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Fluorescence quenching of rhodamine B dye pluronic F127 complex by inorganic anions

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ABSTRACT

In this study we have explored the interaction of a cationic dye Rhodamine B (Rh B), with pluronic block copolymer (F127), both in the presence and absence of the anionic quenchers, (NaI, NaOAc, NaOH, NaCl). We have applied both steady-state and time-resolved spectroscopic techniques along with UV-Visible and Fluorescence spectroscopy to demonstrate the binding of the probe to the pluronic F127 micelles in the presence and absence of quenchers. The probe Rhodamine B(Rh B) penetrates to the more hydrophobic interior of the micellar system of F127 micelle. We have also observed that the partitioning of the ionic solutes between the F127 micelles and aqueous phase changes in the presence of Rh B than in its absence.

Keywords: Rhodamine B, Pluronic F127, Fluorescence quenching, UV- Fluorescence Spectra.

1. INTRODUCTION

Non-ionic pluronic triblock copolymers are known to get considerable attention due to their expanding contributions in the fields of biological and medical science as modern drug delivery carriers[1-7] and also have industrial applications.[8,9] Amphiphilic triblock copolymers having the general formula PEOx-PPOy-PEOx, are composed of hydrophobic poly(propylene oxide) (PPO) block and two units of hydrophilic poly(ethylene oxide) (PEO) block in which PPO block is in the centre.[10-12] Due to their amphiphilic character, these block copolymers display surfactant properties which include the ability to interact with hydrophobic surfaces and biological membranes. In aqueous solutions above critical micelle concentration (CMC) these copolymers self-assemble and form micelles. Due to the presence of unique core-shell structure, polymeric micelles like F127, have the capacity to solubilize hydrophobic drugs without the use of any organic solvent and covalent bond formation. Due to their non-toxicity, they can be safely used for controlled release of drugs. [5,6] A general method for design a drug delivery system is to incorporate the drug within the nanocarrier to enhance solubility. Copolymers are efficient in intracellular delivery because of the presence of the oxyethylene groups in the corona; the hydrophilic PEO corona prevents aggregation and protein adsorption and the hydrophobic PPO incorporate lipophilic drugs[6]. Therefore, pluronic micelles can solubilize many poorly soluble drugs and protect them from inactivation in biological media[7]. Triblock copolymeric micelles are also known to be an excellent delivery system for hydrophobic anti-cancer drugs,[6,7] nucleic acids[3] and are also used in the treatment of multidrug resistant tumors[6]. Rhodamine B is an triphenylmethane dye and an important representative of xanthene dye, widely used as a colorant in textiles and food stuffs, and is also a well-known water tracer fluorescent. It is also known as laser dye[13,14]. They are used as fluorescent probes[15] represents an important biological application due to its low water toxicity if compared with several other organic solvents. But, on the other side, the solubility of laser dyes in water is very low because of hydrophobic interactions between alkyl substituent [16] and water. In this case, dyes tend to aggregate in the form of dimers. In order to overcome this drawback, it is necessary to increase the solubility of organic dyes in aqueous solutions. Rhodamine B is a cationic dye, has been used extensively as a sensitizer in various biological systems where the processes of energy and electron transfer are taking place. They exhibit various photophysical and photobiological applications. A large number of researchers have devoted their attention to advancing the fundamental understanding on the interactions between polymers and surfactants and the aggregations and phase behaviors of

aqueous polymer/surfactant systems using different techniques, like fluorescence quenching [17,18]. The study of selective fluorescence quenching helps to avoid fluorescence from undesired molecules having only slightly different molecular structure, making the analysis easier[19,20]. When combined with a separate method, fluorescence quenching can be used to simplify the qualitative and quantitative analysis of complex samples for selective discrimination against interfering components[21,22]. Dynamic bimolecular quenching where there is negligible overlap of fluorophore emission and quencher absorption spectra can often be explained through the involvement of excited state donor-acceptor interaction .Depending on the ionization energy of the donor, electron affinity of the acceptor and the polarity of the solvent such interaction can lead to complete electron transfer resulting in ion pairs, or partial charge transfer, resulting in exciplexes [23-26]. For quenching processes where there is neither new emission ascribable to exciplexes nor any detectable ion pairs, the donor-acceptor mediated quenching can only be inferred from the application of Waller's equation. The mechanism of quenching was found to be purely dynamic and non-emissive charge-transfer exciplex mediated[27].

A literature survey indicates that a systematic study on the possible mechanism of Pluronic with Rh B dye fluorescence quenching by metal ions has not yet been undertaken. In the present work, a series of alkali metal ions have been used as quenchers. The Fluorescence quenching was thoroughly characterized by UV–Vis absorption spectroscopy, fluorescence spectroscopy and life time spectra. The quenching mechanism for the above system is described.

2. EXPERIMENTAL SECTION

2.1. MATERIALS AND METHODS

Pluronic F127 (PEO_{100} PPO_{65} PEO_{100}, average molar mass of 12,600), Rhodamine B and anionic quenchers NaI, NaOAc, NaOH, NaCl were purchased from sigma chemical Co. (Bangalore, India). Triply-distilled water was used for the experiments.

UV-vis spectra were recorded by Shimadzu 1700 UV-vis spectrometer. Fluorescence measurements were performed using Fluoromax-4 fluorescence spectrophotometer. The time resolved fluorescence measurements were carried out using Horiba JobinYvon TCSPC lifetime instrument. The value of χ^2 in the range 0.99 – 1.3 is usually considered as a good fit.

2.1. Sample preparation

Accurately weighed amount of rhodamine B was weighed accurately and dissolved in methanol in 10 ml smf and then sonicated for 10 min. one ml of this solution was dried and made up with distilled water to bring the final concentration of the rhodamine B to 1×10^{-5}M. From this 4 µM solution was prepared and weighed amount of F127 was dissolved in this solution to bring the concentration to 5% (w/v). The samples were left in refregerator for 1 day for complete dissolution. 0.1M NaI, NaOAc, NaOH, NaCl were prepared. With double distilled water.

Calculated amount of the above salt solution was added to the previously prepared pluronic F127 in rhodamine B dye. The quencher concentration was varied from 0.01 to 0.1M and then characterization was done. The quencher was added to the equilibrated solution just before taking the fluorescence spectrum. The solution was shaken well for uniformity and spectrum was taken immediately to prevent the possibility of any photoreaction.

3. RESULT AND DISCUSSION

3.1. UV–visible Spectroscopy

The UV visible spectra for Rhodamine B with quenchers were taken in the presence and absence of Pluronic F127 in the aqueous solvent. The peak value(λmax) of Rh B and quencher was observed at 553nm figure 1(A) and (B). The incorporation of pF127 did not change the λmax of the spectra

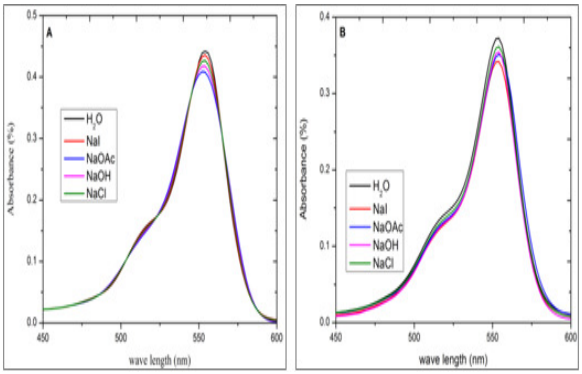

Figure – 1: (A) UV–vis. Absorption spectra of Rhodamine B with Quenchers(NaI, NaOAc, NaOH, NaCl) (B) UV–vis. Absorption spectra of Rhodamine B in PF127 with Quenchers.

The λmax value in UV-spectra did not change for the fluorophore with five different quenchers, Both the absorption spectra were observed to be of same nature with and without pluronic F127 surfactant. All the absorbance values at λmax 553 nm are shown in table 1.

Table - 1: UV–vis. Absorption spectra of Rhodamine B with and without PF127 - Quenchers

Quenchers	Wave length (nm)	Absorbance (a.u.)	Absorbance (a.u.)
		RhB in PF127 with quenchers	RhB with quenchers
H_2O	553	0.373	0.447
NaI	553	0.368	0.441
NaOAc	553	0.367	0.436
NaOH	553	0.360	0.426
NaCl	553	0.365	0.430

The absorption spectrum was observed with increasing concentration of the metal ions. As show in the table, there is no shift in the wavelength maximum only the absorbance value changed. No additional peak was observed to appear. This result predicts that there may not be any interaction between flurophore and metal ions there ground state.

The excitation spectrum of flurophore and the absorption spectrum. Were similar in appearance. Hence any possibility of geometrical changes of the flurophore molecule in the excited state can be ruled out.

3.2. Ionic Strength

Addition of electrolytes such as NaI, NaOAc, NaOH, NaCl, causes the adsorption of surfactants onto the surface of adsorbent. These effects are mostly due to the decreased attraction between oppositely charged species and the decreased repulsion between similarly charged species at higher ionic strength. Both the efficiency and effectiveness of adsorption of surfactants onto charged substrates are increased by an increase in the ionic strength of the aqueous phase[28]. PH of the solution medium plays an important role in this the change in pH of the flurophore on addition of quencher is presented in supplementary(S1).

3.3. Fluorescene Spectroscopy

The emission spectrum of flurophore did not show any shift of wavelength. No additional peak was observed. Hence in all probability there may not be any interaction between PF127 and metal ions in the ground state. The emission spectrum of rhodamine B with increasing quencher concentration displays no change of fluorescence spectra except that there was reduction in fluorescence intensity with order of increasing quencher concentration.

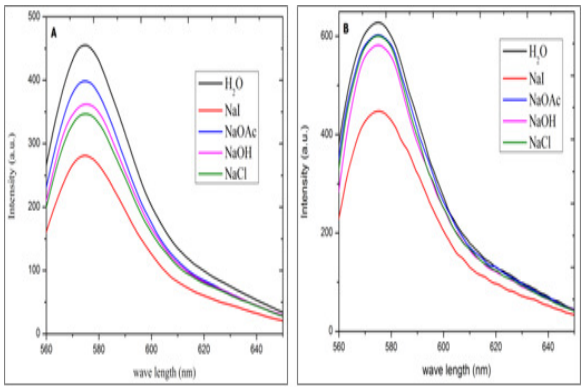

Figure - 2: (A) Fluorescence emission spectra of Rh B with Quenchers (B) Fluorescence emission spectra of Rh B in PF127 with Quenchers.

The Fluorescence spectrum of Rh B containing quenching salts (NaI, NaOAc, NaOH, NaCl) in aqueous solution in presence and absence of pluronic F127 are displayed in the figure 2(B) and 2(A) respectively. The Rh B fluorescence spectra shows prominent band at 575nm which is assigned due to the Rh B monomeric emission. The flurophore having PF127 surfactant shows high emission compared to without it. Hence probably fluorescence energy transfer has taken place. The change in fluorescence energy transfer efficiency due to the presence of quencher has been examined. There is no change in wavelength but the changes only occurred in intensity due to presence of PF127.

Fluorescence is an essential tool in many research areas in protein research, sensors , etc. Since these studies are normally, easy to perform, need only a small amount of sample and are non-destructive, Quenching studies provide a lot of information regarding the location of fluorophore inside its macromolecular structure. This in turn can give structural information about the macromolecule [29-32]. A variety of mechanisms have been proposed. Example, Proton as well as electron transfer, long range transfer of energy, induced conformational changes different intra molecular reactions. The various mechanism of quenching can be categorized as dynamic quenching (collisional encounters) or static quenching (ground state complex formation) between the quenchers and flurophore. In case of collisional quenching emission intensity depends on the quencher concentration [Q] is governed by the well known stern-volmer equation. In this equation, F_0 and F represent the fluorescent intensity, τ_0 and τ are lifetime in the absence of substance presence of quencher respectively and Ksv is the stern-volmer constant [33].

` The quenching mechanism can be studied from the Stern-Volmer plot for the

quenching constant of the flurophore by all the quenchers,

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} = 1 + K_{SV}[Q]\,]$$

All the plots were linear for the system with the slope as Ksv. where F_0 is the fluorescence intensity in the absence and F, the intensity in presence of the quencher [Q]. The slope gives the Ksv which is stern-volmer quenching constant. (Table 2&3). Stern-Volmer Plots Usually holds good if collisional quenching is taking place [34].

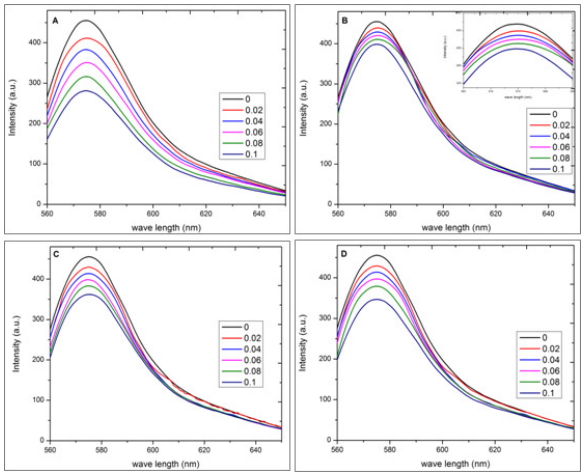

Figure - 3: Fluorescence emission spectra of Rh B with increasing in concentration of Quenchers ((A)NaI, (B)NaOAc, (C)NaOH, (D)NaCl)).

When static quenching occurs it is due to ground-state complexation, the Ksv represents the formation constant of the complex. Table 4 shows the stern-volmer constant Ksv with the fluorescence intensity ratio parameter F_0/F for various concentrations of quencher with and without PF127. As can be seen from the table, the Ksv values are low when the quencher is present in presence of PF127 than without it, viz. For NaOAc the Ksv value is 1.36 in absence and 0.32 in presence of pluronic F127. This means that the quencher salts are bound more strongly to the probe in absence of PF127 than with it. Also it is seen that NaI is the best quencher in this group of all salts chosen having a highest Ksv value of 6.43. if it were a static quenching fluorescence spectrum in presence of quencher would have shown a change and we observed no change in emission spectrum.

The extent of quenching was found to increase as the quencher concentration increases, (Figure 3 & 4) for the present system. (Fo /F) against the quencher concentration [Q] was plotted for absence and presence of PF127 is a straight line with slope as Stern-Volmer quenching constant Ksv.

The stern-volmer plot for flurophore shows linearity and non-linearity with different quenchers. The linear part of plot was observed seen to depend on the type of quencher used. Good quencher displays nonlinear nature.

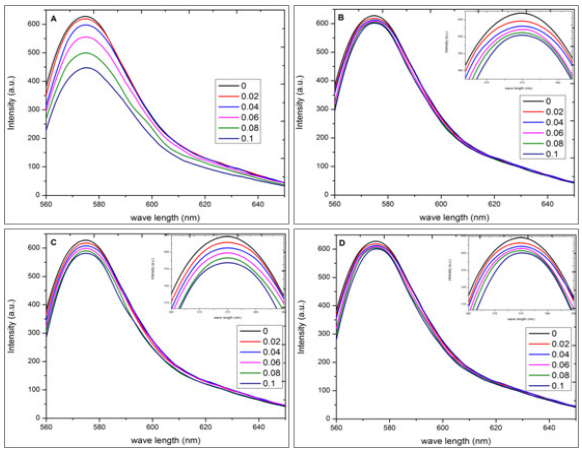

Fig.ure – 4: Fluorescence emission spectra of Rh B in PF127 with increasing in concentration of Quenchers (NaI, NaOAc, NaOH, NaCl).

Table - 2: Fluorescence emission spectra of Rh B with increasing in concentration of Quenchers

Concentration of Quechers[Q] with Rhodamine B without PF127 (M)	Wave length (nm)	Intensity (λ_{max})			
		NaI	NaOAc	NaOH	NaCl
0.00	575	456.89	456.89	456.89	456.89
0.02	575	412.93	440.96	430.26	430.21
0.04	575	384.38	430.26	415.03	15.33
0.06	575	352.22	420.18	398.16	398.16
0.08	575	316.17	410.93	384.38	380.38
0.1	575	281.73	398.16	363.06	348.22

Table - 3: Fluorescence emission spectra of Rh B in PF127 with increase in quencher concentration.

Concentration of Quechers[Q] with Rhodamine B in PF127 (M)	Wave length (nm)	Intensity (λ_{max})			
		NaI	NaOAc	NaOH	NaCl
0.00	575	628.57	628.57	628.57	628.57
0.02	575	619.57	619.32	618.46	619.57
0.04	575	598.73	614.15	608.82	613.16
0.06	575	556.39	610.75	599.05	608.9
0.08	575	500.98	606.82	590.97	604.82
0.1	575	448.18	603.9	582.43	601.32

Here NaOAc plots are linear and NaI and NaOH, NaCl are not linear hence better quenchers. whereas, NaOAc, NaOH, NaCl plots are linear and NaI is a not linear. Hence NaI is a better quenchers in presence of PF127.

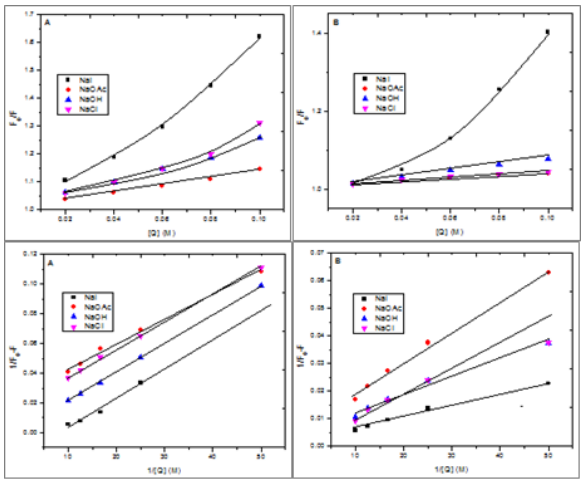

Figure - 5: (A) Stern–Volmer plot for fluorescence quenching of Rh B and quenchers without PF127 (B) Stern–Volmer plot for fluorescence quenching of Rh B and quenchers with PF127 (C) PF127 Lineweaver–Burk plot for Rh B and quenchers with PF127 (D) Lineweaver–Burk plot for Rh B and quenchers without PF127.

There was positive deviations from linearity were obtained in all the salts even at low concentration. This indicates that the quenching process is not purely collisional. Similar inferences have been drown by Behera *et al.* [35] when they observed the fluorescence quenching nature of some rhodamine B derivatives by a number of water soluble quenchers in the anionic micro emulsions in 0 - 0.1 M concentration. There was little change change in the polarity of the medium. The static quenching calculated for Ksv by The Lineweaver–Burk equation was given by equation,

$$\frac{1}{F_0 - F} = \frac{1}{F_0} + \frac{K_D}{F_0[Q]}$$

Quenching can occur through an internal conversion and then charge transfer to singlet excited states, after which it can be followed by an intersystem crossing associated with the charge transfer triplet states[15].

The Association constant is expressed as $K_A = 1/K_D$. The formation constant (K_A, L mol^{-1}) is obtained from the slope of the figure 4(C,D). High association constant(K_A) value and low dissociation constant (K_D) value obtained by this method suggested a reasonably strong binding between Rh B and quenchers in the absence of PF127 from table 5. The Lineweaver–Burk equation shows linear plot for all quenchers and it indicated the energy transfer of the flurophore involved in FRET process. Figure 5(C) and (D) display the linear regression coefficient.

In this univalent metal halides, the constant metal Na^+ ions with different halides ions like I^- ,OAc^- ,OH^- ,Cl^- are studied. NaI was observed to be extremely efficient quencher, NaOH, NaCl are moderate quenchers and NaOAc is poor quencher in this group for the flurophore in presence of pluronic F127.

Table - 4: Stern-volmer quenching constant for different quenchers

Quenchers	RhB in PF127 with quenchers				RhB with quenchers			
	[Q]M	F_0/F	Ksv	Correlation coefficient	[Q] M	F_0/F	Ksv	Correlation coefficient
NaI	0.02	1.01	4.90	0.95	0.02	1.10	6.43	0.97
	0.04	1.04			0.04	1.18		
	0.06	1.12			0.06	1.29		
	0.08	1.25			0.08	1.44		
	0.1	1.4			0.1	1.62		
NaOAc	0.02	1.01	0.32	0.99	0.02	1.03	1.36	0.99
	0.04	1.02			0.04	1.06		
	0.06	1.03			0.06	1.08		
	0.08	1.03			0.08	1.11		
	0.1	1.04			0.1	1.14		
NaOH	0.02	1.01	0.78	0.99	0.02	1.06	2.40	0.98
	0.04	1.03			0.04	1.10		
	0.06	1.05			0.06	1.14		
	0.08	1.06			0.08	1.18		
	0.1	1.07			0.1	1.25		
NaCl	0.02	1.01	0.37	0.98	0.02	1.06	3.00	0.95
	0.04	0.02			0.04	1.10		
	0.06	0.03			0.06	1.14		
	0.08	0.04			0.08	1.20		
	0.1	0.04			0.1	1.31		

Table - 5: Lineweaver–Burk calculation for association constant(K_A) and dissociation constant(K_D)

Quenchers	RhB in PF127 with quenchers				RhB with quenchers			
	1/[Q]	$1/F_0$-F	$K_{A\ L/Mol}$	$K_D \times 10^{-5}$	1/[Q]	$1/F_0$-F	$K_{A\ L/Mol}$	$K_D \times 10^{-5}$
NaI	50	0.111	4.90×10^4	2.040	50	0.022	6.43×10^4	1.555
	25	0.033			25	0.013		
	16.6	0.013			16.6	0.009		
	12.5	0.007			12.5	0.007		
	10	0.005			10	0.005		
NaOAc	50	0.108	0.32×10^4	33.33	50	0.062	1.36×10^4	7.352
	25	0.069			25	0.037		
	16.6	0.056			16.6	0.027		
	12.5	0.045			12.5	0.021		
	10	0.040			10	0.017		
NaOH	50	0.098	0.78×10^4	12.82	50	0.037	2.40×10^4	4.166
	25	0.050			25	0.023		
	16.6	0.033			16.6	0.017		
	12.5	0.026			12.5	0.013		
	10	0.021			10	0.010		
	50	0.111	0.37×10^4	27.02	50	0.037	3.00×10^4	3.333

NaCl	25	0.064	25	0.024
	16.6	0.050	16.6	0.017
	12.5	0.042	12.5	0.013
	10	0.036	10	0.009

3.4. Fluorescence lifetime measurements

Fluorescence lifetime measurements were carried out for the Rh B, quencher system presence of PF127. Figure 6 displays the time resolved fluorescence spectra decay curve for the above system. Table 5 shows the average value for different quenching system.

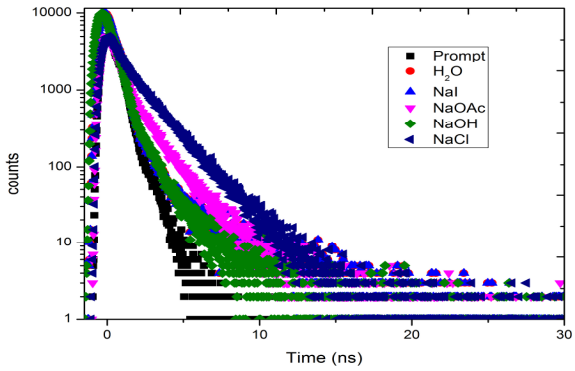

Figure – 6: Time-resolved Fluorescece decay curves for Rh B in PF127 with different impurities of different Quechers

For dynamic quenching,

$$Ksv = (kq.\tau_0)$$

where Kq represents the bimolecular quenching constant ($1mol^{-1}\ s^{-1}$) and τ_0, the excited singlet lifetime of the fluorophore in the absence of quencher. For PF127, τ_0 was obtained from table 6. $\boldsymbol{\tau_{ave}}$ value for NaI is 4.45 which is the lowest in the group. This indicates that NaI is the best quencher in this group. This is in good agreement with our earlier observation that salt of iodides are the best quenchers.

Table - 6: Time-resolved Fluorescece decay for Rh B in PF127 with different Quechers

Quenchers	τ_{ave} ×10^{-11} (ns)	χ2
H_2O	5.1716	1.00
NaI	4.4534	1.15
NaOAc	5.0450	1.04
NaOH	4.6530	1.09
NaCl	4.8990	1.10

The efficiency due to steady state fluorescence is expressed by equation,

$$E(I) = 1 - \frac{F}{F_0}$$

The emission spectrum of the donor (Rh B) along with the absorption spectrum of the acceptor and the distance between the donor and the acceptor energy transfer effect can be obtained by the following equation,

$$E = \frac{R_0^6}{R_0^6 + r_0^6}$$

$$E = 1 - \frac{I_{DA}}{I_D}$$

where, I_{DA} = fluorescence intensity in presence of both donor and acceptor, I_D=fluorescence intensity of donor, r is the distance between the acceptor and the donor and R_0 is the critical distance when the transfer efficiency is 50%, which can be given by the following equation.

$$R = 0.211[k^2 n^{-4} \Phi_D J(\lambda)^{\frac{1}{6}}]$$

where, ϕ_D is the quantum yield of the donor in the absence of acceptor n is the refractive index of the medium, k^2 is the orientation factor ($k^2=2/3$), and J(λ) is the spectral overlap between the emission spectrum of the donor and absorption spectrum of acceptor. J(λ) can be calculated using equation:

$$J = \frac{\int_0^\infty F_D(\lambda)\epsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda}$$

By this equation it is possible to find the distances of positive charge Rh B in the surface of quenchers in presence or absence of PF127. There is interaction of positively charged dye and negatively charged ion of quenchers. Energy transfer is taking place. And that the presence of PF127 affects the binding. This is obvious from the change in Ksv values in Table 4. Since, the lifetime is almost a constant value it can be inferred that static quenching is taking place.

4. CONCLUSIONS

The quenching of Rh B in pluronic F127 by inorganic anions has been evaluated by this study.Out of the four salts of sodium used here, NaI was observed to be the most effective quencher, NaOH and NaCl moderate quenchers, NaOAc poor quencher for this system. The significant steady state fluorescence quenching (Ksv = 6.43×10^{-3}) for KI as quencher in PF127 compared to (Ksv = 4.90×10^{-3}) without pluronic shows that the quencher is strongly bonded to the probe in absence of pluronic. Very little change in the fluorescence lifetime value suggests that

quenching operating between the dye and quencher is of static nature.

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