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Study of Fluorescence Quenching of Coumarin Dye by Dimethyl Aniline In Binary Solvent Mixtures - A negative deviation

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ABSTRACT

Coumarin derivatives are extensively investigated in terms of their photo physical properties to understand excited state in regard to understand and innovate molecules. In this article we study steady state quenching of fluorescence of a coumarin derivative namely 3-Hydroxy-3-[2-oxo-2-(3-oxo-3H-benzo[f]chromen-2-yl)-ethyl]-1,3-dihydro-indol-2-one (3HBCD) in binary mixture of acetonitrile and 1,4 dioxane. Dimethylaniline is used as quencher. A negative deviation is seen with modest quencher concentration in the Stern-Volmer (S-V) plots. The quenching ability of Dimethylaniline in reference to aniline is more due to its higher ionization energies.

Keywords : Fluorescence Quenching; Negative deviation; Coumarin derivatives; solvent mixtures.

I. INTRODUCTION

Coumarin and its derivatives have been well famous for their anti-tumor [1-2], anti-coagulant, anti-viral, anti-oxidant, anti-inflammatory vaso relaxant [3-4], anti-microbial [5] and enzyme inhibition properties [6-9]. These compounds are specifically known to exert an anti-tumor effect and can cause significant changes in the regulation of the immune response, cell growth and differentiation [10]. Various fields like biology, medicine and electronics utilizes Coumarins are widely used due to their high fluorescence overall response. Solvent polarity, solvent viscosity and pH of the solution greatly affect fluorescence properties of coumarin as revealed by studies [11-16]. Fluorescence quenching play a sensory role by molecular identification which serves numerous categories of beneficiary including biology as well as chemical analysis. In biochemistry, fluorescence quenching has been used for a) investigation of accessibility and localization of probes in a membrane or protein [17-19] b) analyzing accessibility of fluorophores to quenchers c) detecting presence of multiple emitting species and many more. Many investigators found interest towards florescence quenching due tremendous novel application [12, 13, 27-30]. Fascinating applications and properties of coumarins and bioanalytical applications of fluorescence quenching inspired us for the present investigation.

In present work, 3HBCD is investigated for its quenching interaction with aromatic amine Dimethylaniline as external quencher. Effect of solvent polarity and viscosity is analysed by doing quenching analysis different solvent mixtures of acetonitrile and 1, 4-dioxane at room temperature.

Negative deviation in S-V plot is observed in all solvent.

II. THEORY

Among various reasons fluorescence quenching of a sample externally added molecule called quencher gives immense information on surrounding medium.

Static quenching (contact quenching), dynamic quenching (collisional quenching) and Fluorescence Resonance Energy Transfer are three main quenching mechanisms that are caused by external molecule. Both of static and dynamic 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

$$I0/I=1+KSV[Q] \quad (1)$$

Here KSV is known as S-V constant. Among linear and positive deviations in S-V plots some of the researchers have also reported a negative deviation in S-V plots (downward curvature) [18-22]. Many Reasons such as heterogeneity of the system, selective quenching, hydrogen bond complex formation, occurrence of reversible photochemical process etc are recognized for the negative deviation [18]. Quenching data arising due to one of these reasons may be analyzed using linear form Lehrer equation [20] given below.

$$I0/\Delta I=1/f+1/(f\,KSV\,[Q]) \quad (2)$$

Here $\Delta I = I0 - I$. A plot of $I_0/\Delta I$ versus $1/[Q]$ is linear with 1/f as intercept and KSV = intercept/slope. Negative deviation in S-V plot is inspected using above mentioned equations. f is the fraction of accessible fluorophores.

III. EXPERIMENTAL DETAILS

3HBCD is synthesized by standard methods [23,24] whose molecular structure is as shown in fig.1.

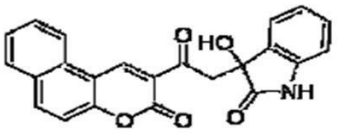


Fig 1: Molecular structure of 3HBCD

The doubly distilled aromatic amine Dimethylaniline is used as quencher. Spectroscopic grade solvents are obtained from S-D-Fines Chemicals Ltd., India. Sample concentration is kept at
1 $x10^{-5}$ M. Mole concentration of quencher is varied from 0.00M – 0.10M for the solution prepared. Absorption spectra are measured at room temperature using double beam UV-VIS Spectrophotometer (Model: Shimadzu UV-1800) where maximum absorbance is found to be 389 nm and the same is used as excitation wavelength. The fluorescence spectra are recorded using fluorescence spectrophotometer (Model: Hitachi F-2700) by keeping operating voltage 400V and the slit width at 5nm. Typical emission spectra of the sample in 20% 1, 4dioxane + 80% acetonitrile without and with different quencher concentrations are shown in fig. 2.

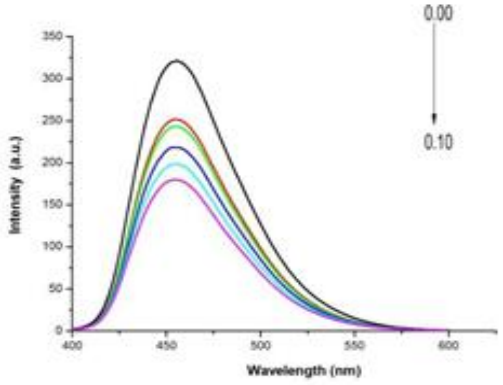


Fig 2: Emission spectra at 20% DX+ 80% CAN for fixed concentration of 1×10^{-5} M with varying quencher concentration of Dimethylaniline

Fluorescence lifetimes (τ0) measurement facility is availed at IISc, Bangalore using Photo physics model of TCSPC nanosecond fluorescence spectrometer HORIBA FLUOROLOG. Lifetime is measured without quencher by exciting the at 389nm.

IV. RESULT ANALYSIS

The fluorescence quenching study is ACN and 1,4DX at room temperature. External quencher Dimethylaniline (DMA) is added. Quenching data is analysed by the most convenient and reliable method i.e., by plotting S-V plots [9-22] using equation (1) and is shown in fig3.

The plots are almost linear in the lower concentration range and at higher quencher concentration (0.06-0.10M), show deviation towards x-axis with unity as intercept. The quenching data related to negative deviation S-V graph is handled by using equation (3) and modified S-V plots are presented as in fig.4.

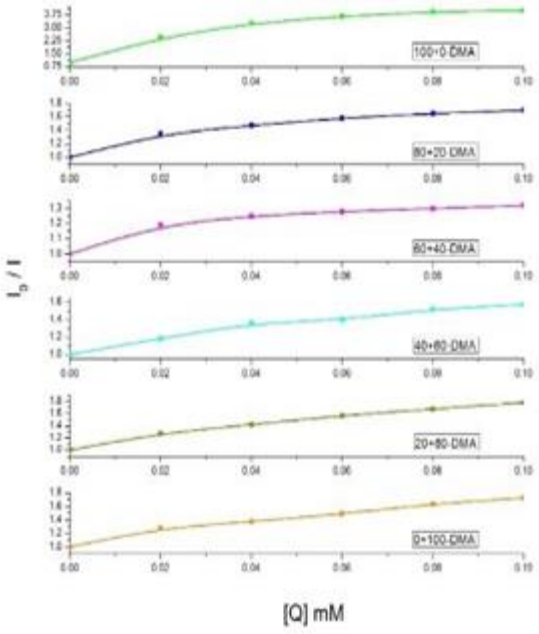


Fig 3: Stern–Volmer plots exhibiting negative deviation in different solvent mixtures

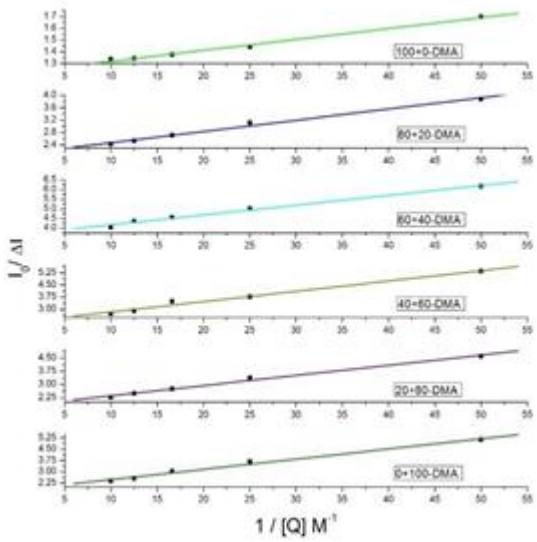


Fig 4: Modified linear Stern–Volmer plots of I0/(I0 –I) versus 1/[Q] in different solvent mixtures with Dimethylaniline

The obtained straight line plots has with f^{-1} as intercept where f lies between 0 and 1 ($0 \leq f \leq 1$) and ^{LH}KSV= intercept/slope.

The bimolecular quenching rate parameters kq was determined with the substitution of the experimentally measured values of ^{LH}KSV and τ0 in the relation kq= ^{LH}KSV/ τ0 (Table 1). From the plots I0/I versus [Q] (i.e. for lower quencher concentration region a linear plot is made) we have calculated S-V constant ^{LH}KSV to analyse different quenching parameters to categorize the quenching mechanism. These values are also tabulated in Table 1.

Overall static quenching is ruled out due to no changes in absorption and emission spectrum of the fluorophore upon addition of quencher and since values ^{LSV}KSV measured from the linear fit are relatively small compared to ^{LH}KSV (Table 1).

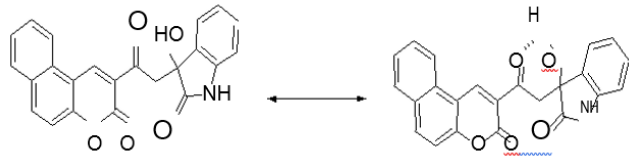
A negative deviation in S-V Plots showed at higher concentrations (from 0.06M to 0.1M) of quencher can be due to the occurrence of a reverse reaction in the photochemical process or the existence of two fluorophores with different accessibility to quencher. photochemical process is ruled out as no new

fluorescence emission band is observed on adding quencher into the mixture solutions of 3HBCD. Hence negative deviation in Fig. 3 would be due to the presence of 3HBCD in two different conformers.

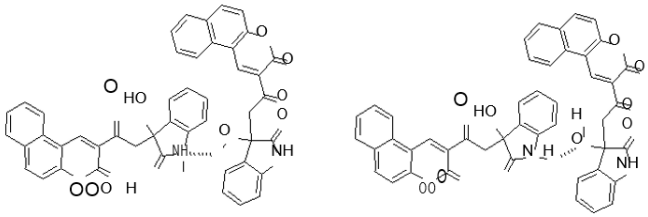
It can proposed that 3HBCD occurs in possibly two different conformers which are accessible for fluorescence quenching in different fractions. Conformer (I) may be due to possibility of intra-molecular hydrogen bonding between the hydrogen atom of hydroxyl group and oxygen atom of the carbonyl group (as shown in Shcme-1). Conformer (II) exists due to inter -molecular hydrogen bonding between –OH group of one coumarin molecule with lactam –NH group of the other coumarin molecule as shown in Scheme-2. Here one of fractions of accessible fluorophores is assumed be conformer (I) due its highest stability[18].

Table 1: Quenching parameters of 3HBCD in solvent mixtures of ACN and DX with fraction of accessible fluorophores (f), S-V constant (KSV), bimolecular quenching rate parameter (kq) and diffusion rate constant (kd)

Solvents	Viscosity η (cP)	Dielectric constant ε	Life time τ (ns)	f	$^{LH}K_{SV}$ (M^{-1})	$^{LSV}K_{SV}$ (M^{-1})	kq 10^9 ($M^{-1}s^{-1}$)	kd 10^9 ($M^{-1}s^{-1}$)
100% Acetonitrile (ACN)	0.34	36	0.35	0.55	26.7	9.35	74.51	31.34
20(DX)+80(ACN)	0.34	29.22	0.40	0.56	30.95	9.5	75.56	30.41
40(DX)+60(ACN)	0.40	22.44	0.62	0.45	34.76	9.02	55.94	26.2
60(DX)+40(ACN)	0.53	15.66	0.61	0.47	58.3	11.8	94.64	20.2
80(DX)+20(ACN)	0.77	8.88	0.73	0.27	71.5	6.22	97.57	10.8
100% 1,4 Dioxane (DX)	1.17	2.1	1.11	0.81	132.1	56.6	118.3	9.11



Scheme-1: Intra-molecular hydrogen bonding formation.



Scheme-2: Inter molecular hydrogen bonding formation between –OH group and –NH group.

By plotting the data according to equation (3) it is observed that S-V constant varies from 26.70 M^{-1} to 132.10 M^{-1} (Table1). Also from fig 4, intercept yields fraction of accessible fluorophore f (i,e. for one of the conformer of 3HBCD) to be < 1 which suggests that both the conformers are partially available for quenching. S-V constant obtained in our previous paper [18] by using aniline as quencher, it is found to be less than the values obtained by using dimethylaniline. Hence the quenching ability of Dimethylaniline in reference to aniline is more due to its higher ionization energies.

The high values suggest efficient quenching of fluorescence. Evaluated values of kd using the formula

$$kd = 4\pi N' DR \qquad (5)$$

is made according to standard procedure and are tabulated in Table 1. Viscosities of the solvent mixtures are obtained from literature [25].

As seen here values of kq is greater than kd suggesting diffusion limited reaction. But from fig 5 ^{LH}KSV is found to be increasing with the increase in viscosity of solvent mixture. Hence it can be inferred

from Inverse dependency of kd on viscosity of the solvents, that quenching is not merely controlled by material diffusion [20]. The value of $^{LH}K_{SV}$ inversely dependent on dielectric constant of the medium. A plot of ^{LH}KSV vs. ε is obtained as in fig 6 which may suggest that the reacting species are of opposite charge. With decrease in acetonitrile percentage in the medium dielectric constant decreases, which destabilizes the re-acting species, and increases the reactivity and the rate of the reaction is enhanced [26].

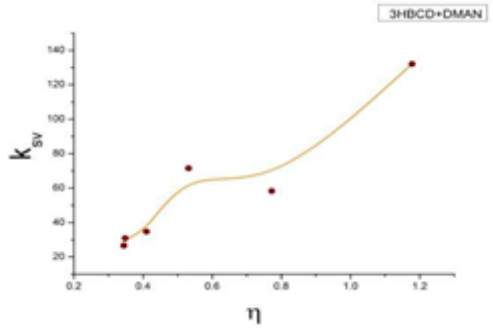


Fig 5: Plot of S-V constant ^{LH}KSV mixtures viscosity

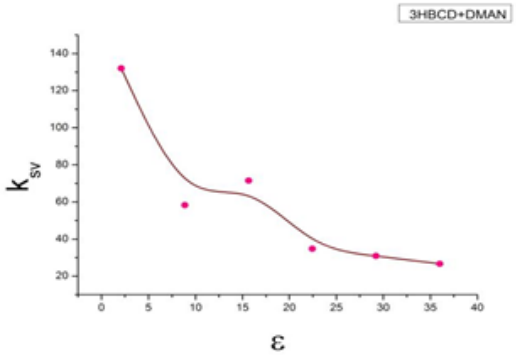


Fig 6: Plot of S-V constant ^{LH}KSV constant of solvent mixtures

V. CONCLUSIONS:

Aprotic binary mixtures are used to study fluorescence quenching mechanism of 3HBCD, using Lehrer equations. Aromatic amine Dimethylaniline is used as external quencher.

Large value for bimolecular quenching rate is obtained representing high quenching efficiency.

With the increase in solvent viscosity diffusion limited rate constant decreases representing that dynamic quenching is not strictly controlled by material diffusion.

Quenching ability of Dimethylaniline in reference to aniline is more due to its higher ionization energies. The presence of 3HBCD in two ground-state conformers due to intermolecular and intra-molecular hydrogen bonding is indicated by negative deviation from the normal Stern–Volmer relationship.

Overall quenching of the fluorescence of 3HBCD is affected considerably by solvent polarity, viscosity of the media and ionization energies of quencher.

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