Solvatochromism Effect of Different Solvents on UV-Vis Spectra of Floureseceine and its Derivatives

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ABSTRACT: *The solvatochromism of fluoresceine and its derivatives was studied in solvents of different Hydrogen Bond Donor (HBD), Hydrogen Bond Acceptor (HBA), Donor Number (DN) and Acceptor Number (AN) by their UV-Vis spectra. Results showed that position, intensity and shape of absorption bands change with type of solvent. These changes can be rationalized by solvatochromic parameters such as α, β, E_T (30), DN and AN using multiple linear regression (MLR) technique. Correlation coefficients of obtained equations were 0.965-0.999.*

KEY WORDS: *Solvatochromism, Fluoresceine and its derivatives, MLR method, Linear Solvation Energy Relationship (LSER).*

INTRODUCTION

Fluoresceine and its derivatives are usually used as sensitive probes in labeling of proteins and lipids in analytical methods [1-5]. Their UV-Vis absorption spectra can be influenced by interaction with solvents. So, they can act as sensitive probes for different solvents. Taft and Kamlet's linear free energy relationship method rationalizes solvent effects in terms of a linear combination, which depends on five fundamental parameters. These solvatochromic parameters used in linear salvation energy relationships (LSER) are as the following general form [6]:

$$(XYZ)_S=(XYZ)_0+ a\alpha+ b\beta + e\ E_T(30) + d\ DN + n\ AN \quad (1)$$

In this equation $(XYZ)_S$, $(XYZ)_0$, α, β[6-8], DN[9] and AN[10] are the property to be correlated (the maximal absorption energy), a constant $(XYZ)_0$ is the solute property in a given solvent or in a hypothetical solvent in which $\alpha = \beta = E_T(30) = DN = AN = 0$), index of solvent hydrogen bond donor (HBD) or acidity, index of solvent hydrogen bond acceptor (HBA) or basicity, donor number scale and acceptor number scale, respectively.

The coefficients a, b, e, d and n are related coefficient for each polarity scale. The $E_T(30)$ scale is based on the negatively solvatochromic pyridinium N-phenolate betaine dye as probe molecule and its values are simply defined as the molar electronic transition energies (E_T) of dissolved betaine, measured in $Kcal.mol^{-1}$ at room temperature (25 °C) and standard pressure (1 bar), according to the following equation:

$$E_T\ (30) = hc\ N_A\ /\lambda_{max} \quad (2)$$

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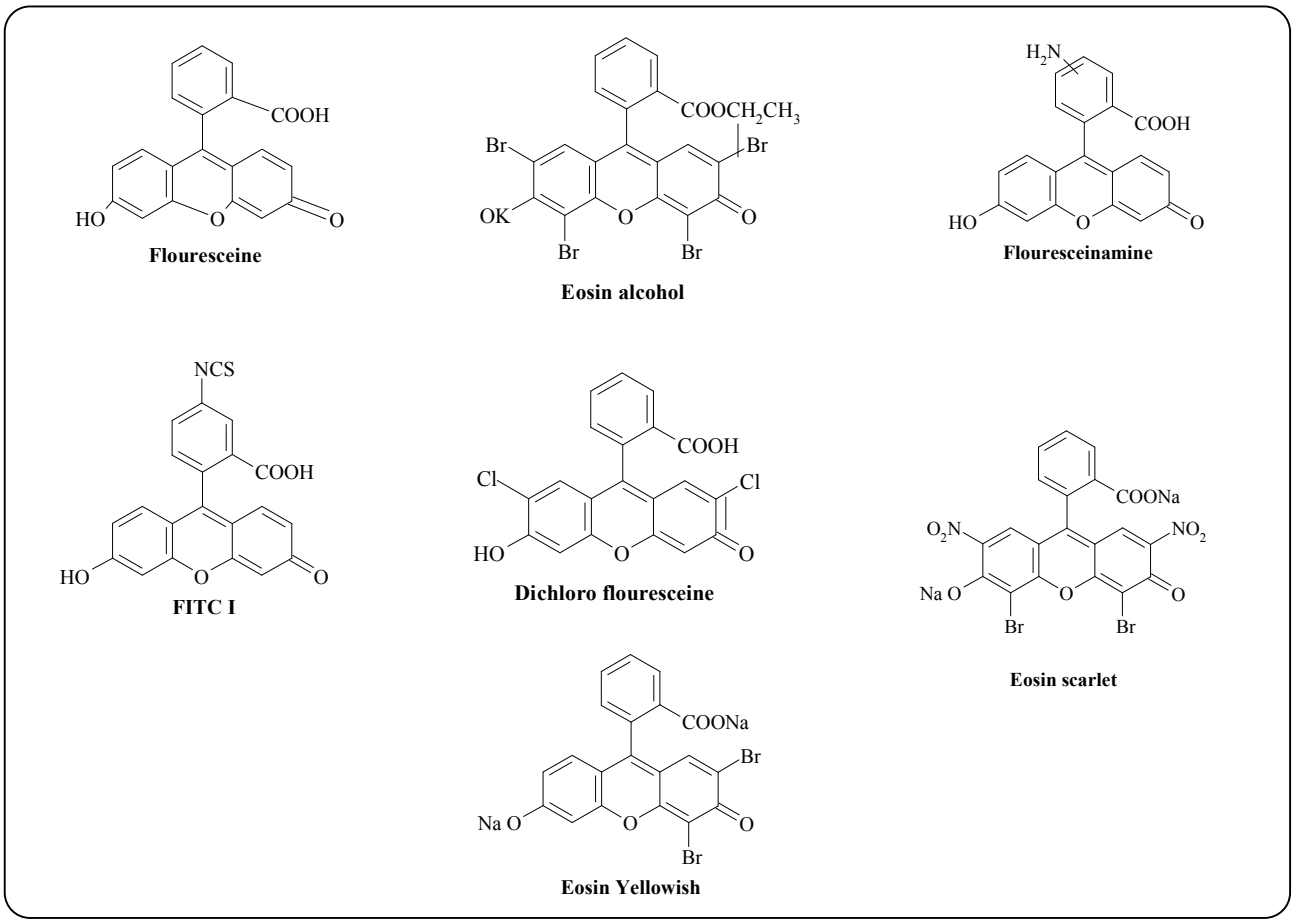

Fig. 1: The structure of Fluoresceine and its derivatives (probes)

In this equation, h, c, λ_{max} and N_A are Plank constant, speed of light, maximum wavelength of the intramolecular charge transfer $\pi \rightarrow \pi^*$ absorption band of betaine and Avogadro number, respectively. Negative solvatochromism of betaine dye is related to the reduction dipole moment of the electronic excited state by a considerable charge transfer from O^- phenolate to the pyridinium part of the betaine molecule [11-14]. In this project, intermolecular hydrogen-bonding intraction, electron donating and accepting between fluoresceine and its derivatives with different solvents were studied. Transition of O^- phenolate of fluoresceine and its derivatives to the π or π^* level was also studied.

EXPERIMENTAL

Apparatus

All absorption spectra were obtained using double beam UV-Vis CECIL 5505 with 0.5cm pathlength cells and photomultiplier tube detector (Cambridge England). Data were analyzed using multiple linear regressions (*backward search*) with SPSS software (Version 11.0, LEAD Technologies, Inc.)

Materials and method

Fluoresceine, 2′, 4′,5′,7′-Tetrabromoeosin ethyl-acetate (eosin alcohol), Fluoresceinamine, 4′,5′-dibromo-2′,7′-dinitrofluorescein disodium salt (eosin scarlet), Fluorescein -5- iso- thiocyanate (FITCI), 2′,4′,5′,7′- tetrabromo fluorescein disodium salt (eosin yellowish) and 2′,7′-dichloro fluoresceine shown in Fig. 1 were purchased from Fluka (Buchs, switzerland) and used without further purification. All solvents including acetic acid, acetone, acetonitrile, benzene, n-butanol (BuOH), chloroform, dichloromethane, diethylether, N,N′-dimethyl formamide (DMF), N,N′-dimethyl sulfoxide (DMSO), dioxane, ethyl acetate, ethanol (EthOH), n-hexane, methanol (MeOH), n-propanol (n-PrOH), l-propanol (l-PrOH), tetra-chloromethane,

toluene, tetrahydrofurane (THF), and p-xylene were UV grade from Fluka (Buchs, switzerland). Water used was deionized doubly distilled.

Stock solutions of 0.0005 M of all probes were prepared separately in each solvent and then diluted to a proper concentration, whenever needed. Each solution was scanned in the range of 190-500 nm.

RESULTS AND DISCUSSION

Solvatochromism of fluoresceine and its derivatives

Solvatochromism is caused by differential solvation of the ground and the first excited state of the light-absorbing molecule. If with increasing solvent polarity, the ground state molecule is better stabilized by solvation than the molecule in the excited state, *negative solvatochromism (blue shift)* will result, or vice versa, better stabilization of the molecule in the first excited state relative to the ground state, with increasing solvent polarity, will lead to *positive solvatochromism (red shift)*. The solvents used were arranged with increasing their dielectric constant as shown in table 1. The maximum absorption wavelength (λ_{max}) obtained for the probes in all solvents, are presented in table 2. The maximal energy, E_{max}(Kcal.mol^{-1}) due to λ_{max} were calculated using the following equation:

$$E_{max} = N_A\, hc / \lambda_{max} \qquad (3)$$

where λ_{max} is the maximum absorption wavelength of solution. In order to show positive or negative solvatochromism for each probe, the wave number related to λ_{max} of the most polar solvent was substracted from that of the most nonpolar solvent (table 3) and considered as $\Delta\bar{\nu}$. Positive and negative sign of $\Delta\bar{\nu}$ is an indication that probe has red or blue shift, respectively.

Relationship between E_{max} and polarity scales of solvents using LSER model

Each of polarity scales in LSER equation was previously obtained from a standard probe and shows a kind of interaction between probe and solvent. It is assumed that the interactions between fluoresceine and its derivatives with solvents used are similar to those of standard probes. The solvent polarity scales (α, β, E_T(30), DN, AN) are reported previously [6-10]. To obtain the best LSER equation for each probe, solvents were divided in two groups using index of solvent hydrogen bond donor (α). The first group includes the solvents with α = 0 and the second group with α ≠ 0. The LSER equation parameters of the backward regression applied to Eq. (1) for all probes in the first and second groups of solvents are presented in tables 4 and 5, respectively.

Table 1: Dielectric constant (D) of solvents.

Solvent	D
Water	78.39
DMSO	46.68
Acetonitril	37.5
DMF	36.71
Methanol	32.70
Ethanol	24.55
Acetone	20.70
n-PrOH	20.33
i-PrOH	19.92
n-BuOH	17.51
Dichloromethane	8.93
THF	7.58
Acetic acid	6.15
Ethylacetate	6.02
Chloroform	4.806
Diethylether	4.335
Toluene	2.379
Benzen	2.275
p-Xylene	2.269
CCl_4	2.238
Dioxane	2.209
Hexane	1.879

As shown in table 4, E_{max} shows correlation with the β for probes 3,4,5,6 and 7, while for probes 1 and 2, no statistical significance was observed since the coefficient values for β are smaller than the error calculated from SPSS software [15]. The β parameter for the second group of solvents was observed for probes 2, 4 and 6 (table 5). The absence of correlation E_{max} with β could be due to low acidity of the probe.

Probes 4, 6 and 7 are moderately sensitive to the HBD ability (α) of the second group of solvents. The correlations give similar coefficients for these probes,

Table 2: The maximal absorption wavelength, λ_{max} of Fluoresceine and its derivatives.

solvent	$\lambda_{max\ 1}$	$\lambda_{max\ 2}$	$\lambda_{max\ 3}$	$\lambda_{max\ 4}$	$\lambda_{max\ 5}$	$\lambda_{max\ 6}$	$\lambda_{max\ 7}$
Acetic acid	246.7	250.2	248.5	248.2	251.3	249.5	247.5
Acetone	204.7	202.5	203.7	204.2	204.2	203.5	211.8
Acetonitril	221.4	204.5	225.5	193.5	193.4	242.7	193.5
Benzen	-	-	-	-	-	-	283.2
n-BuOH	231.2	259.5	236.2	230.2	230.2	260.7	233.5
Chloroform	236.9	-	238.0	-	-	-	246.7
Dichloromethane	-	-	211.0	-	-	-	236.0
Diethyl ether	223.6	-	240.7	-	-	235.9	229.2
DMF	277.9	272.9	278.7	270.0	270.0	269.7	285.5
DMSO	278.9	272.7	278.5	258.9	258.9	268.7	286.5
Dioxane	225.6	212.0	261.0	191.2	191.2	194.2	228.0
Ethylacetate	-	252.7	276.5	250.7	250.7	-	283.2
EthOH	223.5	193.2	223.9	192.7	192.7	194.2	229.2
Hexane	-	223.5	204.7	239.0	239.0	192.2	-
MeOH	224.7	198.5	229.2	195.2	195.2	197.7	228.5
n-PrOH	225.0	213.7	225.7	194.2	194.2	195.0	228.7
i-PrOH	225.7	200.2	224.5	194.5	194.5	194.2	229.0
CCl_4	-	-	-	-	-	-	-
Toluene	-	-	-	-	-	-	283.0
THF	238.7	250.9	245.2	239.0	239.0	234.0	238.02
Water	235.7	254.5	225.5	-	-	193.7	255.5
p-Xylene	-	-	-	-	-	-	-

1: Fluoresceine, 2: Eosin alcohol; 3: Fluoresceinamine, 4: Eosin scarlet, 5: Fluoresceine iso-thiocyanate, 6: Eosin yellowish, 7: Dichloro fluoresceine:

Table 3: The solvatochromism of fluoresceine and its derivatives

Compound	$\bar{\nu}_{max}$ most nonpolar solvent (cm^{-1})	$\bar{\nu}_{max}$ most polar solvent (cm^{-1})	$\Delta\bar{\nu}$ (cm^{-1})	Solvatochromism
Fluoresceine	$4.43*10^4$	$4.24*10^4$	$+ 0.19*10^4$	+
Fluoresceinamine	$3.83*10^4$	$4.43*10^4$	$- 0.6*10^4$	-
Eosin alcohol	$4.72*10^4$	$3.93*10^4$	$+ 0.79*10^4$	+
Fluoresceine iso-thiocyanate	$3.62*10^4$	$3.63*10^4$	$- 0.011*10^4$	-
Dichloro Fluoresceine	$4.38*10^4$	$3.91*10^4$	$+ 0.47*10^4$	+
Eosin scarlet	$4.28*10^4$	$3.86*10^4$	$+ 0.42*10^4$	+
Eosin Yellowish	$5.136*10^4$	$5.163*10^4$	$- 0.027*10^4$	-

Table 4: The LSER equation prarmeters of probes with first group of solvents (α = 0).

Polarity scale	1	2	3	4	5	6	7
	coefficient	coefficient	coefficient	coefficient	Coefficient	coefficient	coefficient
Constant	151.60	347.66	298.69	517.19	164.72	148.60	462.62
β	-	-	-66.09	33.98	-	-	125.96
β^2	-	-	151.64	-	124.66	-132.12	-
1/β	-	-	-	-	-	-	-
$E_T(30)$	-	-7.11	-5.10	-12.73	-	-	-11.94
$E_T(30)^2$	-	-	-	-	-0.06	-	-
$1/E_T(30)$	-	-	-	-	-	-	-
DN	-1.48	-	-	-	-	-	-
1/DN	-	-	-	-	-	-	-
AN	-0.37	4.10	-	7.47	-	1.58	4.32
AN^2	-	-	-	-	-0.05	-	-
1/AN	-	-	-	-	-	-	-
R^*	0.981	0.990	0.995	0.988	0.999	0.989	0.965

*1: Fluoresceine, 2: Eosin alcohol; 3: Fluoresceinamine, 4: Eosin scarlet, 5: Fluoresceine iso-thiocyanate, 6: Eosin yellowish, 7: Dichloro fluoresceine, *Correlation coefficient.*

Table 5: The LSER equation parameters of probes with the second group of solvents (α ≠ 0).

Polarity scale	1	2	3	4	5	6	7
	coefficient	coefficient	coefficient	coefficient	Coefficient	coefficient	coefficient
Constant	88.82	64.67	86.11	-65.60	116.38	-174.50	282.09
α	-	-	-	-	-	-66.30	-69.17
α^2	-	-	-	-73.14	-	-	-
1/α	-	-	-	-	-	3.15	-1.13
β	-	-167.51	-	-83.07	-	-	-
β^2	-	-	-	-	-	-	-
1/β	-	-	-	-	-	40.90	-
E_T (30)	0.69	1.97	-	4.62	-	-	-
$E_T (30)^2$	-	-	0.01	-	-0.006	0.05	-
1/ E_T (30)	-	-	-	-	-	-	-5626.87
DN	0.27	4.18	0.36	2.73	0.52	5.29	0.39
1/DN	-	-	19.17	-	-	-	-7.07
AN	-0.38	-	-0.32	-	-	-	-
AN^2	-	-0.02	-	-	-	-	-
1/AN	267.20	-	396.36	-	335.96	-	-
R*	0.982	0.983	0.973	0.994	0.950	0.982	0.960

*1: Fluoresceine, 2: Eosin alcohol; 3: Fluoresceinamine, 4: Eosin scarlet, 5: Fluoresceine iso-thiocyanate, 6: Eosin yellowish, 7: Dichloro fluoresceine, *Correlation coefficient.*

implying similar basic characteristics for these solutes. Correlation between E_{max} of all probes with the E_T (30) scale of solvents show the intramolecular transition of O^- phenolate to the π or π^* level.

As seen in table 5, in all probes, E_{max} shows correlation with DN of second group of solvents. Thus, in the interaction between these probes and solvents, the nucleophilic property of solvents has an important role.

E_{max} for probes 3 and 6 do not have correlation with AN of first group of solvents. For the second group of solvents, there is no correlation between E_{max} and AN for probes 4, 5, 6 and 7 which means that there is no unshared electrons in the probes and all electrons participate in resonance structures.

The magnitude of the coefficients of solvatochromism parameters of Eq. (1) shown in table 4 and 5 represent the measured of the special effect of those parameters, in interaction between probes and solvents [16].

Positive sign of the coefficient shows an increase in E_{max} by stabilization of ground state relative to the excited state, whereas coefficient with negative sign indicates a decrease in E_{max} by stabilization of excited state relative to the ground state. The polarity scales for the mixture of solvents could be estimated from plot of E_{max} vs. each polarity scales having λ_{max} of a probe in the mixture of solvents.

CONCLUSIONS

The UV-Vis absorption spectra of fluoresceine and its derivatives (probes) are influenced by the surrounding medium. The solvent can bring about a change in the position, intensity and shape of absorption bands. The interaction between probe and solvent can be described by linear solvation energy relationship (LSER) equation. The obtained equations by Multiple linear regression (MLR) technique and SPSS software showed that polarity parameters (α, β, E_T (30), DN and AN) play an important role in the mechanism of the interactions between probe and solvent. The coefficients derived by SPSS software are a measure of special effect of interaction between probe and solvent (change in λ_{max}).

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