CHAPTER - II

Spectroscopic study of 5-Ethoxycarbonyl-4-cinnamyl-6-methyl-3,4-dihydropyrimidine-2(1H)-one

2.0 INTRODUCTION

Fluorescent probe has been widely used in the field of biological and organic material science [1]. Some dihydropyrimidine derivatives are fluorescent materials that possess many valuable photophysical properties. They have received considerable interest due to their important activities such as antibacterial, antiviral, antihypertensive, antitumor, anti-inflammatory [2] etc.. They have numerous applications in many different biological processes such as calcium channel blockers [3], α-1-a antagonists and neuropeptide antagonists [4]. Recently some marine alkaloids such as dihydropyrimidine -5-carboxylate have been synthesized and used as fluorescent probes. They exhibit interesting biological activities like potent HIV-gp-120-CD_4 inhibitors as well as anti- HIV agents [5].

One of the important fluorescent probe BODIPY, boron dipyrromethane was used to understand the properties such as chemical and photostability relatively high absorption coefficients and fluorescence quantum yields [6-9]. Its photophysical properties have been widely studied by absorption, fluorescence spectra and fluorescence life time measurements [10-14]. Solvent polarity and the local environment have profound effects on the emission spectral properties of fluorophore molecules. The effect of solvent polarity is one origin of the Stokes shift, which is one of the observations in fluorescence. In addition to specific solvent-fluorophore interaction, many fluorophore can form an internal charge transfer (ICT) state [15]. Therefore the role of solvent polarity is not only to lower the energy of excited state due to general solvent effects but also to govern which state has the lowest energy.

The development of photodevices for monitoring various structure and its surroundings is an important research target for understanding biological events accompanying interbiomolecular interactions such as replication, transcription, expression activation and inactivation [16]. Specifically monitoring the change of local microenvironments such as dielectric properties in DNA and its binding proteins is highly important for understanding interbiomolecular interactions. An ideal probe for monitoring various structure

and dynamics of DNA and its surrounding should be sensitive to its local microenvironments and should be incorporated site specifically throughout any DNA sequence of interest. Therefore we need to develop a novel bioactive molecule like DHPM in which the fluorophores are tethered by rigid linkers and show unique absorption and fluorescence emission spectra. The 5-Ethoxycarbonyl-4-cinnamyl -6-methyl-3, 4-dihydropyrimidine-2(1H)-one (DHPM) is a fluorophore with high charge transfer (CT) and sensitive to solvent polarity.

The absorption spectra of DHPMs are characterized by intense long-wavelength bands, which is obtained by (π, π^*) transition $S_1^{FC} \leftarrow S_0$. As the methane group increases, the probability of the $S_1^{FC} \leftarrow S_0$ transition increases. In the case of asymmetric ionic cyanines, both transition probability and the band shift strongly depend on the electronic asymmetry of the dye [17]. Upon electronic excitation, the dipole moment of DHPM can either increase or decrease considerably which affects the solvatochromic behaviour of DHPM molecule. An increase of excited state dipole moment with respect to that of ground state corresponds to a positive solvatochromism (bathochromic band shift upon the increase of solvent polarity) where as a decrease leads to a negative solvatochromism (hypsochromic band shift when solvent polarity increases)

The fluorescence quantum yield (Φ_F) of some laser dye DCM [18,19], increases with solvent polarity, while quantum yield of fluorine–substituted derivatives of DCM decreases [19,20]. It has been also revealed that Φ_F of DCM and DA-substituted stilbenes increase at low temperatures and by inclusion into a polymeric matrix [19,21]. In the singlet excited fluorescent S_1^F state, the excited state dipole moment of DCM exceed that of the ground S_0 state by 20 D and large Stokes shift (in 5000 cm^{-1}) between the maxima of the absorption and fluorescence spectra is observed in polar solvents [22].

The fluorescence quenching of organic molecules in solution by various quenchers like aniline, bromobenzene, halide ion, metal ion, carbon tetrachloride etc. has been studied by several investigators by steady state [23-

27] and transient methods [28-30]. In almost all the cases, experimental results follow the linear Stern-Volmer relation as follows,

$$I_0/I = 1 + K_{SV}[Q]$$

Where, I_0 and I are the fluorescence intensities in absence and presence of the quencher respectively. K_{SV} is the Stern-Volmer quenching rate parameter and Q is the quencher concentration. But in some cases, it has been observed that the experimental result show positive deviation from linear S-V relation [25,26]. This positive deviation is attributed to various processes like intersystem crossing, formation of charge transfer complexes both at ground and excited states, static and dynamic quenching etc. apart from this, the polarity and change in quencher concentration are expected to play a role in this mechanism.

2.1 EXPERIMENTAL

2.1.1 *Materials:*

All solvents used for synthesis and spectroscopic measurements were of analytical or spectroscopic grade. Triple distilled water was used whenever required. Anthracene was supplied by Aldrich in 99% purity and used as received. The 5-Ethoxy-carbonyl-4-cinnamyl-6-methyl-3,4-dihydropyrimidine-2(1H)–one was synthesized in accordance with the method reported in literature [31] and were purified by recrystallization from ethanol and identified by IR, proton NMR spectral data and physical constants.

IR 3115, 3039, 1710, 1678, 1615, 1250, 750 cm^{-1}

NMR (DMSO d_6)δ, 1.5 (t , 3H , -OCH_2-**CH_3**), 4.1 (q , 2H , -**OCH_2**CH_3), 2.1(s, 3H, vinyl methyl), 5.1(1H, benzylic H), 5.8 (d, 1H,-CH=C**H**-), 7.1(m, 5H, Ar-H), 7.38 (**d**, 1H, -C**H**=CH-), 8.5 (br. s, 1H, NH), 9.1 (br. s, 1H, NH).

2.1.2 *Spectroscopic measurements:*

The UV-Visible absorption spectra were measured on Elico model SL-177 UV-Visible spectrophotometer. Fluorescence spectra were recorded on PC

based Spectrofluorophotometer (JASCO Japan FP-750). Life time was measured on Time Resolved Fluorimeter. The IR spectrum was recorded on Perkin Elmer spectrophotometer using KBr pellets and NMR spectrum was obtained on Brucker 300 MHz instrument using DMSO as an internal standard. The DHPM solution of concentration 1x 10^{-3} M was used in determination of UV-Visible absorption and fluorescence spectra. The anthracene was used as fluorescent probe in the quenching experiments performed with DHPM quencher. Quantum yields Φ were calculated using as a reference quinine sulphate in 0.1 N H_2SO_4 (Φ =0.54) with the correction made for different refractive indices of solvents [32].

2.2 RESULTS AND DISCUSSION

2.2.1 *Absorption and fluorescence measurements:*

The studies of absorption and fluorescence spectra of DHPM (1 x 10^{-3} mole/lit) were measured in different solvents at room temperature and estimated the spectroscopic data such as molar absorption coefficients, absorption and emission maxima, fluorescence quantum yields, Stokes shifts are presented in Table 1. The fluorescence emission occurs in region 385-600 nm with maximum emission at 489 nm, some of fluorescence spectrum shown in fig.2.2. The emission spectrum of the compound exhibit gradual shift from deep blue (λ_{max} = 476 nm) in 1,4 dioxan to red in butanol (λ_{em} = 489 nm). The absorption peak of DHPM in various solvents shows slight shift from 352 nm in THF to 358 nm in formamide, which indicates that the large dipole moment in the excited state is higher than in the ground state because of internal charge transfer.

The fluorescence quantum yields (Φ) and Stokes shift (υ) were determined by the following equation,

$$\Phi = \frac{\Phi_{std} . FA_{std} n^2}{F_{std} An^2_{std}} \qquad \text{---------- (1)}$$

Where,

F & $F_{std.}$ = Peak areas of sample & standard solutions respectively.

A & $A_{std.}$ = Absorbance at excitation wavelengths of sample & standard solutions respectively.

n & $n_{std.}$ = Refractive index of sample and standard solutions respectively.

Φ & $\Phi_{std.}$ = Quantum yields of sample & standard solutions respectively.

The fluorescence quantum yields of the compound was seen slight difference with solvent polarity which showed that the negative and positive solvatokinetic effect. For negative solvatokinetic effect, quantum yield increases with a suitable enhancement of intramolecular charge transfer (ICT) which involves $n\pi$ electron configuration while reduction in quantum yield by strong ICT is called positive solvatokinetic effect. As Table I shows that the highest fluorescence quantum yield was observed for 1,4-dioxan and lower for butanol solvent. The interaction between the solvent and fluorophore affect the energy difference between the ground and excited state. To a first approximation, this energy difference (cm^{-1}) is a property of refractive index (n) and dielectric constant (ϵ) of the solvent which is usually explained by the Lippert-Mataga equation [33-34].

$$\Delta\nu = \bar{\nu}_{abs.} - \bar{\nu}_{em.} = \frac{2(\mu_e - \mu_g)^2}{4\pi.\epsilon_0 .h.c.a^3}\Delta f.(\epsilon .n) + constant \qquad \text{.....2}$$

$$\Delta f = \frac{(\epsilon - 1)}{(2\epsilon + 1)} - \frac{(n^2 - 1)}{(2n^2 + 1)}$$

Where,

υ = Stokes shift in cm^{-1}

h = 6.6262 x 10^{-34} J is Planck's constant.

c = 2.99 x 10^8 $m.s^{-1}$ is the velocity of light.

ϵ_0 = the permittivity of vacuum (8.8542 x 10^{-12} $C^2.N^{-1}.m^{-2}$) .

The sensitivity of this fluorophore to solvent polarity is due to a charge shift. The solvent sensitivity of fluorophore has been estimated by a Lippert-Mataga plot. The plot of Stokes shifts versus the polarity function Δf (fig 2.3)

has shown the good correlation coefficient of Stokes shifts with solvent polarity. This result indicates that the DHPM has an ICT characters in the excited state and also the dielectric solute-solvent interactions are responsible for the observed solvatochromic shift for the present molecule.

2.2.2 *Fluorescence quenching studies:*

Fig.2.4 shows the fluorescence quenching of anthracene (D) with the addition of DHPM (A). From the figure, it is observed that the fluorescence intensity of the donor (anthracene) decreased regularly with increasing concentration of DHPM, indicating non-radiative energy transfer between the excited donor and the accepter. The kinetics of quenching of anthracene was studied by using Stern-Volmer relation. The plot of F_0/F versus concentration of quencher is shown in fig. 2.5, where F and F_0 are the fluorescence intensities in presence and absence of DHPM respectively. The Stern-Volmer plot is straight line with intercept having value one on Y-axis and indicates validity of Stern-Volmer equation given below [35,36].

$$F_0/F = 1 + k_q \tau [Q] = 1 + K_{sv} [Q] \qquad \ldots\ldots (3)$$

From the linear portion of the curve, the value of quenching rate constant k_q obtained by following relation

$$k_q = K_{sv}/\tau \qquad \ldots\ldots (4)$$

Where , K_{sv} is the Stern-Volmer constant obtained from slope i.e. 2.52×10^3 mol^{-1} dm^3 and The value of τ = 3.53 ns (1 ns = 1×10^{-9} S) was measured on Time Resolved Fluorimeter. The estimated value of k_q was found to be 7.145 $\times 10^{11}$ $dm^3 mol^{-1} s^{-1}$.

2.2.3 *Energy transfer between DHPM and Anthracene:*

FRET is the distance- dependent transfer of energy from a donor molecule to an accepter molecule. According to Forster's theory, there are many factors that influence resonance energy transfer. The primary conditions that need to be met in order to occur FRET are relatively few. a) The donor and accepter molecules must be in close proximity to one another (typically 10-100 ^{0}A) b) The absorption or excitation spectrum of the accepter must overlap with

the fluorescence emission spectrum of the donor. c) The donor and accepter transition dipole orientations must be approximately parallel. These conditions have been optimized as shown in the fig 2.6. Using Forster non-radiative energy transfer efficiency, E depends not only on distance (r) between D and A but also on the critical energy transfer distance (R_o) expressed by the following relation [32].

$$E = 1 - (F/F_0) = \frac{R_0^6}{R_0^6 + r^6} \qquad \text{.....(5)}$$

Where, F and F_o are the fluorescence intensities of anthracene in presence and absence of DHPM, R_o is the critical distance, which is estimated from following equation,

$$R_0^6 = 8.8 \times 10^{-25} \times K^2 \times N^{-4} \times \Phi \times J \qquad \text{.....(6)}$$

Where,

K^2 = 2/3 = the factor expressing the spatial orientation of dipole,

N = Refractive index of medium (donor)

Φ = the fluorescence quantum yield of the donor,

J = the overlap integral of fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor is given by,

$$J = \int \frac{f(v)\, E(v)\, dv}{v^4} \qquad \text{.....(7)}$$

Where, F (v) is the fluorescence intensity of donor at wavelength λ,

E (v) is the molar absorption coefficient of acceptor at wavelength λ. From the overlapping of absorption spectra of acceptor and the fluorescence spectra of donor, $J = 6.009 \times 10^{-14}$ cm^3 L mol^{-1} can be determined by integrating the spectra. In the present case K= 2/3, N= 1.4203, Φ = 0.4102 according to equation (5-6), we could calculate the R_o = 5.23 nm, E = 0.23 and r = 6.39 nm. For Anthracene-DHPM system, the average distance, r < 8 nm which indicates

that non-radiative energy transfer from anthracene to DHPM occurs with high probability [37].

2.3 CONCLUSION

The titled compound 5- Ethoxycarbonyl-4-cinnamyl -6-methyl-3, 4-dihydropyrimidine-2(1H)-one (DHPM) was synthesized and characterized by NMR , IR spectral data. The photophysical properties of DHPM were investigated by UV-Visible, fluorescence spectra and fluorescence life time measurements in various solvents. The quantum yield of the compound differs slightly with solvent polarity. The fluorescence of anthracene was found to be quenched and quenching is in accordance with Stern-Volmer relation. The Stern-Volmer constant (K_{sv}) 2.52 x 10^3 mol^{-1} dm^3, quenching rate constant (kq = 7.145 x10^{11} dm^3mol^{-1} s^{-1}) and using FRET, distance (r = 6.39 nm) between Anthracene (donor) and DHPM (acceptor) using FRET were calculated.

2.4 TABLE AND FIGURES

Table I - Spectroscopic data of 5- Ethoxycarbonyl-4-cinnamyl -6-methyl-3, 4-dihydropyrimidine-2(1H)-one (DHPM) in various solvents:

Solvent	Solvent polarity, (ΔF)	$\lambda_{abs(nm)}$	λ_{em} (nm)	Є,dm^3 $cm^{-1}mol^{-1}$	$\Delta v^{-}cm^{-1}$ Stoke's shift	Quantum yield, Φ
1,4-Dioxan	0.0211	356	476	1.034×10^3	7081.48	0.0508
Chloroform	0.1498	356	484	2.130×10^3	7428.73	0.0127
THF	0.2243	352	481	2.393×10^3	7619.07	0.0144
Butanol	0.2641	354	489	3.059×10^3	7798.68	0.0071
Formamide	0.2756	358	486	2.232×10^3	7356.82	0.0150
Propanol	0.2775	354	488	2.037×10^3	7756.78	0.0100
2-Methyl propanol	0.2857	354	489	2.151×10^3	7798.68	0.0117
Ethanol	0.2890	356	488	2.260×10^3	7598.08	0.0108
Acetonitrile	0.3056	352	488	2.061×10^3	7917.28	0.0112

FIGURES:

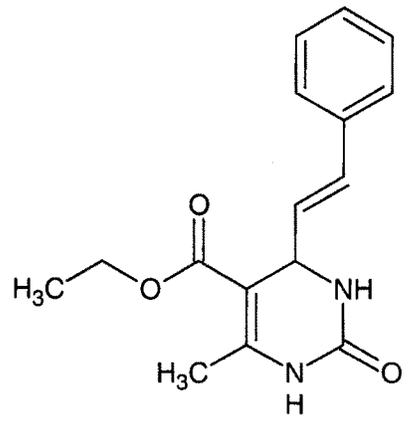

Fig.2.1: Molecular Structure of 5-Ethoxy carbonyl-4-cinnamyl-6-Methyl-3, 4-dihydropyrimidine-2(1H)-one (DHPM)

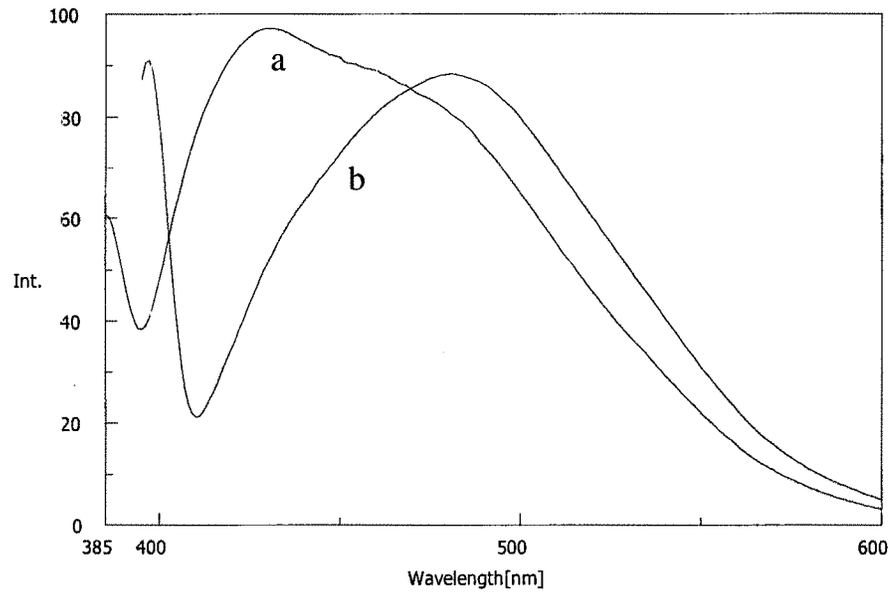

Fig.2.2 : Fluorescence spectra of DHPM. (a) 1 x 10^{-3} M in 1,4 dioxane monitored at 383 nm excitation wavelength. (b) 1 x 10^{-3} M in THF at 394 nm excitation wavelength.

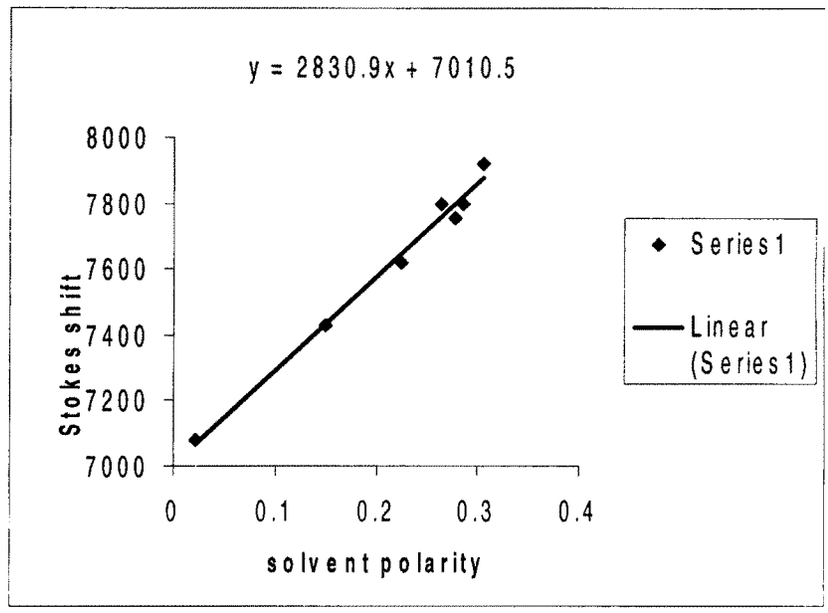

Fig. 2.3: A plot of Stokes shift Vs solvent polarity

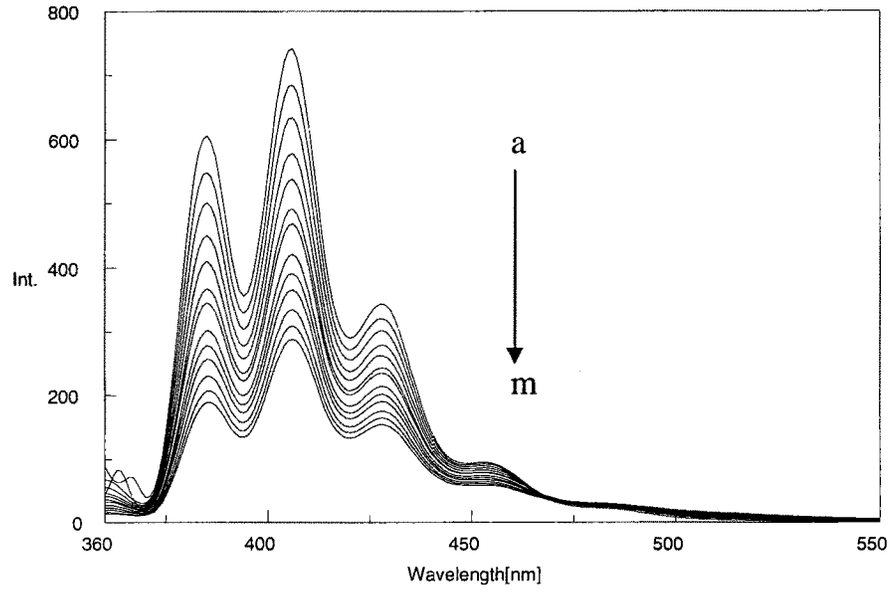

Fig. 2.4: Fluorescence quenching of 10^{-5}M anthracene (1ml) with 10^{-3} M DHPM,

a) 0 ml DHPM , b) 0.25 ml DHPM, c) 0.5 ml DHPM, d) 0.75 ml DHPM, e) 1 ml DHPM, f) 1.25 ml DHPM, g) 1.5 ml DHPM, h) 1.75 ml DHPM, i) 2 ml DHPM, j) 2.25 ml DHPM, k) 2.5 ml DHPM, l) 2.75 ml DHPM, m)3 ml DHPM.

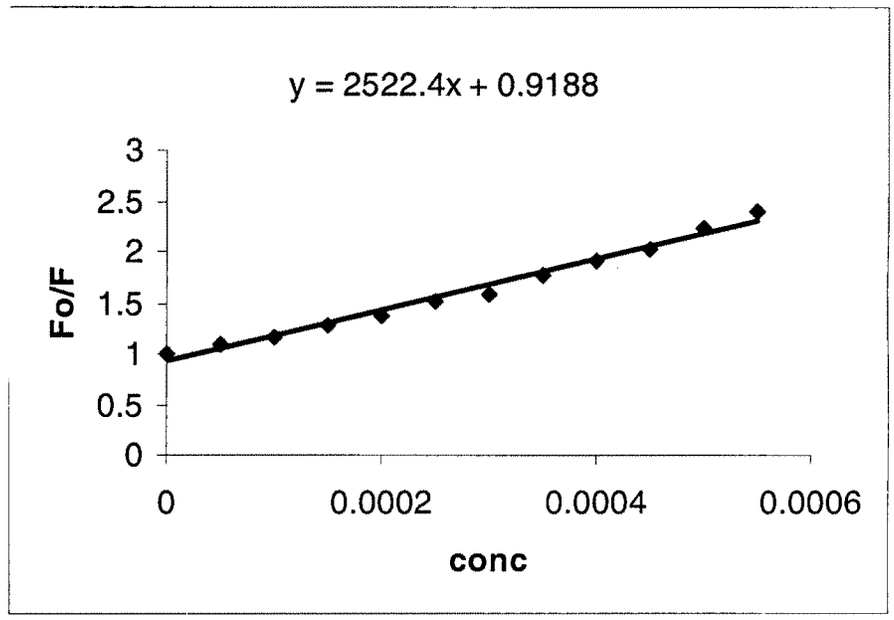

Fig. 2.5: Stern – Volmer plot

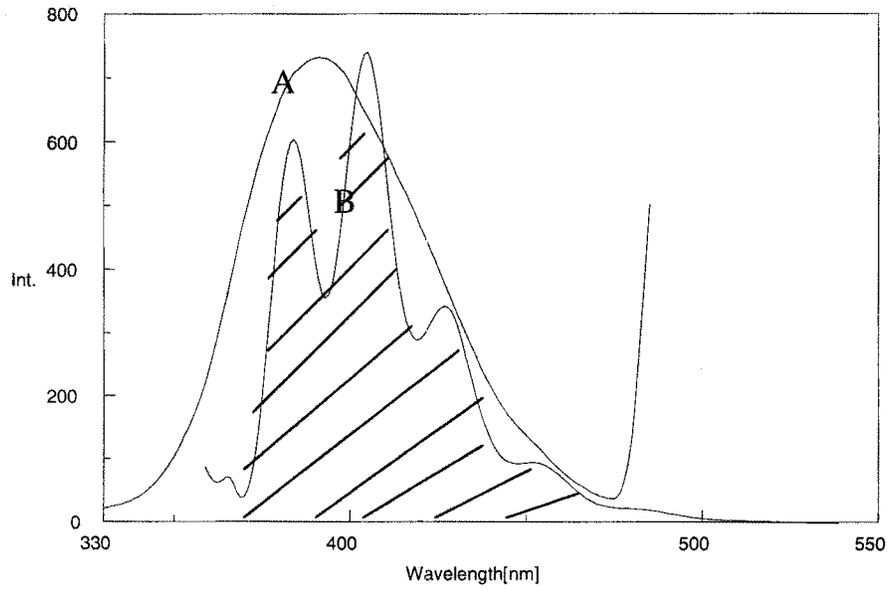

Fig.2.6: Region of overlap between emission spectrum of anthracene (B) and excitation spectrum of DHPM (A).

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