



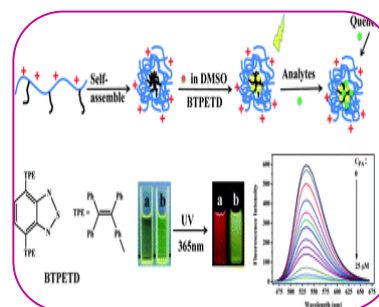
PHOTOPHYSICAL STUDY BETWEEN ANTHRACENE AND PROFLAVINE HEMISULPHATE IN AQUEOUS CATIONIC MICELLAR SOLUTION

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ABSTRACT

Photophysical study between anthracene and proflavine hemisulphate is studied in micellar solution of Cetyl trimethyl Ammonium bromide (CTAB). The fluorescence of anthracene is quenched by proflavine hemisulphate (PF) and quenching is accordance with Stern-Volmer relation. The efficiency of energy transfer found to depend upon concentration of proflavine hemisulphate. The value of critical energy transfer distance (R_0) calculated by using Foster relation is 18.1 Å and as it is less than 50 Å indicates efficient energy transfer in present system. The analytical relation was established between extent of sensitization and concentration of proflavine hemisulphate, which helped to estimate proflavine hemisulphate directly from pharmaceutical drugs.



KEYWORDS : Photophysical study, Fluorescence quenching, Anthracene, Proflavine Hemisulphate, Cetyl trimethyl Ammonium bromide (CTAB).

INTRODUCTION

Surfactants are substances with molecule structures consisting of a hydrophilic (water soluble) and a hydrophobic (water insoluble) group. The hydrophobic group is usually a hydrocarbon moiety. The hydrophilic groups give surfactants the primary classification, and they are anionic, cationic and non-ionic in nature. The cationic form of an amine product. Cationic surfactants are frequently used as antimicrobial, emulsifying, anticorrosion and softening agents in pharmaceutical preparations, cosmetic formulations, cleaning products, disinfectants and other industrial products [1,2]. The process generally results in simultaneous quenching of donor fluorescence and electronic excitation of acceptors [3,4]. According to Forster Theory the rate of energy transfer depends upon the extend of spectral overlap between the donor emission and acceptor absorption, the quantum yield of donor, relative orientation of donor and acceptor transition dipoles and the distance between the donor and acceptor transition dipoles. The extend of quenching can be related with the concentration of the acceptor. One of the highly fluorescent polynuclear aromatic hydrocarbons (PAH's), anthracene is widely used as a fluorescent probe in homogenous solution systems for fluorimetric detection and estimations of bio-molecules [5,6]. The aqueous insolubility of anthracene limits its use as probe for many water soluble bio-molecules and drugs. The anthracene solubility can be increased by its incorporation in micelles in surfactant solution. The aqueous insolubility of anthracene limits its use as probe for many water soluble bio-molecules and drugs. The absorption spectrum of PF has strong overlap with emission spectrum of anthracene. This has enabled the use of anthracene as fluorescent probe and PF has a quencher.. The present study includes the quenching of fluorescence of anthracene by propflavine hemisulphate (PF) in CTAB micellar solution and its application on the detection of PF. The earlier methods used for detection and estimation of this drug includes Liquid Chromatography,

Stripping Voltametry and direct Fluorimetry analysis [7-9]. These methods are complex and destructive while the analytical method based on FRET technique developed in present studies is more selective, sensitive, reproducible and nondestructive.

2. EXPERIMENTAL:

2.1. Chemicals:

Anthracene (Merck- Schuchardt) and Proflavine hemisulphate (Hi media) were used after purity testing. Cetyl trimethyl Ammonium Bromide (CTAB) procured from S.D. fine Chem. Limited was used as received. The critical micelle concentration (CMC) of CTAB is 2.0×10^{-3} M. Double distilled water was used to prepare all solutions.

2.2. Preparation of solutions:

The anthracene solution in CTAB was prepared by dissolving the calculated quantity in CTAB solution and stirring overnight. The concentration of anthracene was kept constant to 8×10^{-5} M. The stock solution of PF of concentration 1×10^{-4} M was prepared in SDS and during quenching experiment was varied from 2×10^{-6} M to 14×10^{-6} M. The solutions were deaerated before recording their fluorescence spectra.

2.3. Equipment:

The fluorescence spectra and Fluorescence excitation spectra of the solutions were recorded on PC based Spectrofluorophotometer JASCO, Japan (Model FP-750). The excitation and emission slit width used during spectral measurements were 10 nm.

3. RESULT AND DISCUSSION:

3.1 Fluorescence Quenching Studies:

The excitation and fluorescence spectra of PF and Anthracene in CTAB solution are presented in Fig. 1. The excitation spectrum of PF shows sharp band in the range between 400 nm to 500 nm. The fluorescence spectra of PF is peaking at 510 nm. The anthracene dissolved in CTAB micellar solution was found to exhibit the sufficient fluorescence intensity, which can be absorbed by PF because of the large integral overlap seen between emission spectrum of Anthracene and excitation spectrum of PF in Fig.1.

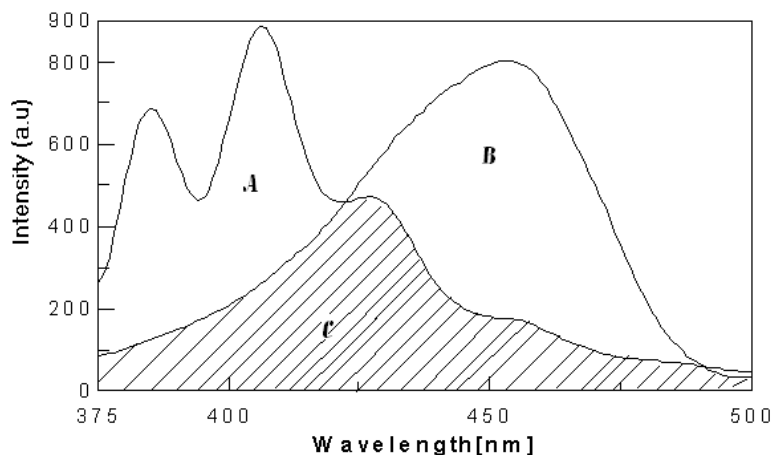


Fig. 1: Emission spectrum (A) of Anthracene, Excitation spectrum of Proflavine (B), Integral overlap area of Anthracene and Proflavine Hemisulphate

Overlap region between Anthracene and PF is an indication of efficient energy transfer takes between the donor-acceptor pair. The region of integral overlap is used to calculate the critical energy transfer distance (R_0) between anthracene (donor) and PF (acceptor) using Förster relation [10]

$$R_0^6 = \frac{9000k^2\Phi_D \ln 10}{128\pi^5 n^4 N_A} J \tag{1}$$

In above equation.. k^2 is the orientation factor of the dipole of the donor and acceptor, n is refractive index of the medium, Φ is the fluorescence quantum yield of the donor in the absence of acceptor and J is the overlap integral which gives degree of spectral overlap between the donor emission and acceptor excitation. The value of J is given by equation,

$$J = \frac{\left(\sum F(\lambda)\right)\epsilon_A(\lambda)\lambda^4\Delta\lambda}{\sum F(\lambda)\Delta\lambda} \tag{2}$$

where, $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at wavelength λ , $\epsilon(\lambda)$ is the molar absorption coefficient at wavelength λ . Under experimental condition $k^2 = 2/3$, $n = 1.334$ and $\Phi = 0.009$

Fig.2. Shows quenching of fluorescence of anthracene with gradual increase in concentration of proflavine and simultaneous enhancement of fluorescence of PF appeared at 510 nm. An isoemissive point is seen in figure at 446 nm. The observed quenching is explained by considering the fluorescence resonance energy transfer because the fluorescence spectrum of anthracene and excitation spectrum of PF shown in the fig.1 reveals larger spectral overlap between them. Fluorescence data was analyzed according by Stern-Volmer equation.

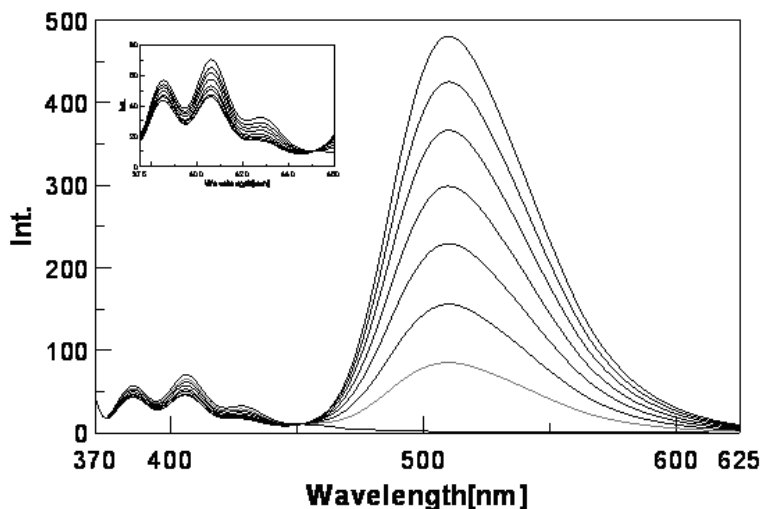


Fig.2.: Quenching of anthracene Fluorescence by Proflavine hemisulphate in CTAB Micellar solution (above CMC)

3.2. Kinetics of quenching of anthracene fluorescence:

$$\frac{F_0}{F} = 1 + K_{sv} [Q] \tag{3}$$

$$K_{sv} = \tau k_q \tag{4}$$

$$k_q = K_{sv} / \tau \tag{5}$$

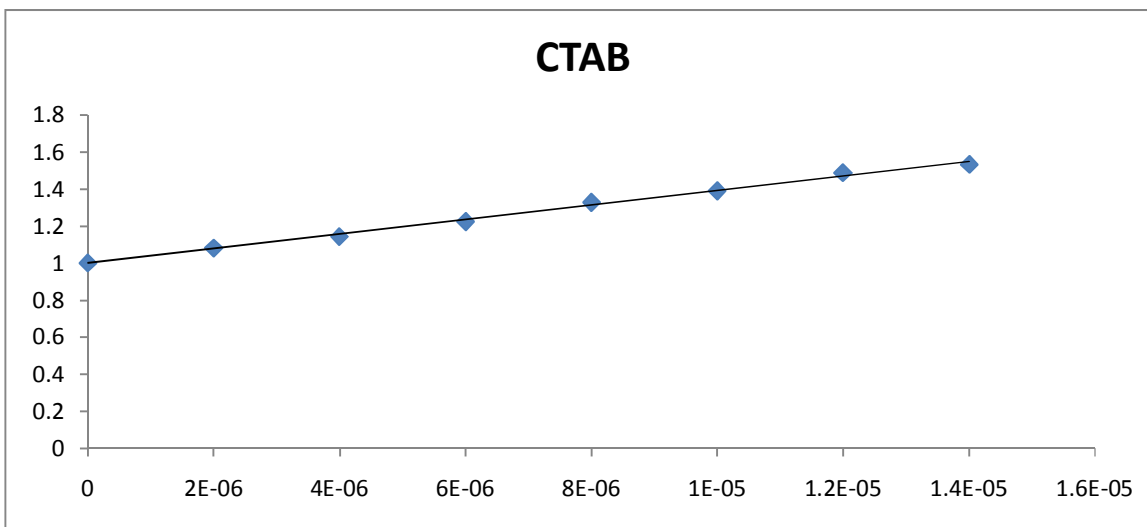


Fig. 3. Plot of F_0/F versus [PF] in aqueous CTAB (2 mmol dm⁻³),

Fig. 3 shows a plot of F_0/F Vs concentration of proflavine hemisulphate, where F and F_0 are the fluorescence intensities of anthracene with and without PF. The straight line nature of plot with intercept 1 on y-axis indicates the validity of Stern-Volmer equation [11]. The values of K_{sv} , k_q obtained from above equation and life time (τ) are given in Table. 1. The efficiency of the energy transfer is calculated in micellar environment. Förster's theory is used to calculate η as per the equation No. 6

$$\eta = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r_0^6} \tag{6}$$

where, r is the distance between the donor and acceptor and R_0 is the critical energy transfer distance, for 50% excitation energy transfer to the acceptor and given by equation no.1 (14).

Solvents	Life Time (ns)	Forster Radius (R_0 Å)	K_{sv}	Distance between Donor and Acceptor ($r = \text{nm}$)	Efficiency of energy transfer	k_q
CTAB	4.9	18.1	3.92×10^4	2.75	0.1834	7.991×10^{12}

Table No. 1. Critical Energy Transfer (R_0), K_{sv} and Distance between donar and acceptor

The value of r found to be 2.75 Å in CTAB micellar solution shown in table no. 1. This value is too far to transfer electron from Anthracene to PF molecule but satisfy the conditions of fluorescence resonance energy transfer [12]. The estimated values of R_0 is 18.1 Å in CTAB. The value of R_0 is less than ≈ 50 Å are an indication efficiency of energy transfer between the donor-acceptor pair [13]. The R_0 values < 50 Å indicates

efficient energy transfer between anthracene and PF. The higher values of K_{sv} for quenching studies in ethanol suggest dynamic quenching due to collision between Anthracene and PF

3.3. Stability of the system:

The present Donor-Acceptor fluorescent system is photostable for a period of one hour.

3.4. Effect of foreign substances and method of analysis:

The effect of foreign substances on the fluorescence determination of Proflavine with proposed method was investigated. An initially foreign substance to the proflavine solution is taken in large excess and measured the fluorescence intensities. When interference was found to be intensive, the tests were repeated with successive smaller amount of foreign substances. According to proposed system the metal ions have small effect on determination of Proflavine. The tolerance limits for the ions are given in the Table No. 2.

Interfering substance	Concentration (micro gm .L ⁻¹)	Change in Fluorescence Intensity (%)
Al ⁺³	0.01	2.25
Ba ⁺²	0.19	0.35
Mn ⁺²	0.01	1.75
Bi ⁺²	0.058	2.00
Zn ⁺²	11.53	5.5
EDTA	0.096	1.015
CuCl ₂	0.002	3.25

Table No. 2 .Effect of interfering substances on fluorescence

3.5. Estimation of PF from pharmaceutical Sample:

Fluorescence Technique was used for estimation of proflavine hemisulphate from ointment Lorexane made, commercial available in the market by Virbac Animal Health India, Pvt. Ltd appropriate amount of sample diluted with CRTAB solution and were assayed by the standard procedure. For the accuracy of the proposed method three different concentrations of sample solutions were used in quenching experiment to determine the values of F₀/F in presence of anthracene. The amount of PF present in the ointment was calculated and given in the Table 3.along with its real composition used during formulation. From these results to confirm the reliability of the results, standard deviation and relative standard deviation in present were calculated and given in the same Table 2. The negligibly small value of RSD i.e. 2.25% indicates that the proposed method gives accurate results.

Name of Sample	Amount of Proflavin Hemisulphate (%)		RSD %	SD
	Certified value	Found value		
Lorexane Virbac Animal Health India, Pvt. Ltd	0.1 W/W	0.092 W/W	2.25	0.0015

4. CONCLUSION:

The fluorescence Resonance Energy Transfer studies between anthracene and proflavine hemisulphate in micellar environment is more efficient due to aqueous solubility of anthracene. The kinetic studies showed the validity of Stern-Volmer relation. The analytical method based on FRET developed for

estimation of proflavine hemisulphate from pharmaceutical ointments is simple and reproducible. The close agreement between observed and actual values in the sample proves the suitability of the method for routine analysis of proflavine hemisulphate in real samples.

REFERENCE:

1. Cross J, Singer E J (1994) Cationic surfactants – analytical and biological evaluation, surfactant science series, vol. 53. Marcel Dekker, New York, p 3, chapter 1
2. Jinshui Liu, Xin Wang, and Lun Wang, *Microchim Acta* 151, 123–126 (2005)
3. De, S.; Girigiswami, A. *J. Colloid Interf. Sci.* **2004**, 271, 485.
4. Liu, B. S.; Liu, Z. C.; Jing, G. *Anal. Lett.* **2005**, 38, 1367.
5. He, Y, Y.; Wang, X.C; Fan, X. Y; Zhao, B.; Jin, P.K. *Chines Journal of Chemistry* (**2008**) 26, 1373
6. Skupiriska, K.; Zylm, M.; Misiewicz, I.; Kasprzycka-Guttaman, T. *Acta Biochemical Polonica*, (**2006**) 53, 101
7. Vasile, D.; Rael, V.P.; Marius M. ; Monica, C. I ; Jose M. C. *Revue Roumaine de Chimie*, **2003**, 48, 287.
8. Plakas, S. M.; El said, K. R.; Jester, L. E; Bencsath, F. A.; Hayton W. L. *Journal of AOAC International*. **1997**, 80, 486.
9. Girousi, S. T.; Alexiadou D. K.; Ioannou, A. K. *Microchimica Acta*, **2008**, 160 ,435
10. Bhattar, S. L.; Kolekar G. B.; Patil S. R. *J Luminescence* .**2008**,128, 306.
11. T. Z Janosi J Korppi-Tommola³ Z Csok, L Kollar, P Myllyperki and J Erostyak., Hindawi Publishing Corporation *Journal of Spectroscopy* Volume 2014, Article ID 708739, 8 pages
12. L. Karnam, L. Brambilla, M. Del Zoppo and C. Bertarelli, *Phys. Chem. Chem. Phys.* DOI: 10.1039/c7cp01553d
13. J. Bravo., F Mendicuti, E Saiz., W, L. Mattice., *Macromol. Chem. Phys.* 197, 1349-1360 (1996).