



EFFECT OF HYDROGEN BONDING ON FLUORESCENCE QUENCHING OF QUINOLIN-8-OL - ANALYSIS USING NEGATIVE STERN-VOLMER PLOTS

V.T. MUTTANAVAR^a, RAVEENDRA.MELAVANKI^{b*}, KALPANA SHARMA^b,
P. BHAVYA^c, RAVIRAJ. KUSANUR^d AND L. R. NAIK^e

^aDepartment of Physics, JSS Science RSH PU College, Vidyagiri Dharwad-580003, Karnataka, India

^bDept. Of Physics, M S Ramaiah Institute of Technology Bangalore-560054, Karnataka, India

^cDept. Of Physics, New Horizon College of Engineering, Bangalore, Karnataka, India

^dDepartment of Chemistry, R.V. College of Engineering, Bangalore, Karnataka, India

^eDepartment of Physics, KarnatakUnivesity Dharwad-580003, Karnataka, India

ABSTRACT

Photophysics of fluorescent organic compounds give a better knowledge of the excited state properties which in turn will help in the design of newer molecules and understanding their performance in specific applications. Here we present the fluorescence quenching study of a Quinolin-8-ol (QO) in toluene and butanol solvents by steady state fluorescence measurements. Aniline is the quencher. Negative deviation in the Stern – Volmer (S-V) plots has been observed with moderate quencher concentration. The downward curvature in the S-V plot is interpreted in terms of existence of different conformers of the solute in the ground state. The formation of intermolecular and intra molecular hydrogen bonding is found to be responsible for the conformational changes in the ground state of the solute. Quenching data is analyzed by modified Stern-Volmer equation or Lehrer equation.

KEYWORDS: *Quinolin-8-ol (QO), fluorescence quenching, modified Stern–Volmer (Lehrer) equations and hydrogen bonding.*



RAVEENDRA.MELAVANKI*

Dept. Of Physics, M S Ramaiah Institute of Technology Bangalore-560054,
Karnataka, India

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INTRODUCTION

The compounds bearing a quinoline moiety are well known due to their broad biological activity¹ In particular, 8-hydroxyquinoline and its derivatives were introduced into antifungal clinical use and novel compounds of this type are still investigated.²⁻⁴ A series of compounds derived from 8-hydroxyquinoline as potential HIV-1 integrase inhibitors were also synthesized⁵. These compounds show a significant similarity to some novel antifungal agents, homoallylamines, which possess potent antifungal activity. Fluorescence quenching is a bimolecular reaction in which the fluorescence intensity decreases upon adding an external molecule called 'quencher'. Quenching study is useful to extract information about (a) proteins in complex system, (b) accessibility of fluorophores to quenchers, (c) presence of multiple emitting species and many more^{6,7}. Molecular biologists are trying to explore new techniques for gene discovery and expression analysis based on the fluorescence quenching study. New fluorescent probes have been suggested^{8,9}. Because of many such novel applications, many investigators have taken up this study¹⁰⁻¹⁴.

Theory

Fluorescence intensity of a sample reduces due to various reasons like, light scattering, inner filter effect, photo bleaching, self-quenching due to high concentration and collisional interaction between

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

Here K_{SV} is known as S-V constant. It is also called as dynamic constant for dynamic mechanism and association constant for static mechanism. It is just equal to the reciprocal of quencher concentration $[Q]$ required to quench 50% of the fluorescent intensity. It is given by the slope of a linear S-V plot of above equation. S-V plot is linear if the quenching mechanism is either only dynamic or only static. Sometimes there exists a combined effect of static and dynamic mechanisms. In such case S-V plot shows a positive deviation or upward swing, especially in the higher $[Q]$ values. Data

$$I = (1 - f)I_0 + \frac{fI_0}{1 + K_{SV}[Q]} \quad (2)$$

Where I_0 and I are the fluorescent intensities of solute molecules in the absence and in the presence of quencher, $[Q]$ is the concentration of the quencher, f is the fraction of accessible fluorophores, K_{SV} is the S-V constant. The linear form of this equation is

$$\frac{I_0}{\Delta I} = \frac{1}{f} + \frac{1}{fK_{SV}[Q]} \quad (3)$$

Here, $\Delta I = I_0 - I$. A plot of $I_0/\Delta I$ versus $1/[Q]$ is linear with $1/f$ as intercept and $K_{SV} = \text{intercept/slope}$.

fluorophore and externally added molecule called quencher. Heavy atoms like halogens or halogen containing substances, aromatic and aliphatic amines, acrylamide, nitrous oxide, pyridinium hydrochloride etc. are some example for quenchers. Dynamic quenching (collisional quenching), Static quenching (contact quenching) and Fluorescence Resonance Energy Transfer (FRET) are three main quenching mechanisms that are caused by external molecule. In dynamic quenching the quencher diffuses to the fluorophore in its excited state within the lifetime of the fluorophore and develops contact with it. Now the fluorophore becomes non radiative and hence fluorescence intensity decreases. Dynamic quenching not only affects the fluorescence intensity but also reduces the excited life time of the fluorophore. Static quenching is associated with either the formation of ground state complex or the existence of 'quenching sphere'. The complex formed in the ground state is non-fluorescent and intensity reduces. However the life time of the uncomplexed fluorophores will not change. Their excited state properties remain unaffected. In the case of FRET, energy of a donor molecule in its electronically excited state is transferred to acceptor molecule through nonradiative dipole-dipole coupling¹⁵. Both dynamic and static quenching processes are explained using Stern-Volmer (S-V) equation and Stern-Volmer plots (S-V plots). The fluorescence intensities before (I_0) and after (I) adding quencher are related as

related to positive S-V plot is pursued using modified S-V equations¹⁵. Negative deviation of S-V plots (downward curvature) is also reported by many researchers^{10,13,14,16}. Many Reasons such as heterogeneity of the system, selective quenching, hydrogen bond complex formation, occurrence of reverse reaction in photochemical process etc are identified for the negative deviation. Quenching data which are expected to arise from one of these reasons may be represented by modified 'Stern – Volmer' equation or Lehrer equation^{10,17,18} given below.

In the present paper we report the quenching of a newly synthesized Quinolin-8-ol (QO) in toluene and butanol solvents. Aniline is used as quencher. Negative deviation in S-V plot is examined using above mentioned equations.

Experimental methods

A newly synthesized Quinolin-8-ol (QO) whose molecular structure is as shown in fig.1 is quenched by doubly distilled quencher namely aniline in solvents of toluene and butanol. Spectroscopic grade solvents are obtained from Merck India. The absorption spectra are measured at room temperature using double beam UV-VIS Spectrophotometer (Model: Shimadzu UV-1800) with a wavelength accuracy of 0.5nm. The concentration of the solution is maintained at 1×10^{-5} M in order to avoid self-absorption process and aggregation formation. The solutions are prepared by varying the quencher concentration (0.00M – 0.10M). The excitation wavelength is chosen based on maximum absorbance and it is found to be 320 nm. The fluorescence spectra are recorded using fluorescence spectrophotometer (Model: Hitachi F-2700) with standard quartz cuvettes at room temperature with perpendicular geometry. Typical emission spectra Quinolin-8-ol (QO) in toluene solvent with different quencher concentrations is shown in fig. 2. Fluorescence lifetimes (τ_0) of the solute without quencher are measured in toluene solvent using TCSPC nanosecond fluorescence lifetime spectrometer Photophysics model of TCSPC nanosecond fluorescence spectrometer HORIBA FLUOROLOG K U Dharwad, Karnataka, India.

RESULTS AND DISCUSSIONS

The fluorescence study is carried out in solvents of toluene and butanol. Emission intensity is more when the sample is dissolved in butanol. This indicates that solute exhibits more fluorescence nature in polar atmosphere. Fluorescence intensity is further quenched with the addition of quenchers viz, aniline. S-V plots are constructed using equation (1) and are given in fig.3. They are nearly linear in the lower concentration range and show negative deviation at higher quencher concentration (0.06-0.10M) with intercept equal to unity. The

$$k_d = 4\pi N' DR$$

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

linearity in the lower concentration range is ascribed to diffusion limited dynamic quenching. The negative deviation in the plot may be due to any of the reasons as mentioned earlier. One of the reasons is the presence of two fluorophores with different accessibility to quencher. For example, in the case of fluorescing tryptophans in structured proteins, the tryptophan residues are buried within the protein and as such give rise to heterogeneous quenching. Some residues which are involved in the binding site of a ligand acting as a quencher are readily available for quenching; others which are not involved in binding may not be quenched at all. This condition of heterogeneous population of fluorophores is seen in the S-V plot as a negative deviation from the linearity¹⁴. Modified S-V plots or Lehrer plots obtained using equations (3) are as shown in fig. 4. The value of f is nearly equal to 1 ($f \leq 1$) and S-V constant (K_{SV}) varies between 72.1 M^{-1} to 53.6 M^{-1} in the case of toluene and butanol respectively. The proper interpretation of quenching data and the determination of K_{SV} require identification of the quenching mechanism. From the absorption and emission spectra the negative deviation in the S-V plot rules out the formation of non-fluorescent complex between the aniline and Quinolin-8-ol since no new characteristic peak is observed. Even if static quenching occurs due to the presence of aniline molecules in the vicinity of fluorophores at the time of excitation, it is expected to be very weak because K_{SV} values calculated from the linear fit of I_0/I versus lower part of $[Q]$ are relatively small compared to K_{SV} calculated from equation (3) in most of the cases. These values are presented in Table.1. The bimolecular quenching rate parameter k_q is calculated using the equation $K_{SV} = k_q \tau_0$ with K_{SV} representing the slope of Lehrer equation and τ_0 being the lifetime of the fluorophore in the absence of quencher. The higher values of k_q suggest the efficient quenching of fluorescence. The efficient quenching may take place before the complete formation of an exciplex/conformer and the diffusion limited quenching is expected to increase with the decrease in solvent viscosity. In order to understand the effect of solvent viscosity (η), the diffusion-limited rate constant k_d is estimated using the equation

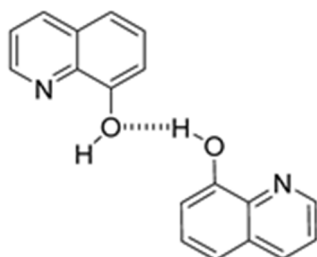
$$(6)$$

radii (in Å) of the solute (S) and the quencher (Q), respectively¹⁹. The diffusion coefficients are calculated using Stokes-Einstein equation²⁰

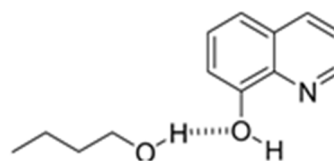
$$D = \frac{kT}{a\pi\eta R} \quad (7)$$

Here k is Boltzmann constant, T is the absolute temperature, η is the solvent viscosity (in cP), R is the radius of solute or quencher molecule as the case may be and 'a' is Stoke – Einstein number. Its value is 6 for solute and 3 for quencher. The calculated values of k_q and k_d in both the solvents are given Table.1. In case of toluene, it is found that k_q is $20.61 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ and k_d is $20.51 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ whose viscosity is 0.496cP and $15.34 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ and $5.13 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ in butanol whose viscosity is 1.982cP. This indicates that both k_q and k_d decreases with the increase in solvent viscosity. The diffusion-limited rate constant k_d and bimolecular quenching rate k_q increases with the decrease in viscosity of the solvents. Hence it is surmised that the quenching mechanism is not solely controlled by material diffusion²¹. Also with increase in dielectric constant K_{sv} increases representing charge transfer character of the excited complex. Observation of negative deviation in S-V

plots for Quinolin-8-ol is due to existence of the solute initially in two different conformers at ground state. In Type I there will be intermolecular hydrogen bonding between hydrogen atom of one Quinolin-8-ol molecule with oxygen atom of other Quinolin-8-ol molecule as shown in Scheme I. In type II there is also a possibility of intermolecular hydrogen bonding with the alcoholic solvent butanol. The formation of intermolecular hydrogen bonding with butanol is as shown in Scheme II. Due to the presence of stable intermolecular hydrogen bonding of these compounds between themselves in toluene where less viscosity favours Quinolin-8-ol movement and hydrogen bonding with alcohol molecule in butanol make the compound to exist as two different conformers. The existence of two conformers may be the reason for negative deviation in S-V plots. These observations may serve as useful information in designing the newer molecules as sensors.



Scheme -I



Scheme II

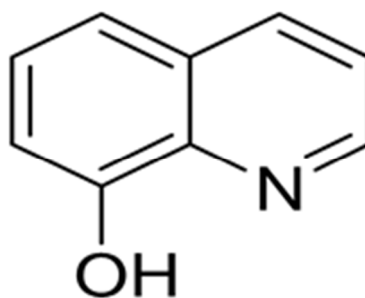


Figure 1
Molecular structure of Quinolin-8-ol

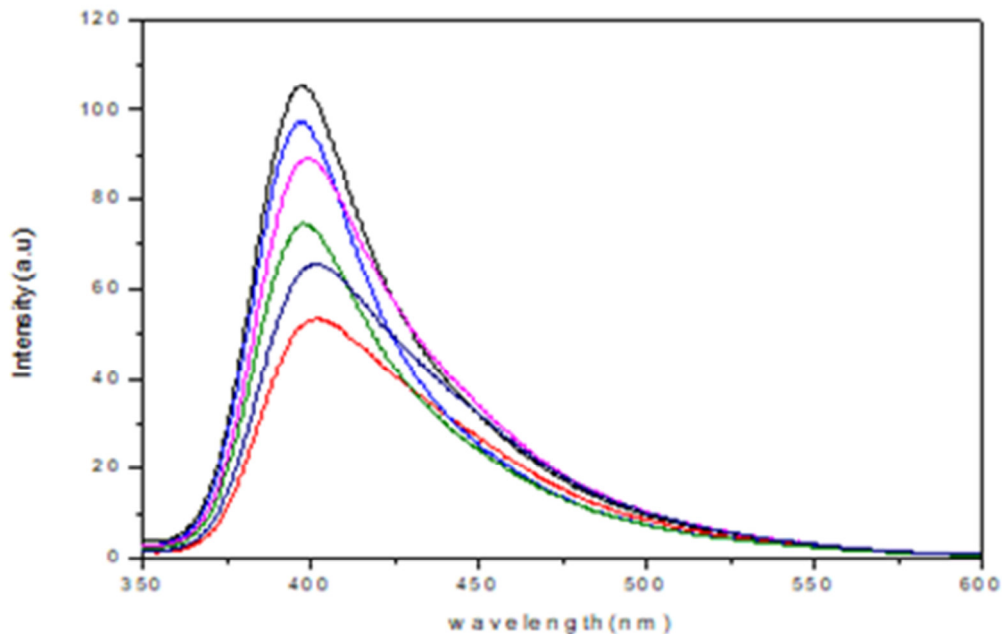


Figure 2
Emission spectra of the solute in Toluene at fixed concentration of $1 \times 10^{-5} M$ with varying quencher concentration of aniline

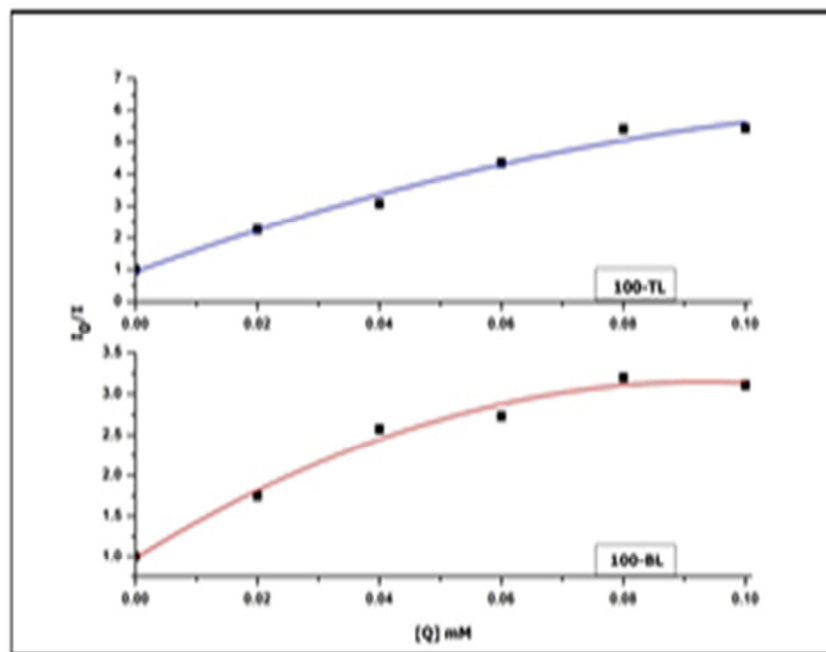


Figure 3
Stern-Volmer plots of I_0/I versus $[Q]$ exhibiting negative deviation in toluene and butanol

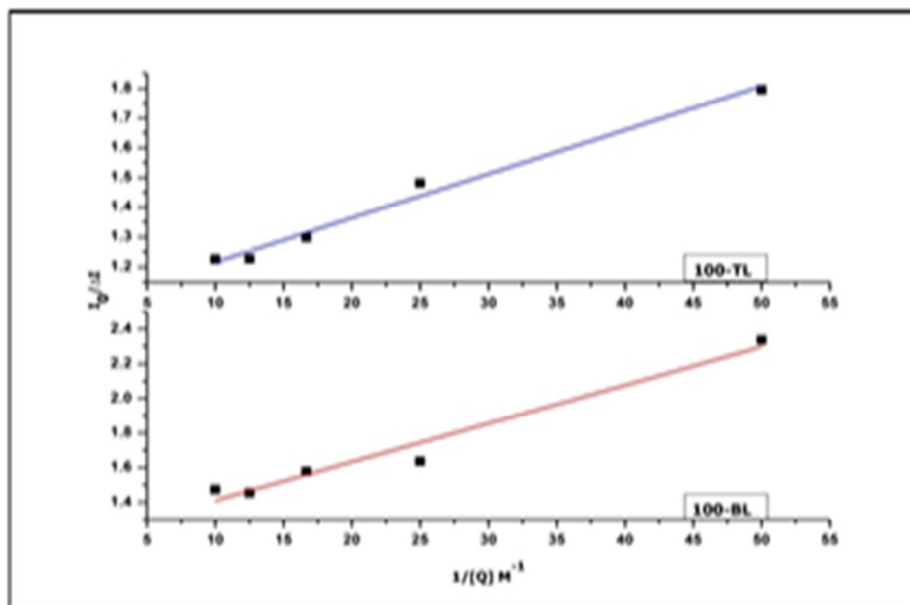


Figure 4
Modified linear Stern–Volmer plots of $I_0/(I_0-I)$ versus $1/[Q]$ in toluene and butanol with Aniline as quenchers

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)

| Solvents | η (cP) | Dielectric constant(ϵ) | f | $^{LH} K_{SV}$ (M^{-1}) | $^{LSV} K_{SV}$ | $k_q \times 10^9$ ($M^{-1}s^{-1}$) | $k_d \times 10^9$ ($M^{-1}s^{-1}$) |
|--------------------|-------------|-----------------------------------|--------|-----------------------------|-----------------|--------------------------------------|--------------------------------------|
| 100% toluene (TL) | 0.496 | 2.38 | 0.9365 | 72.147 | 41.066 | 20.61 | 20.51 |
| 100% 1butanol (BL) | 1.982 | 17.565 | 0.8407 | 53.677 | 41.268 | 15.34 | 5.13 |

CONCLUSIONS

Fluorescence quenching of Quinolin-8-olby aniline in toluene and butanol solvent 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 Quinolin-8-ol continue to draw the attention in designing newer molecules for sensing applications.

AUTHOR CONTRIBUTION STATEMENT

Author V.T. Muttannavar is the research student who's contribution is conduction of experiment and preparation of manuscript.

Author Raveendra. Melavanki is the corresponding Author of this manuscript and has reviewed the article.

Author Kalpana Sharma contribution is to calculate different quenching parameters.

Author Raviraj. Kusanur is the chief chemist who's contribution is to supervise the synthesis and yield and checked all the chemical tests.

Author P. Bhavya is the research student who's contribution is to synthesize the title molecule

Author L. R. Naik is research supervisor of first author and helped in preparation of the manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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