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EFFECT OF HYDROGEN BONDING ON FLUORESCENCE QUENCHING OF 2-PYRAZOLE CHROMONES ANALYSIS USING NEGATIVE STERN-VOLMER PLOTS.

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ABSTRACT: In order to create a newer molecules and understanding the performance in particular applications, it is helpful to have a better excited state properties through the study of the photophysics of fluorescent organic compounds. Here, we provide a study of fluorescence quenching of a 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II] in benzene and ethanol solvents by steady state fluorescence measurements, aniline is used as a quencher. With a moderate quencher concentration, Stern-Volmer (S-V) graph shows a negative deviation has been observed. The reason for the downward curvature in the Stern-Volmer plot is considered to be the existance of several conformers of the solute being in the ground state. It has been noticed that the conformational changes in the solute's ground state are produced by the establishment of intra molecular and inter molecular of hydrogen bonds. The modified Stern-Volmer equation or the Lehrer equation is utilized to analyse the quenching data.

KEYWORDS: Benzene, ethanol, fluorescence, quenching, aniline

I.INTRODUCTION:

The compounds bearing a CHPPC-I and CCHPPC-II is a result of their extensive biological function, moiety are well known[1].In particular, 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II]: and its derivatives were placed into use as antifungal medications, and researchers are still looking into new molecules of this kind [2-4]. A number of compound produced from 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II]: also produced as possible HIV-1 integrase inhibitors [5]. These compounds have similarities to several recently discovered antifungal drugs called homoallylamines, which have strong antifungal action. The bimolecular reaction of a Fluorescence quenching in which the quantity of fluorescence intensity reduces when an additional molecule is added to a quencher.The results of quenching experiments can be used to learn about a variety of information. (a)Including the existance of numerous emitting organisms (b). fluorophores' availability to quenchers, and (c) proteins in systemic complexity [6-7]. Based on the fluorescence quenching study, biologists of molecules are investigated to trying new methods for gene finding and analysis of expression. They have been new fluorescence probes proposed [8–9]. Many researchers have started this work since it has a number of useful methods [10–14].

Theory:

A sample of fluorescence intensity decreases for a variety of reasons, like a collisional interaction between the fluorophore and an externally added quencher molecule such as Light absorption, light scattering, and inner filter effect, photo bleaching, self-quenching as a result of high concentration. Halogens and their compounds like a examples of including acrylamide, nitrous oxide, pyridinium hydrochloride, aromatic and aliphatic amines, and other heavy atoms. These are few examples of quenchers. There are three basic quenching methods that are brought on by external molecules: Fluorescence Resonance Energy Transfer (FRET), Dynamic quenching (collisional quenching) and Static quenching (contact quenching). The fluorophore is quenched dynamically during its lifetime, in the excited state and reaches coming into contact with it. The intensity of the fluorescence now decreases. when the fluorophore stops radiating. In addition to altering fluorescence intensity, dynamic quenching also reduces the fluorophore's excited life time. The development of a complex or ground state in the presence of a "quenching sphere" are related to static quenching. The complex that forms in the ground state reduces intensity and becomes non-fluorescent intensity, the simple fluorophores' lifetime will not be change, because there excited state properties remain unaffected.By nonradioactive dipole-dipole coupling, an acceptor molecule receives energy from a donor molecule that is electronically excited. in the case of

FRET [15]. Stern-Volmer equation is used to explain the Stern-Volmer graphs, Shows a both dynamic and static quenching processes (S-V plots).The relation between the fluorescence intensity before (I_0) and after (I) applying quencher is

$$\frac{Io}{I}= 1+\mathrm{Ksv}[Q] \qquad (1)$$

Here K_{SV} is refered to as Stern-Volmer constant. For a dynamic system, it is also known as a static mechanism of dynamic constantas in association with solute molecule.

This value is exactly equal to the reciprocal quencher concentration's [Q]. The fluorescence's intensity had to be quenched at 50%. It is determined by the linear S-V plot of the above mentioned equation of a slope. If the quenching mechanism is only dynamic or only static, the S-V plot will be linear. There is sometimes a combined effect between dynamic and static quenching. In this case, the Stern-Volmer plot shows an upward swing or positive deviation, especially at higher [Q] values. Using modified Stern-Volmer equations, data relevant to positive S-V plots are investigated [10,13]. The number of researchers have also reported that S-V graphs can vary negative deviation (curve downward) [14,16]. The negative deviation can be attributed to a variety of factors, including the system's heterogeneity, selective quenching, the production of hydrogen bond complexes, the occurrence of the photochemical process's reverse reaction, etc. The modified " Lehrer equation " equation or Stern-Volmer equation.I [17,18] .

$$I = (1-f)I_o+\frac{fIo}{1+Ksv[Q]} \qquad (2)$$

It can be used to express data quenching that are expected to come about by one of these reasons, as described below

$$\frac{I0}{\Delta I} = \frac{1}{f} + \frac{1}{fKsv[Q]} \qquad (3)$$

Where f is the fluorophores of fraction that are accessible.The fluorescence intensities of the solute molecules in the presence I and absence Io of the quencher respectively. is the Stern-Volmer constant is KSV and [Q] is the concentration of quencher. The linear form of this equation is

Here, $\Delta I = I_0 - I$. A plot of $I_0/\Delta I$ versus $1/[Q]$ is linear with $1/f$ as intercept and K_{SV} = *intercept/slope*. They discuss the quenching of a newly synthesised material in the present work 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-choloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^2) [CCHPPC-II] in solvents like ethanol and benzene. A aniline is taken as quencher. Using the above mentioned equations, Investigated is the negative deviation of the S-V plot.

III. Experimental methods:

A newly synthesized 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II]: pyrazole molecular structure is as shown in fig.1.An aniline is taken as quencher that has passed in doubly distilled and is dissolved in benzene and ethanol .Spectroscopic grade solvents are obtained in Merk India. A double beam UV-VIS Spectrophotometer (Model: Shimadzu UV-1800) with a accuracy of wavelength is 0.5 nm is used to measure the absorption spectra at room temperature. To prevent self-absorption and the formation of aggregations, the concentration of the solution is kept around1×10^{-5}M. The solutions are prepared by changing the concentration of the quencher (0.00M – 0.10M). The excitation wavelength is found to be 320 nm based on maximum absorption. The spectra of fluorescence are captured using a fluorescence spectrophotometer (Model: Hitachi F- 2700) at room temperature with perpendicular geometry and standard quartz cuvettes. The Typical emission spectra 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II]: with different quencher concentrations of benzene and ethanol solvent asshown in fig. 2.

Using the TCSPC spectrometer, fluorescence lifetime will be calculated in nanosecond fluorescence lifetimes (Ʈ0) of the solute without a quencher are determined in Solvent of benzene and ethanol. TCSPC spectrometer fluorescence in nanosecond of photophysics model HORIBA FLUOROLOG K U Dharwad, India, in the state of Karnataka.

IV. RESULTS AND DISCUSSIONS:

Benzene and ethanol are used as solvents for the fluorescence experiment.When the sample is dissolved in ethanol, the intensity of the emission increases. This shows that in polar atmospheres, solutes exhibit a greater degree of fluorescence. The addition of quenchers, like aniline, considerably reduces fluorescence intensity. S-V graphs are made using equation (1) and are shown in figure 3. They exhibit a negative deviation with an intercept of unity at higher quencher concentrations (0.06-0.10M), but are essentially linear in the lower concentration range (0.00-0.04M). It is suggested that diffusion is restricted dynamic quenching is responsible for the linearity in the lower concentration range. Any of the above mentioned causes could be to account for the plot's negative deviation. One of the causes is the presence of two fluorophores that have differing levels of quenching accessibility. For instance, tryptophan residues that fluorescence in structured proteins are buried within the protein and as a result produce heterogeneous quenching. The plot's negative deviation could have any of the above causes. Two fluorophores that have differing levels of quenching accessibility are one of the causes. For instance, tryptophan residues that fluorescence in structured proteins and are buried within the protein result in heterogeneous quenching because they are buried within the protein. In the case of benzene and ethanol, respectively, the S-V constant (KSV) ranges from 115 M-1 to 88.8 M-1 and the value of fraction of flurophore is almost equal to 1. Identification of the quenching mechanism is necessary for accurate data interpretation and KSV calculations. From the emission and absorption spectra the negative deviation in the S-V plot rules out the formation of non-fluorescent complex between the aniline 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II] and since no new characteristic peak to be observed. In most cases, K_{SV} values calculated from the linear fit of I_0/I versus [Q] the lower part of are relatively small compared to K_{SV} calculated from equation (3), so even if static quenching occurs as a result in the presence of aniline molecules nearby fluorophores at the time of excitation, it is very weak to be predicted. These values are presented in Table.1.

By Calculating the rate parameter of bimolecular quenching kq the equation

$$K_{SV}=k_q\tau_0 \qquad (4)$$

Where K_{SV} stands for the Lehrer equation's slope, and τ0 represents the fluorophore's lifetime in the absence of a quencher.

$$k_q = \frac{KSV}{\tau o} \qquad (5)$$

The value of kq getting an effective quenching of fluorescence,before an exciplex or conformer has fully developed the efficient quenching may occur. Diffusion-limited quenching is expected to increase as solvent viscosity decreases. The equation is used to calculate the diffusion-limited rate constant kd, which is essential for understanding the impact of solvent viscosity (η).

$$k_d = 4\pi NDR \qquad (6)$$

Here N is Avogadro number in per millimole, $D = D_S + D_Q$ and $R = R_S + R_Q$ represents the sum of the diffusion coefficients (in cm^2s^{-1}) and the molecular radii (in Å) of the solute (S) and the quencher (Q), respectively [19]. The diffusion coefficients are calculated using Stokes-Einstein equation.[20].

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

Here, R is the radius of the solute or quencher molecule , k is the Boltzmann constant, T is the absolute temperature, η is the solvent viscosity (in cP), and 'a' is the Stoke-Einstein number. The value 3 for the quencher and 6 for the solute.

The calculated kq and kd values for both solvents are given Table.1. In case of benzene, it is found that k_q is 132.79 × $10^9 M^{-1}s^{-1}$and k_d is 59.36× 10^9 $M^{-1}s^{-1}$ whose viscosity is 0.56cP and 113.63× 10^9 $M^{-1}s^{-1}$and 48.89× 10^9 $M^{-1}s^{-1}$in ethanol, viscosity is 1.02cP. This indicates that as solvent viscosity increases, both kq and kd decreases.

As the viscosity of the solvents decreases, the both bimolecular quenching rate and the diffusion-limited rate constant kd value becomes increases. Thus, it is assumed that material diffusion is not the only factor affecting the quenching mechanism [21]. Also, as the dielectric constant increases, Ksv is also increases, indicating the excited complex's charge transfer characteristics. The solute's initial existence in two residues at the ground state is the reason of the observed negative deviation in the S-V plots for CHPPC-I and CCHPPC-II. There will be hydrogen bonds between one hydrogen atom and others in the 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II] molecule with oxygen atom of other molecule 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II] as shown in figure 1. Using the alcoholic solvent ethanol, intermolecular hydrogen bonding is also a possibility. Figure1 shows how ethanol causes intermolecular hydrogen bonds to develop, In benzene, where there is less viscosity, these compounds have stable intermolecular hydrogen bonds with one another. The 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one(HL^2) [CHPPC-II] Ethanol may exist in two different conformers due to movement and hydrogen bonds with alcohol molecules. Negative deviation in S-V plots may be caused by the presence of two conformers. These observations could be helpful in developing the more recent molecules such as sensors. Stern-Volmer (S-V) plots of the effective of fluorescence quenching exhibited upward slopes from linearity in all solvents for different molarities [22]. Many organic molecules that have a significant impact on our daily lives were a gift from nature to humanity. Many organic compounds have gained potential uses in the biomedical, pharmaceutical, and electronics industries. The fluorescence quenching analysis shows that the Stern-Volmer plot linearly depends on the solvent's dielectric constant. It displays how dynamic quenching reactions are in nature. The quenching reactions involved in the quenching has been identified using a variety of quenching parameters. [23].The Organic molecules will undergo the photochemical and photophysical fluorescence quenching process in crystalline form, and this form will be used in the sensing framework [24].

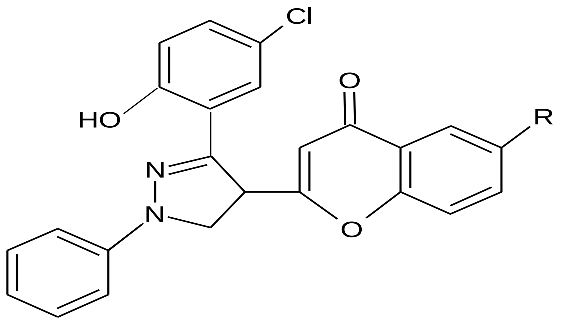


Figure 1. *Molecular structure of CHPPC-I (R=H) and CCHPPC-II(R= CH_3)*

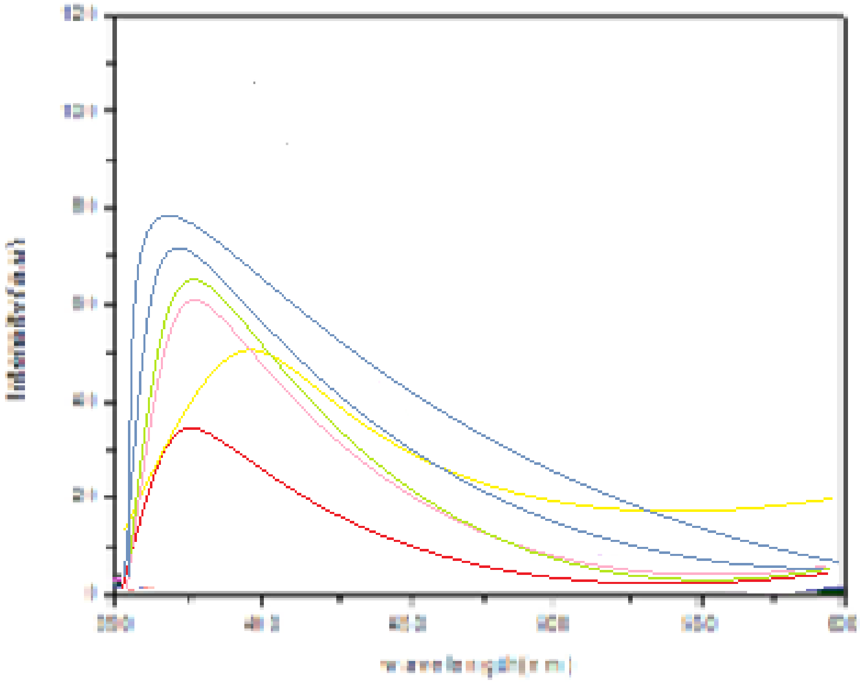
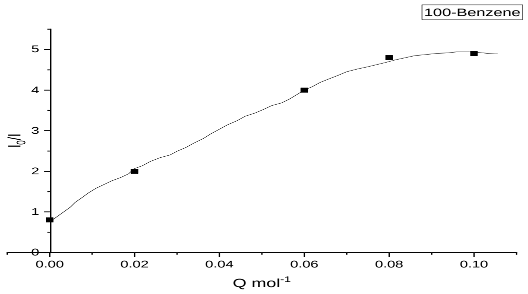


Figure 2: *Emission spectra of the solute in benzene at fixed concentration of 1×10^{-5} Mwith varying quencher concentration of aniline.*



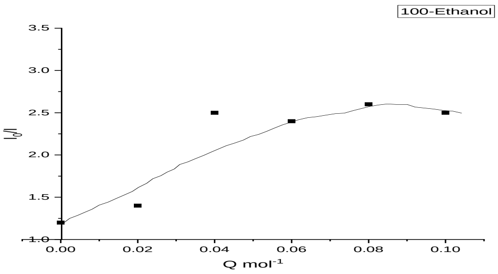


Figure 3: *Stern–Volmer plots of I_0/I versus [Q] exhibiting negative deviationin benzene and ethanol.*

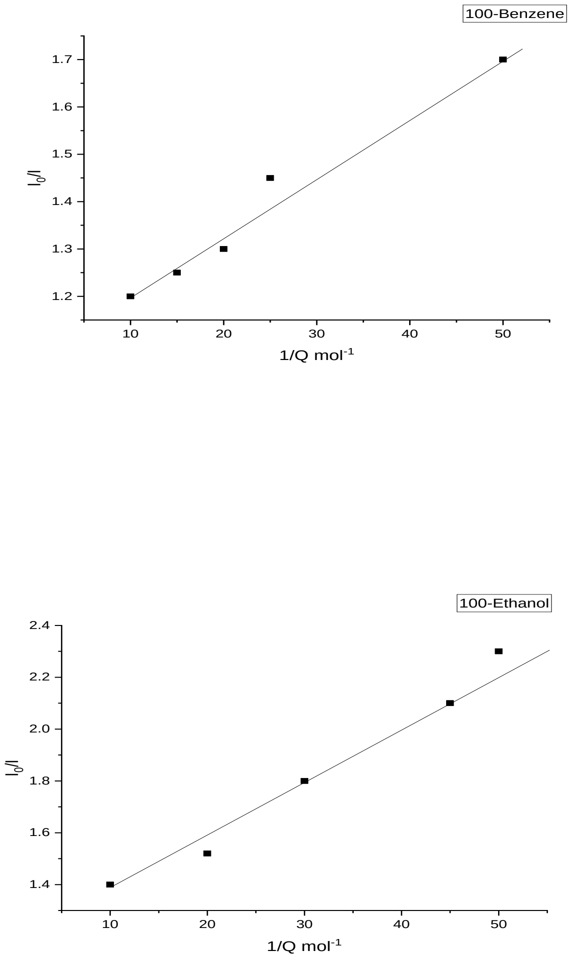


Figure 4

Modified linear Stern–Volmer plots of $I_0/(I_0-I)$ versus 1/[Q] in benzene andethanol with anilline as quencher.

Table 1: Viscosity (η), Dielectric constant(ε), the fraction of accessible fluorophores (f), S-V constant (K_{SV}), bimolecular quenching rate parameter (k_q) and diffusion rate constant (k_d)

Solvent	***Viscosity(η) in Cp***	***Dielectric constant(ε),***	**f**	$^{LH}K_{SV}(M^{-1})$	$^{LSV}K_{SV}(M^{-1})$	$k_q \times 10^9$ $(M^{-1}S^{-1})$	$k_d \times 10^9$ $(M^{-1}S^{-1})$
Benzene	0.56	2.15	0.869	115	57.5	132.79	59.36
Ethanol	1.02	24.55	0.990	88.8	25	113.63	48.89

CONCLUSIONS:

Fluorescence quenching of CHPPC-I and CCHPPC-II moiety are well known due to their broad biological activity [1]. In particular, 2-(3-(5-chloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^1) [CHPPC-I] and 6-chloro-2-(3-(5-choloro-2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (HL^2) [CCHPPC-II] by aniline in benzene and but anolsolvent environment is carried out at room temperature. The efficient quenching mechanism is explained by calculating some of the quenching parameters using Stern – Volmer kinetics and the mechanism is credited partly to diffusion limited. The diffusion limited rate constant (k_d) is compared with the quenching rate parameter (k_q) and the comparison suggests that the quenching mechanism is not entirely due to material diffusion. The negative deviation observed in the S-V plot is explained in terms of intermolecular hydrogen bond formations. From the observed results one can arrive at the conclusion that the studied CHPPC-I and CCHPPC-II to draw the attention in designing newer molecules for sensing applications.

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