Fluorescence Quenching Studies on Some Substituted Amines by Phenolphthalein

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Abstract : *Fluorescence spectroscopy has assumed a major role in analysis, particularly in the determination of trace contaminants is our environment, industries and bodies, because for applicable compounds fluorescence gives high sensitivity and high specificity. In this present work the quenching experiment was done in two different solvents, Diethyl ether and Methanol. Phenolphthalein is taken as the quencher. The fluorescers used for the quenching experiments are some substituted amines [Ethylamine, Diethylamine, Diethylene triamine]. Experimental solutions were prepared by adding fixed aliquot of stock solution of fluorescer to 5 ml of the solvent, so that concentration of resulting solution is about 10^{-5} M. Quencher solutions were added to these solutions. The fluorescence spectra of fluorescers (Amines) were measured with JASCO model FP-550 spectrofluorometer, operating with 150W Xenon lamp as light source. The various parameters like Stoke's shift. Ionization potential, electron affinity, molar extinction coefficient and solvent parameter are calculated and tabulated. Based on the observations from the results obtained, it was found that the Phenolphthalein is the best quencher for amines.*

Key words - Fluorescence, amines, Phenolphthalein, quenching.

I. Introduction

The analytical technique based on the absorption of infrared, visible and ultraviolet light has found extensive applications in physics. Many solutions when irradiated with visible or ultraviolet light, not only absorb this light, but re-emit light of different wavelengths. This effect is known as fluorescence and its exploitation opens up possibilities in the discrimination and accurate determination of many substances in very dilute solutions. Fluorometric methods can detect concentrations of substances as low as one part in 10 billion, sensitivity 1000 times greater than that of most spectrophotometric methods.

This increased sensitivity is because in fluorescence, the emitted radiation is measured directly and can be increased or decreased by altering the intensity of the exciting radiant energy.

The temperature of the sample has a considerable effect upon the fluorescence. The fluorescent yield is increased by cooling the sample. The pH of the sample solution is critical for many substances. In some instances highly fluorescent materials becomes completely non-fluorescent even when the pH is altered by few units [1].

A molecule during electronic excitation goes to a higher energy state. The excited molecule may undergo a photochemical reaction or it may return to the ground state by losing its energy. As given by Jablonski diagram[2] there are many unimolecular Photophysical processes available to the excited molecule for the dissipation of the excitational energy. One such photophysical pathway is the radiative fluorescence emission. This radiative emission may be quenched by a bimolecular quenching process. Hence the quenching process is defined as one, which completes with spontaneous emission process and there by shorten the lifetime of the emitting molecule. Quenching of fluorescence gives us much information about bimolecular interactions between excited molecules and quenchers[3].

The common types of quenching observed are,
1. Temperature quenching
2. Oxygen quenching
3. Concentration quenching
4. Impurity quenching

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5. Self-quenching
6. Chemical quenching
7. Static quenching and
8. Dynamic quenching

Bimolecular processes such as quenching either by molecules of the same thing (Self quenching) to by added substances inhibit emission because frequency or bimolecular collisions in gases as well as in solutions ($K= 10^{10}$ sec^{-1}) can compete with fluorescence emission for a molecule in the excited state, direct contact between two interacting molecules may not be necessary and effective cross section for optical collisions may be much larger than those for kinetic collisions. The optical collision theory cannot be applied to liquid solution because of cage effect. In solutions two solute molecules close together and hemmed in by solvent molecules make many repeated collisions with each other before drifting away. This is called an encounter, usually involving 20-100 collisions.

Quenching mechanism has wide applications as a mechanistic tool in photochemical reactions and in photochemical synthesis. Hammond and co-workers [4-6] have developed a very useful technique using quenching process which involves energy transfer between molecule for synthetic and mechanistic studies in organic photo-chemistry. This work was carried out to study the fluorescence quenching of some substituted amines by phenolphthalein.

The quenching experiment was carried out in two solvents of different polarity and viscosity to find outstoke's shift, molar extinction co-efficient, ionization potential, electron affinity and solvent parameter (Z).

The quenching experiment was done in two different solvents, Diethyl ether and Methanol. Phenolphthalein is taken as the quencher. The fluorescers used for the quenching experiments are some substituted amines [Ethylamine, Diethylamine, Diethylene triamine].

Phenolphthalein is a colourless solid. It is insoluble in water, but dissolves in alkalis to form deep red solutions. This is due to the formation of a disodium salt, the ion of which is coloured because of resonance. When excess of strong alkali is added, the solution of phenolphthalein becomes colourless. Phenolphthalein is used as indicator in acid-base filtrations [7]. It is an extremely powerful laxative and this accounts for its widespread use as a denaturant for laboratory alcohol.

Ethylamine is found in foods, drink, tobacco smoke, also produced by marine algal. It is highly toxic and irritant. It is extremely flammable [8]. It is used in manufacturing of resins, rubber etc. It has strong ammonia like odour and a bitter taste.

II. Materials And Methods
2.1 Materials
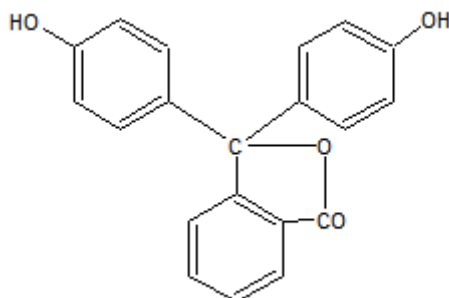The materials used for our quenching experiments are,

2.1.1. Quencher
Phenolphthalein

2.1.2. Fluorescers
(i)   Ethylamine
(ii)   Diethylamine and
(iii)   Diethylene triamine

2.1.3. Solvents
(i)   Diethylether and
(ii)   Methanol

Quencher: Phenolphthalein[7]

$$CH_3 - CH_2 - NH_2$$

Fluorescer (i): Ethylamine[8]

$$C_2H_5 - NH - C_2H_5$$

Fluorescer (ii): Diethylamine[8]

$$CH_2 - CH_2 - NH_2$$

NH

$$CH_2 - CH_2 - NH_2$$

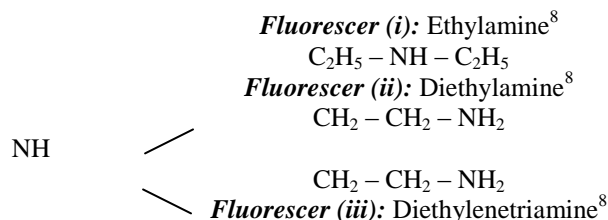***Fluorescer (iii):*** Diethylenetriamine[8]

Figure 2.1 Structure of the Quencher and Fluorescers

2.2 Sample Preparation

The quenching experiment was done in two solvents i.e., Diethyl ether and Methanol. The stock solution of phenolphthalein in each solvent was prepared.

The desired quantity of the quencher (phenolphthalein) was weighed and put into 100 ml volumetric flask, dissolved in the solvent Diethyl ether and made up to the mark to constitute usually a 0.001 M solution. In a similar manner, the quencher stock solution was prepared in Methanol. The stock solutions of fluorescers (0.01 M concentration) in each solvent were prepared.

Experimental solutions were prepared by adding fixed aliquot of stock solution of fluorescer to 5 ml of the solvent, so that concentration of resulting solution is about 10^{-5} M. Quencher solutions were added to these solutions. The volume of the quencher solution added depends on its concentration and its quenching efficiency. The fluorescence measurements were made immediately after mixing the solution. Fluorescent intensities were measured at their emission maximum for each solvent.

2.3 Instrumentation

The fluorescence spectra of fluorescers (Amines) were measured with JASCO model FP-550 spectrofluorometer, operating with 150W Xenon lamp as light source. The wavelength range is from 0-850 nm for every 50 nm on the scale. But in practical, the spectra can be measured only in the range from 220-750 nm. The spectral bandwidth is 10 nm with a scanning speed of 50 nm/min. The sample container is made up of fused quartz cell. The detector used in the instrument is the R-372 photomultiplier tube and the spectra were recorded using a JASCOJC –50 desk top recorder.

The light from the light source L passes through the window W_1 is condensed via the spherical mirror M_1 onto the enhance slit S_1 of the excitation side monochromator, and decomposed into monochromatic rays by the concave grating G_1. These monochromatic rays are made intermittent ray by the chopper CH and part of them are reflected by and the other part pass through the quartz plate B.S. The reflected rays are scattered by the diffuser D.G. and detected by the detector PM_1. The rays coming through B.S are condensed onto the sample by the triodal mirror M_2. The fluorescence emitted from the sample is condensed onto the entrance slit of the fluorescence–side monochromator via the troidal mirror M_3 and plane mirror M_4, and decomposed into monochromatic rays, which pass through the exit slit S_4 and are then condensed onto the detector PM_2 by the spherical mirror M_5.

The signals detected by the two photomultiplier tubes PM_1 and MT_2 are respectively amplified. The signal which has been detected by the excitation-side detector PM_1 and then amplified is transmitted into the control circuit for the high voltage applied on the photomultiplier and used as a monitor under which impressed voltages are fed back to the two photomultiplier tubes so that the output of PM_1 may be kept constant. This system determines the ratio of the fluorescence-side to excitation-side signal, thereby eliminating noises due to fluctuation of the light source. Since the excitation side signal is monitored for keeping the PM_1 output constant, excitation spectra obtained have already been through energy compensation.

The absorption spectra of donors (Amines) were measured using a JASCO-UVIDEC-650 spectrophotometer. The source used is a tungsten or deuterium lamp for ultraviolet irradiation on the sample. The wavelength range of the apparatus is 195-900nm with an accuracy of ± 0.3nm. The sample cell is made up of glass or quartz. Photomultiplier tube acts as a detector and the resultant spectrum is recorded using a heat sensitive graphic printer.

2.4 Methods

The absorption spectra of the fluorescers were taken against a reference of the same solvent containing the same concentration of quencher. The excitation wavelength was usually chosen at absorption maxima. As far as practicable, narrow excitation and emission slit widths were chosen. The excitation spectra were taken usually at fluorescence maxima. While measuring fluorescence intensity at a fixed wavelength for series of solutions, necessary correction was for small variation of absorbance at the exciting wavelength. Absorption and fluorescence spectra were taken at the beginning as well as at the end of a given set of experiments and they were matched in order to check for the possibility of any photoreaction during the course of experiment.

Absorption spectra of the experimental solutions were scanned for all solutions. One of the matched pair of quartz cell (1 cm in width) was rinsed with the reaction mixture, then filled with the same and placed in the cell compartment of the spectrophotometer. The other cell contained the solvent as the reference; the absorbance reading was taken for every acceptor solution. The donor spectra were recorded in each solvent. The spectra of CT complex in different solvents were also recorded by taking the acceptor and donor in 1:1 ratio.

The ratio of $[I_0/I]$ was determined and they were plotted against quencher concentration $[Q]$. The correlation co-efficient and the slope of the plot were determined from regression analysis. The Stern-Volmer constant K_{sv} was obtained from the slope calculated from regression analysis.

III. Results And Discussion

The concentration of fluorescer solution was kept constant (0.01M) in each experiment, by adding fixed aliquot of stock solutions prepared in the respective solvents. Quencher in the respective solvent was added to the above solution in small volumes (0.1ml to 0.5ml) and the fluorescent intensities were measured. Care had been taken to measure the fluorescent intensities immediately after mixing the solution.

The various parameters like Stoke's shift. Ionization potential, electron affinity, molar extinction coefficient and solvent parameter are calculated and tabulated in table 3.1.

Table 3.1 The various parameters like Stoke's shift. Ionization potential, electron affinity, molar extinction coefficient and solvent parameter.

Solvents	Donors	Energy, Ionization Potential and Electron Affinities of Charge Transfer Complexes				Stoke's Shift			Molar Extinction Co-efficient	Solvent Parameter (z value in K cal / mole)
		λ_{ct} max (nm)	hn_{ct} (ev)	$I_D$ev	E_A	$\square_{abs}$ (nm)	$\square_{flu}$ (nm)	Stoke's Shift $\square\square$ (cm^{-1})	$01 \times \varepsilon^5$	Z value $\acute{}$ 10^{10}
Diethyl ether	Ethylamine	430	2.889	8.862	1.2487	280	330	5411	280	66.488
	Diethylamine	450	2.761	8.701	1.3803	300	350	4761	300	63.533
	Diethylenetriamine	455	2.73	8.662	1.4115	295	360	6120	295	62.835
Methanol	Ethylamine	465	2.672	8.59	1.4716	295	390	8257	295	61.483
	Diethylamine	470	2.647	8.559	1.4966	300	390	7692	300	60.829
	Diethylenetriamine	460	2.705	8.631	1.437	280	380	9398	280	62.152

3.2. Quenching in Diethyl Ether

Absorption spectrum of the quencher in diethyl ether and ethylamine in diethyl ether are given in below figure respectively.
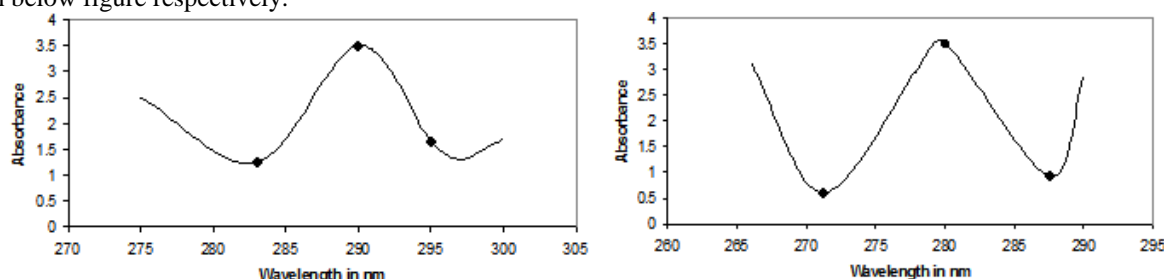

Figure 3.1 Absorption spectra of the quencher in diethyl ether and ethylamine in diethyl ether

The fluorescent intensities of ethylamine without phenolphthalein (quencher) and with different concentrations of phenolphthalein were measured at 330nm. The excitation wavelength used was 280nm. The phenolphthalein concentration was varied from 0.1ml to 0.5ml. The typical fluorescence spectrum of ethylamine in diethyl ether without phenolphthalein and with various concentrations of phenolphthalein is given in Fig. 3.2.

Table 3.2 Fluorescent intensity ratios of amines at different phenolphthalein concentrations in diethyl ether (I_0/I)

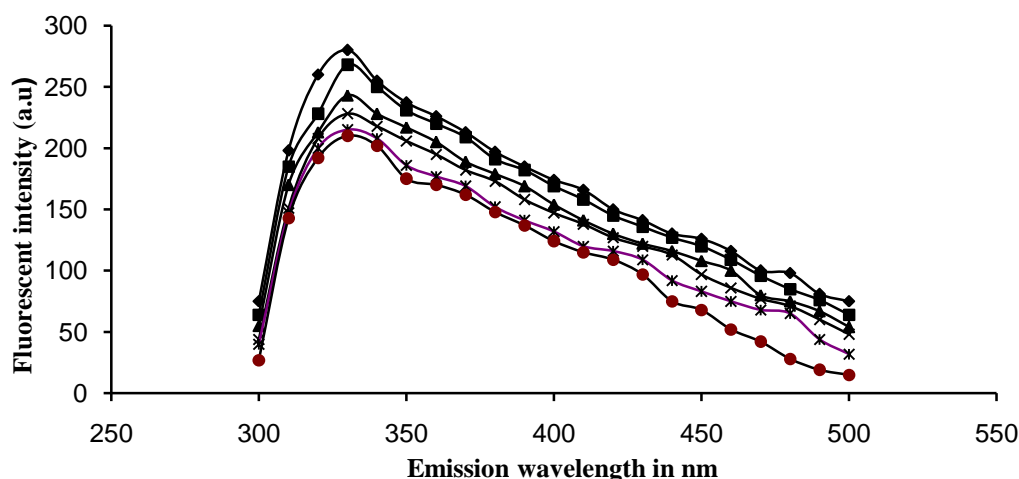| S. No.	[Q] $\times 10^{-5}$ M / L	Fluorescent intensity ratios of amines at different phenolphthalein concentrations in diethyl ether [I_0/I]		
		Ethylamine	Diethylamine	Diethylenetriamine
1	2	1.0447	1.089	1.1814
2	4	1.1522	1.176	1.393
3	6	1.228	1.375	1.6667
4	8	1.3023	1.629	1.8543
5	10	1.3333	2.075	2.568

Figure 3.2 Fluorescence spectrum of ethylamine in diethyl ether without phenolphthalein

The quenching ratios (I_0/I) for ethylamine, diethylamine and diethylenetriamine are plotted against quencher concentration (Q). From the plotted graph Regression analysis for Stern-Volmer plot has been carried out and the values of regression coefficient (r) are tabulated in table 3.4. The slope of the Stern-Volmer plot gives Stern-Volmer constant K_{sv}.

Table 3.4 Regression coefficient (r) for the quenching ratios (I_0/I) for ethylamine, diethylamine and diethylenetriamine against quencher concentration (Q)

S. No.	Name of the amine	Regression Coefficient	Stern-Volmer constant Ksv
1.	Ethylamine	0.9834	0.363 X 10^3
2.	Diethylamine	0.9384	0.12125 X 10^3
3.	Diethylenetriamine	0.9614	0.1632 X 10^3

Table 3.5 Fluorescent intensity ratios of amines at different phenolphthalein concentrations in Methanol (I_0/I)

S. No.	[Q] $\times 10^{-5}$ M / L	Fluorescent intensity ratios of amines at different phenolphthalein concentrations in Methanol [I_0 / I]		
		Ethylamine	Diethylamine	Diethylenetriamine
1	2	1.1718	1.1088	1.5771
2	4	1.3888	1.2538	2.1562
3	6	1.5957	1.4424	3.1362
4	8	2.3437	2.0375	3.8873
5	10	3.0000	2.3970	5.0181

Table 3.6 The values of regression coefficient (r) for the quenching ratios (I_0/I) for ethylamine, diethylamine and diethylenetriamine against quencher concentration (Q) in methanol.

S. No.	Name of the amine	Regression Coefficient	Stern-Volmer constant Ksv
1.	Ethylamine	0.9633	0.2305 X 10^3
2.	Diethylamine	0.9695	0.1680 X 10^3
3.	Diethylenetriamine	0.9948	0.4306 X 10^3

The Stern-Volmer plots for all the quenching processes are linear and the regression analysis of all the curves gives a very good correlation. The regression co-efficient(r) is above 0.96 for all curves. The linearity of Stern-Volmer plot shows,

a) Only one quenching mechanism is operative.
b) Quenching is bimolecular

From the absorption spectrum of phenolphthalein it is clear that phenolphthalein does not has the fluorescence effect. So, we could not record the fluorescence spectrum of phenolphthalein but we can use the phenolphthalein as a quencher. From fig 3.2 we came to understand that if we increase the quencher concentration the fluorescent intensity values decreases. Similar results were obtained for all other fluorescers in different solvents diethyl ether and methanol.

Dwividi and Banger [9] in their work on aromatic hydrocarbon donors with DDQ with chloroform has observed that the λ_{CT} value decreases with increase of ionization potential of the donor and our results agree well with this observation.

Stoke's shift ($\Delta\nu$), Molar extinction co-efficient ($\in$) and solvent parameter are calculated and are presented in table. Absorption spectra of the amines in the solvents diethyl ether and methanol are not affected by the addition of quencher in the concentration range used in fluorescence quenching experiment. This reveals that there is no complex formation or association of the amines with the quencher in the ground state and the quenching occurs only due to the interaction of excited amines and quencher, hence the quenching is not static but dynamic in nature. It could be stated that phenolphthalein is one of the best quenchers for amines.

IV. Conclusion

Fluorescence quenching studies on some substituted amines (ethylamine, diethylamine and diethylenetriamine) by phenolphthalein in two different solvents diethyl ether and methanol have been successfully carried out. The Stern-Volmer plots for all the quenching processes are linear and the regression analysis of all the curves gives a very good correlation. The Stern-Volmer constants for all the quenching processes have been determined. The quenching is found to be dynamic in nature as there was no change in the absorption spectra of the amines in the solvents diethyl ether and methanol by the addition of quencher phenolphthalein. We conclude that phenolphthalein is one of the best quenchers for the amines.

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