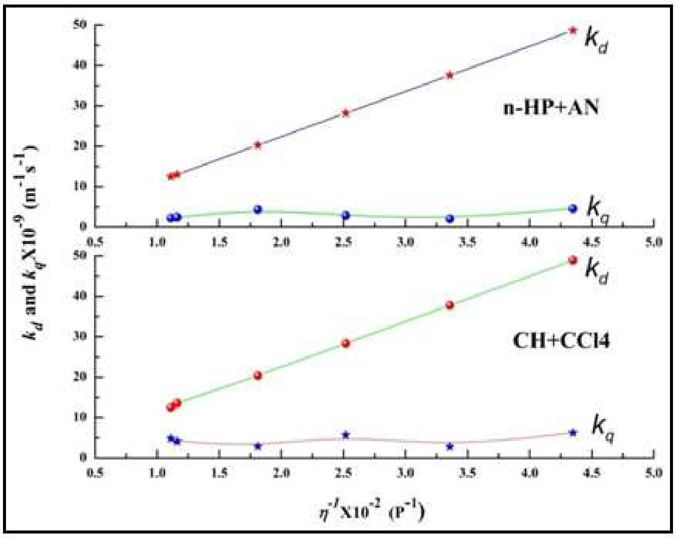

Figure 13

Variation of k_d and k_q as a function of inverse of the viscosity $\eta^{-1}X10^{-2}$ (P^{-1}) of n-heptane+aninline (HP+ANILINE) and cychlohexane+CCl_4 (CH+CCl_4)

Table .1 ***Fluorescence lifetime as a function of quencher concentration at a fixed solute concentration in cyclohexane at room temperature (298 K)***

Solvents + quencher	Quencher concentration [Q] M	τ ns	τ_0/τ
n-heptane + aniline	0.00	2.130	-
	0.02	1.968	1.082
	0.04	1.827	1.165
	0.06	1.684	1.265
	0.08	1.616	1.318
	0.10	1.495	1.425
cyclohexane + CCl_4	0.00	1.081	-
	0.02	0.989	1.093
	0.04	0.927	1.166
	0.06	0.864	1.251
	0.08	0.794	1.361
	0.10	0.748	1.445

Table.2 ***The fluorescence lifetime, fluorescence decay rate constant kf, S–V quenching constant Ksv, quenching rate parameter kq as a function of temperature and the activation energy for collisional quenching process Eq***

Solvent +Quencher	Temperature (°C)	Lifetime $\tau(t)$ (ns)	Decay rate constant k_fX10^{-9} (s^{-1})	K_{sv} (M^{-1})	k_q X 10^{-9} (M^{-1}s^{-1})	E_q (kJmol^{-1})
n-heptane + aniline	30	2.213	0.452	6.08	2.213	32.403
	40	2.204	0.458	6.106	2.770	
	50	2.170	0.461	9.598	4.423	
	60	2.136	0.468	12.85	5.682	
cyclohexane + CCl_4	30	2.085	0.479	5.790	2.777	18.413
	40	1.871	0.534	6.509	3.479	
	50	1.842	0.542	7.596	4.124	
	60	1.577	0.634	7.655	4.854	

Table 3 ***The values of inverse of viscosity η^{-1}, slope ($k_q\tau_0$), quenching rate parameter k_q and quenching probability per encounter p.***

Quenchers	Solvents	η^{-1}X10^{-2} (P^{-1})	k_qX τ_0 (m^{-1})	k_qX10^{-9} (m^{-1}s^{-1})	p
aniline	cyclohexane	1.111	4.801	2.254	0.181
	n-decane	1.161	5.193	2.438	0.188
	n-heptane	2.519	6.085	2.857	0.101
	n-hexane	3.356	4.360 [K'_{SV}=4.195]	2.047 [k'_q =1.969]	0.055 [p'=0.070]
	n-pentane	4.348	9.666	4.538	0.093
	toluene	1.812	9.231	4.334	0.214
CCl_4	cyclohexane	1.111	5.195 [K'_{SV}=4.500]	4.806 [k'_q =4.100]	0.385 [p'=0.328]
	n-decane	1.161	4.416	4.085	0.300
	n-heptane	2.519	6.103	5.646	0.199
	n-hexane	3.356	3.034	2.807	0.074
	n-pentane	4.348	6.722	6.218	0.127
	toluene	1.812	3.086	2.855	0.140

Table 4 ***The values of diffusion coefficients D_Y and D_Q of the solute and quencher, respectively, diffusion rate parameter k_d, activation energy for diffusion E_d, and activation energy for quenching E_a.***

Quenchers	Solvents	D_YX10^5 (cm^{2}s^{-1})	D_QX10^5 (cm^{2}s^{-1})	K_dX10^{-9} (M^{-1} s^{-1})	E_d (Kcal mol^{-1})	E_a (Kcal mol^{-1})
^{a}aniline	cyclohexane	0.557	1.718	12.424	3.23	6.995
	n-decane	0.582	1.796	12.987	5.98	9.629
	n-heptane	1.264	3.896	28.179	2.19	7.643 E'_a =8.642
	n-hexane	1.683	5.191	37.540	2.07	9.163
	n-pentane	2.181	6.725	48.637	3.57	9.251
	toluene	0.909	2.803	20.272	2.66	5.905
$^b CCl_4$	cyclohexane	0.557	1.745	12.495	3.23	4.398
	n-decane	0.582	1.824	13.594	5.98	7.372
	n-heptane	1.264	3.958	28.344	2.19	5.218 E'_a =5.019
	n-hexane	1.683	5.273	37.756	2.07	8.373
	n-pentane	2.181	6.831	48.916	3.57	7.976
	toluene	0.909	2.847	20.387	2.66	7.188

$^aR_Q = 2.84$ Å $^bR_Q = 2.79$ Å $R_Y = 4.379$ Å

4. CONCLUSION

From the ongoing discussion, we find that S–V plots are linear in all the solvents. The value of probability p is less than unity in all the solvents. The value of Ea (E'_a), activation energy for the quenching process is greater than E_d, activation energy for diffusion in all the solvents, which confirms the fact that the fluorescence quenching of [ECNCTTC] by aniline and CCl_4 are not solely controlled by the material diffusion. From the values of S_1 and T_2, there is a possibility of intersystem crossing from $S_1 \rightarrow T_2$, which may be the main non-radiative deactivation process responsible for the decrease in the Fluorescence lifetime (τ) with increase in temperature, whereas fluorescence quenching rate parameter k_q increases with increase in temperature.

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