SOLVENT -DEPENDENT SPECTRAL BEHAVIOR AND DIPOLE MOMENT ANALYSIS OF A FLUORESCENT PROBE.

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Abstract : *The influence of aprotic and protic solvents that are used on the dipole moments and absorbance as well as emission spectra of the Eosin Blue have been carefully examined in an assortment of solvents. The dipole moment can be discovered to have a higher level of excited state than in the ground state. The ground state and excited state dipole moment were computed experimentally using the salvatochromic shift in the emission and absorption spectra associated to the dielectric constant (ε) and refractive index (n). In various solvents, the Stokes shift was investigated for the Eosin blue molecule. For the solvents utilized, this causes the molecule to be compared to the ground state, the excited state is more polar.*

Keywords: *Solvatochromic method, dipole moment, stokes shift , fluorescent probe etc.*

1.Introduction: It is a well-known fact that the characteristics of the solvents used in chemical reactions have an impact. These consist of the dielectric constant, dipole moment, refractive index, polarity, and the type of solute-solvent interactions [1-2]. Polarity of the solvent is the most important characteristic that allows solvating any other molecular species or a solute molecule added to the solvent milieu to change the location of the absorption or emission band of molecules**.** However, since fluorescent dye molecules are complex chemical compounds that may contain a charge centre, they are susceptible to changes in absorption in different media [3–10]. The dipole moments of the ground and excited states of organic (dye) compounds can be used to figure out the electrical and geometrical form of molecules. Analysing the solvatochromism of fluorescence and absorption is the easiest and most common method of estimating the dipole moment experimentally. The solvatochromic approach depends on a linear relationship between the solvent polarity function, incorporating the dielectric constant (ε) and refractive index of the medium, and the wave numbers of the fluorescence and absorption maxima [11].Eosin Blue is one kind of bright red dye. Eosin Y and Eosin B are two very similar compounds that make up this synthetic fluorescein derivative. Eosin dyes have been used for decades as histology stains [12–15], but eosin B has been utilized to stain proteins on acid-urea polyacrylamide gels [16–20].

2. Theoretical Underpinnings of the Solvatochromic Shift Method for Ground and Excited State Determination The dipole moment

The ground state and excited state dipole moments of the Eosin blue molecule have been determined using quantum mechanical perturbation theory [21–23] of absorbance ($\bar{\upsilon}_a$) and fluorescence ($\bar{\upsilon}_f$) band shifts (in wave numbers) in several solvents with different permittivity (ε) and refractive index (n). The ensuing equations are as follows. These are symbolized by the following equations:

$$\bar{\upsilon}_a - \bar{\upsilon}_f = m_1 F_{LM}(\varepsilon, n) + constant \qquad \text{Lippert-Mataga [LM] equation} \qquad (1)$$

$$\bar{\upsilon}_a - \bar{\upsilon}_f = m_2 F_{BKS}(\varepsilon, n) + constant \qquad \text{Bakshiev's [BKS] equation} \qquad (2)$$

$$\frac{\bar{\upsilon}_a + \bar{\upsilon}_f}{2} = m_3 F_{KCV}(\varepsilon, n) + constant \qquad \text{Kawski's -Chamma-Viallet [KCV] equation} \qquad (3)$$

while the solvent polarity parameters, denoted as F_{LM}, F_{BKS} and F_{KCV}, are expressed as

$$F_{LM}(\varepsilon, n) = \left[\left(\frac{(\varepsilon-1)}{2\varepsilon-1}\right) - \left(\frac{n^2-1}{2n^2-1}\right)\right] \qquad (4)$$

$$F_{BKS}(\varepsilon, n) = \left[\frac{(2n^2-1)}{(n^2+2)}\right]\left[\left(\frac{\varepsilon-1}{\varepsilon+2}\right) - \left(\frac{n^2-1}{n^2+2}\right)\right] \qquad (5)$$

$$F_{KCV}(\varepsilon, n) = \left\{\frac{1}{2}\frac{(2n^2+1)}{(n^2+2)}\left[\frac{\varepsilon-1}{\varepsilon+2} - \frac{n^2-1}{n^2+2}\right] + \frac{3}{2}\left[\frac{n^4-1}{(n^2+2)^2}\right]\right\} \qquad (6)$$

where "n" and "ε," the refractive index and dielectric constant, respectively, are the absorption maxima wavelength and fluorescence maxima wavelength in cm^{-1}, respectively. Equation (1), (2), and (3) have been used to plot the graphs that follow: ($\bar{\upsilon}_a$ - $\bar{\upsilon}_f$) vs F_{LM} (ε, n), ($\bar{\upsilon}_a$ - $\bar{\upsilon}_f$) vs F_{BKS}(ε, n) and $\frac{\bar{\upsilon}_a+\bar{\upsilon}_f}{2}$ vs F_{KCV} (ε,n). The consequent slopes are m_1,m_2 and m_3 respectively.

$$m_{LM} = \frac{2(\mu_e-\mu_g)^2}{hca^3} \qquad (7)$$

$$m_{BKS} = \frac{2(\mu_e-\mu_g)^2}{hca^3} \qquad (8)$$

$$m_{KCV} = \frac{2(\mu_e^2-\mu_g^2)}{hca^3} \qquad (9)$$

where μ_e and μ_g denote the dipole moments of the ground and excited states of molecules, respectively. The Onsager cavity radius of the molecule is represented by the initial "a," while the numeric values the letters "h" and "c" represent Plank's constant and the speed of light in vacuum, respectively. The atomic increment approach developed by Edward is used to determine the value of "a." Formulae (8) and (9) provide a foundation for the following equations (10) and (11).

$$\mu_g = \frac{m_{KCV} - m_{BKS}}{2}\left[\frac{hca^3}{2m_{BKS}}\right]^{\frac{1}{2}} \quad \text{For } (m_{KCV} > m_{BKS}) \qquad (10)$$

$$\mu_e = \frac{m_{KCV} + m_{BKS}}{2}\left[\frac{hca^3}{2m_{BKS}}\right]^{\frac{1}{2}} \qquad (11)$$

3. Materials and Methods:

The Eosin Blue has been utilized precisely as supplied by SISCO Research Laboratories PVT LTD. This study employed commercially available spectroscopic-grade solvents from Sigma Aldrich, such as propanol, butanol, octanol, decanol, benzoene, chlorobenzene, toluene, THF, DMSO, and acetone. The solutions were carefully produced at concentrations of 0.1mM to avoid self-quenching mechanisms and enable characterizations across various solvent mediums. Electronic absorption spectra have been collected using a Hitachi model 150-20 UV-visible spectrophotometer, and the spectra of fluorescence were acquired using a JASCO FP-8250 Spectrofluorometer [24–25].

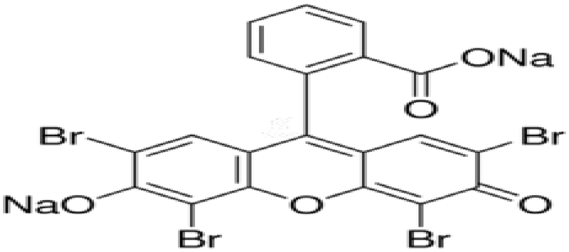

Fig 1. Eosin Blue dye's molecular structure

4.Results and Discussion:

The emission and variation in absorption of Eosin blue in different Solvents were observed. The polarity functions of the solvent F_{LM} (ε,n), F_{BKS}(ε,n), and F_{KCV}(ε,n) were calculated using Equations (4), (5), and (6) for a set of different solvents with different polarities (given in Table 2). The maximum fluorescence of the fluorescent probe show a bathochromic change

for Eosin blue, as shown in Figure 2, nonlinear interactions between solutes and solvents may have an impact on the charge distribution in the excited state.

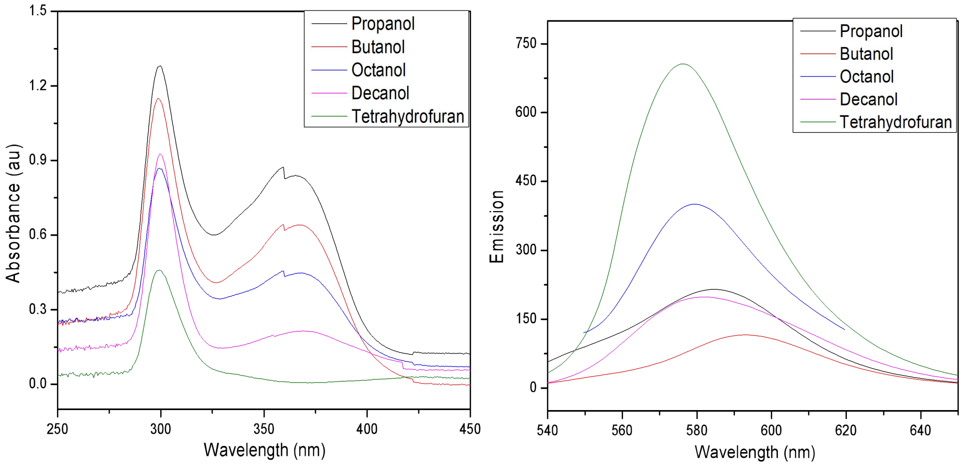

Fig 2: Spectra of absorbance and fluorescence of Eosin Blue in different solvents.

Table 1: Experimental fluorescence and absorption properties and Stokes shift of Eosin Blue in different solvents.

Solvents	λ_{abs} (nm)	λ_{emi} (nm)	$\overline{\upsilon}_a$ (cm^{-1})	$\overline{\upsilon}_f$ (cm^{-1})	($\overline{\upsilon}_a$-$\overline{\upsilon}_f$) (cm^{-1})	($\overline{\upsilon}_a$+$\overline{\upsilon}_f$)/2 (cm^{-1})
Propanol	298	585	33557	17094	10463	28325.5
Butanol	299	593	33444	16863	10451	28218.5
Octanol	299	579	33444	17271	10350	28269
Decanol	300	582	33333	17182	8019	28884.5
Benzene	309	414	32362	24154	8208	28258
Chlorobenzene	298	416	33557	24038	9519	28797.5
Toluene	305	477	32786	20964	11822	26875
THF	299	576	33444	17361	16239	26933
DMSO	302	487	33112	20533	12579	26822.5
Acetone	358	487	27932	20533	7399	24232.5

Table 2: Solvent polarity functions and solvent parameters

Solvents	ε	n	F_{LM}	F_{BKC}	F_{KCV}	E_T^N
Propanol	18.5	1.39	0.2689018	0.762776	0.646533	0.617
Butanol	13.45	1.399	0.2514619	0.700347	0.621317	0.586
Octanol	10.3	1.429	0.2255941	0.626758	0.60441	0.537
Decanol	7.93	1.435	0.2040895	0.550959	0.570468	0.525
Benzene	2.28	1.5589	-0.001193	-0.03143	0.359218	0.111
Chlorobenzene	5.62	1.524	0.14297	0.51147	0.268262	0.2
Toluene	2.379	1.497	0.01311	0.02879	0.34980	0.0987
THF	7.6	1.407	0.2088773	0.546618	0.549905	0.207
DMSO	46.7	1.479	0.263	0.841	0.699	0.444
Acetone	20.7	1.358	0.24650	0.79028	0.63952	0.3549

Subsequently, The absorption transition is controlled by the emissions transition and which raises the excited state's dipole moment. Furthermore, alterations in the Stokes shifts and the fine spectral band show the intramolecular charge transfer (ICT) coming from the singlet state's π→π* transition. The maximal absorption (Eq 1), fluorescence emission maximum (Eq 2), and Stokes shift (Eq 3) were developed by comparing their spectroscopic properties with those of Lippert-Mataga, Bakshiev, Kawski-Chamma-Viallet, and Reichardt. The Eosin blue solvatochromic plots can be shown in Figure 3, and a slope intercepts and coefficients for correlation are listed in Table 3.

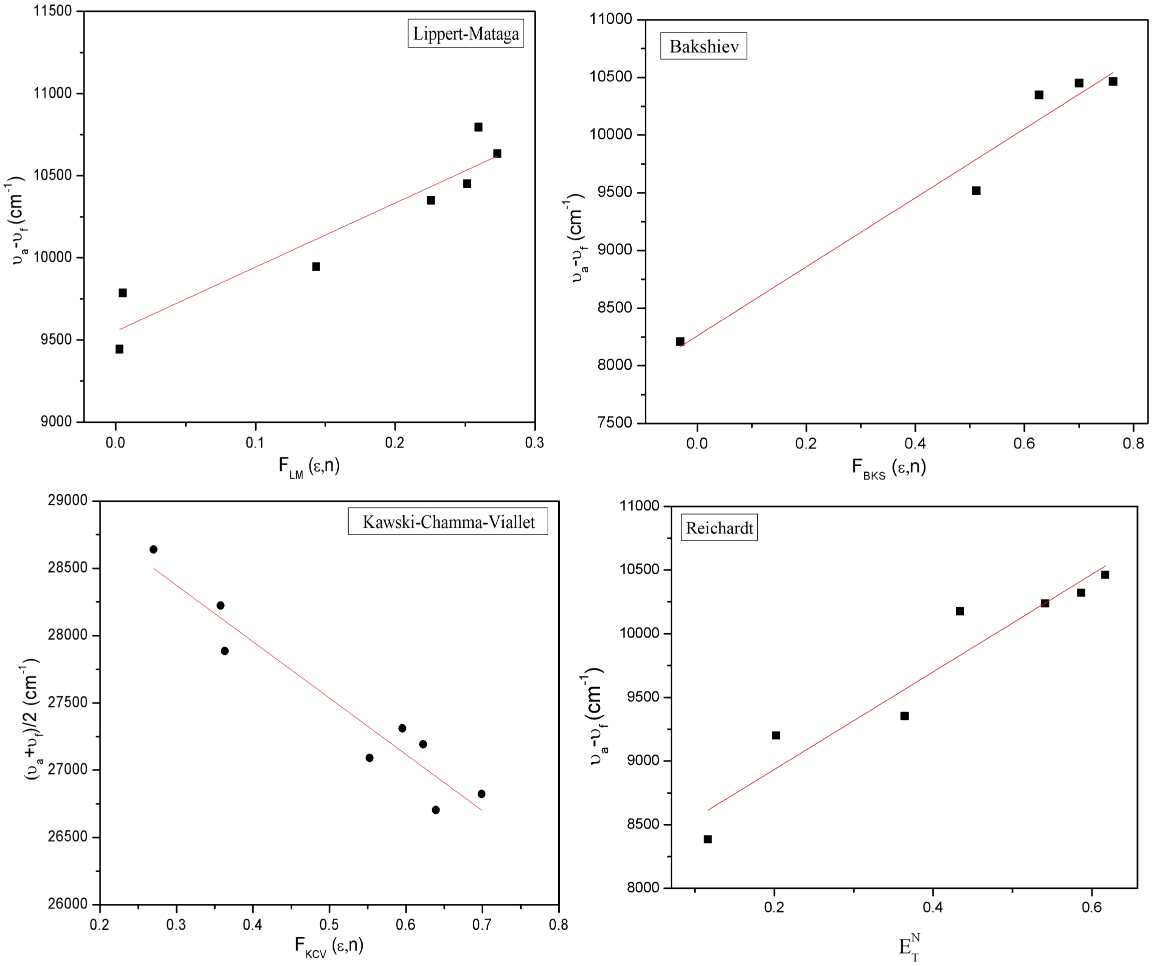

Fig 3: Linear progression plots using stokes shifts, $(\upsilon_a+\upsilon_f)/2$ and solvent polarity function for Eosin Blue molecule

Table 3 shows ground state (μ_g), excited state (μ_e), and the excited to ground state dipole moment ratio ($\frac{\mu_e}{\mu_g}$), and with changes in dipole moments ($\Delta\mu_e$) of the Eosin blue, that are were established using an array of methodologies. The relevance disparity between the fluorophore's ground and excited state dipole moments demonstrates that an internal charge transfer process contributes for the considerable distribution of charges the within the single excited state. The excited state's dipole moment exceeds that compared to the ground state, as illustrated in Table 4, indicating that the Eosin blue molecules exhibit greater polarity.

and solvent-sensitive in the excited state.

Table 3: Analysis of statistical data and correlation of the spectral shifts of molecule.

Method	Slope (cm^{-1})	Intercept (cm^{-1})	Correlation coefficient (r)	No. of data points
Lippert- Mataga	m_1=4696	9703	0.38495	7
Bakshiev	m_2=2992	8260	0.98172	5
Kawski-Chamma-Viallet	m_3=4233	29619	0.92293	8
Reichardt	m_4=3830	8168	0.90114	7

Table 4: Onsager cavity radius of the molecule's ground and excited state dipole moments

Onsager cavity radius 'a' (Å)	μ_g^a (D)	μ_e^b (D)	μ_e^c (D)	μ_e^d (D)	μ_e^e (D)	μ_e^f (D)	Δ (D)	μ_e^g	$\frac{\mu_e}{\mu_g}$

3.885	0.87	5	6.10	5.8	5	3.468	4.2	5.82

μ_g^a Ground state dipole moment
μ_e^b The Kawski-Chamma-Viallet Equation is used for computing the excited state dipole moment.
μ_e^c Bakhshieve's equation is used to compute the excited state dipole moment.
μ_e^d The Lippert-Mataga Equation is used to compute the excited state dipole moment.
μ_e^e The E_T^N Equation is used to compute the excited state dipole moment.
μ_e^f The McRae Equation is used to compute the excited state dipole moment.
$\Delta\mu_e^g$ Change relative dipole moment between μe and μg.
$\frac{\mu_e}{\mu_g}$ The ground dipole moment to excited state ratio

5.Conclusions:

In this investigation, the solvatochromic properties of Eosin blue were studied using Spectroscopy involving absorption and emission. The measured solvent effect on the absorbed and peak emission values in the recorded spectrum data showed substantial relationships between the molecules of the solute and solvent. The emission spectra exhibited a distinct bathochromic shift when the polarity of the solvent approached.. This indicates that the excited condition has steady with relation to the ground state.

The Lippert-Mataga, Bakshiev, Kawski-Chamma-Viallet, and Reichardt equations produced good correlations, confirming the continuum solvent model's suitability for the current system. Different equations were accustomed to determine the excited and ground state dipole moments.The intermolecular charge transfer was confirmed by an observation implies the dipole moment in the excited state 4.2 Debye, while the dipole moment in the ground state is 3.4682 Debye. In brief, the results suggest Eosin Blue has substantial solvatochromism and that the polarity of the solvent has an impact and dipolar effects dominate the interactions.

The study reveals crucial information into the charge transfer in Eosin Blueand's excited state, and the results justify the compound's applicability as a probe in the research of solvents and microenvironmental effects in photophysics and spectroscopy.

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