Environment Polarity Effects on Spectral Properties and Dipole Moments of Fluorescein Disodium

Mahsa Khadem Sadigh

Department of Laser and Optical Engineering, University of Bonab, Bonab, Iran

Corresponding author email: mahsa.sadigh@ubonab.ac.ir

Regular paper: Received: Apr.20, 2023, Revised: July 22, 2023, Accepted: July 26, 2023, Available Online: July 28, 2023, DOI: 10.61186/ijop.17.1.11

ABSTRACT— Xanthene and its derivatives are important in medical diagnosis and laser technology. In this work, spectral features of fluorescein disodium were investigated in different environments with different polarity characteristics. Hence, the absorption and fluorescence spectra of this molecule were studied in different environments. Our results show that spectral features of fluorescein disodium depend strongly on substituents in its structure and molecular interactions. To investigate the contribution of various molecular interactions on the spectral behavior of the used sample, the linear solvation energy relationship concept is used. Moreover, the dipole moment variations from ground to excited states were estimated using molecular spectral features in different solvent media. According to the results, the dipole moment value in the excited state is higher than that in the ground state.

KEYWORDS: Dipole moment, Fluorescein disodium, Interaction, Polarity.

I. INTRODUCTION

We live in a world that is surrounded by colorful materials such as dyes. These soluble materials are produced naturally from plants and animals or synthesized artificially by chemists and classified according to their solubility and chemical properties. Investigations have indicated that the photo-physical properties of dyes can be affected in different environments [1]. For this reason, different types of dyes for application in various fields of science have been investigated [2-7]. Despite extensive efforts yet empirical studies in this area continues [8-10]. In recent years, xanthene dyes have been considered due to their applications as a probe in studies of biological systems and sensors, medicine, gain medium in laser dyes, imaging and diagnosis of some tumors [11-17]. Their spectral behaviors depend on the features of their surrounding environment. Depending on the chemical structure of dyes, environment molecules can play a significant role in photo-physical behaviors of them [18-23]. Fluorescein disodium as a member of this group of dyes with high absorption and emission in visible range of electromagnetic spectra is examined in solvent environments with different polarities in this work.

Solvent-dependent mechanism originate from either general (dielectric enrichment) or specific (hydrogen bonding) molecular interactions. Solvent-induced effects can be described using their polarity scales and solvatochromic parameters [1]. There are various phenomena that single solvent polarity parameter cannot explain completely the nature and degree of molecular interactions, so multi-parameter solvent polarity scales are used, commonly. This method is based on a linear free-energy relationship, and known as Kamlet-Abboud-Taft [21] formalism:

$$\Delta\nu = \Delta\nu_0 + a\alpha + b\beta + s\pi^*, \quad (1)$$

where π^*, β, and α (dimensionless parameters) indicate dipolarity/polarizability [24], hydrogen bond acceptor (HBA) basicity [25], and hydrogen bond donor (HBD) acidity [26], respectively. $\Delta\nu_0$ is also a regression value in the reference solvent. The other parameters (a, b and s coefficients) are obtained by multi-

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linear regression analysis, and determine the contribution of solvents in the spectral behavior of solute molecules. Moreover, the spectral shifts of prepared samples in solvents are used as a precise method for calculating the ground and excited states' dipole moments. The data give considerable result about the electronic and geometrical structure of the under-study molecules in both ground and excited states that can be useful in designing nonlinear optical materials. In general, solvent molecules can surround solute molecules in the ground and excited state. Under this condition, molecular interactions between dye molecules and solvent molecules can change solute molecules charge distribution and molecular configuration. These interactions can increase π conjugate length and enhance molecular nonlinear responses.

The aim of this study is the investigation of spectral behaviors of a xanthene dye in environments with different polarities. Hence, absorption and fluorescence spectra of fluorescein disodium were recorded at room temperature. For calculating the dipole moments of the used sample, the solvatochromic method is also used. Dipole moment variations and spectral properties of selected sample in solvents with different polarities will give important information about its function in various optical phenomena.

II. EXPERIMENTAL SECTION

A. *Materials*

Fluorescein disodium (Fig. 1) was prepared in the form of powder and used without further purification. All the used solvents in the study were of the highest available purity from Merk and their features were listed in Table 1.

B. *Absorption and emission spectroscopy*

First, the diluted solution of fluorescein disodium was prepared in various solvents. Then Double beam Shimadzu UV-2450 Scan Spectrophotometer and JASCO FP-6200 Spectrofluorometer were used to record the spectral features of the used sample, respectively. Quartz cuvettes were also used for spectroscopic measurements in solution via 1 cm optical length.

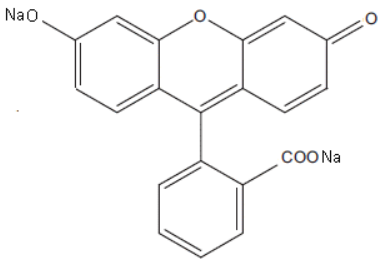

Fig. 1. Chemical structure of fluorescein disodium.

Table 1. Polarity parameters, and polarity functions of employed solvents.

Solvent	α	β	π^*	$f(\varepsilon,n)+2g(n)$	$f(\varepsilon,n)$
Water	1.17	0.47	1.09	1.364	0.912
(DMSO)	0.00	0.76	1.00	1.470	0.840
(DMF)	0.00	0.69	0.88	1.420	0.838
Methanol	0.98	0.66	0.60	1.302	0.854
Ethanol	0.86	0.75	0.54	1.302	0.812
Acetone	0.08	0.48	0.62	1.280	0.792
2-Propanol	0.76	0.84	0.48	1.290	0.778
1.Butanol	0.84	0.84	0.47	1.292	0.750
1.Hexanol	0.67	0.94	0.40	1.252	0.686
1.Decanol	0.7	0.85	0.45	1.145	0.553

C. *Estimation of dipole moments*

Calculation of dipole moment of molecules in ground and excited states give valuable data about the molecular charge distribution. In spite of different techniques, solvent-induced spectral shifts are used for measuring the dipole moments. Kawski and coworkers explained a quantum mechanical second-order perturbation theory for absorption (ν_a) and fluorescence (ν_f) band shifts in solvent environments with different permittivity and refractive index [27-34]. Such solvent effects for difference and the sum of absorption and fluorescence maxima have been defined as follows:

$$\underline{\underline{\upsilon_a}} - \upsilon_f = m_1 f(\varepsilon, n) + \text{const.}, \quad (2)$$

$$\underline{\underline{\upsilon_a}} + \upsilon_f = -m_2 \left[f(\varepsilon, n) + 2g(n) \right] + \text{const.}, \quad (3)$$

$$m_1 = \frac{2\left(\mu_e - \mu_g\right)^2}{hca^3}, \quad (4)$$

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$$m_2 = \frac{2\left(\mu_e{}^2 - \mu_g{}^2\right)}{hca^3}. \quad (5)$$

In general, there is a linear relation between the difference and sum of wave numbers and solvent polarity functions $f(\varepsilon,n)$, g(n), so m_1 and m_2 can be obtained from slopes of the straight lines of absorption and fluorescence band shifts (Eqs. (2) and (3)). Solvent polarity parameters are also expressed as follows:

$$f(\varepsilon,n) = \frac{\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}}{\left[1-\frac{2\alpha(\varepsilon-1)}{a^3(2\varepsilon+1)}\right]\left[1-\frac{2\alpha(n^2-1)}{a^3(2n^2+1)}\right]^2} \quad (6)$$

$$g(n) = \frac{\frac{n^2-1}{2n^2+1}\left[1-\frac{2\alpha(n^2-1)}{a^3(2n^2+1)}\right]^2}{\left[1-\frac{2\alpha(\varepsilon-1)}{a^3(2\varepsilon+1)}\right]} \quad (7)$$

where ε, n, a, and α are dielectric permittivity, refractive index, spherical cavity radius of solute and average polarizability, respectively. For solute molecules with isotropic polarizability, the condition of $2\alpha/a^3 = 1$ is usually satisfied. Therefore, the Eqs. (6) and (7) are converted to Bakhshiev relations.

$$f(\varepsilon,n) = \frac{2n^2+1}{n^2+2}\left[\frac{\varepsilon-1}{\varepsilon+2} - \frac{n^2-1}{n^2+2}\right] \quad (8)$$

$$g(n) = \frac{3}{2}\left[\frac{n^4-1}{\left(n^2+2\right)^2}\right] \quad (9)$$

If the ground and excited state's dipole moments can be considered parallel, it can be written as follows [28-33]:

$$\mu_g = \frac{m_2 - m_1}{2}\left[\frac{hca^3}{2m_1}\right]^{1/2} \quad (10)$$

$$\mu_e = \frac{m_2 + m_1}{2}\left[\frac{hca^3}{2m_1}\right]^{1/2} \quad (11)$$

$$a = \left[\frac{3M}{4\pi\rho N}\right]^{1/3} \quad (12)$$

where h, c, ρ, and N are Plank's constant, the light velocity in vacuum, molecular weight, and Avogadro constant, respectively. μ_g and μ_e are the dipole moment of ground and excited states, respectively. In this study, spectral properties of fluorescein disodium were studied for measuring its ground and excited states' dipole moments.

III. Results and Discussion

A. *Polarity effects on the absorption and fluorescence spectra*

According to Fig. 1, and data in Table 2, fluorescein disodium has an absorption band in the region of 480-550 nm of visible area of electromagnetic spectra in solvents with different polarity. The absorption and fluorescence spectra of the used sample were recorded at room temperature in various organic solvents with different polarity (Fig. 1). Observed data in Table 2 show that the position of maximum absorption and emission of fluorescein disodium is affected by the solvent polarity. Despite small bathochromic shifts in the absorption spectrum, hypsochromic shifts were observed in the emission spectrum by increasing solvent polarity. Maximum shifts in absorption and emission processes are about 1596.16 cm^{-1} and 1072.89 cm^{-1} by changing solvent polarity in fluorescein disodium, respectively.

Solvent polarity and the local environment have profound effects on the emission spectral properties of fluorophores. The effects of solvent polarity are one origin of the Stokes shift. This phenomenon is used for designing precise sensors that molecular surrounding environment properties have an important role in their physical, chemical, and biological behavior.

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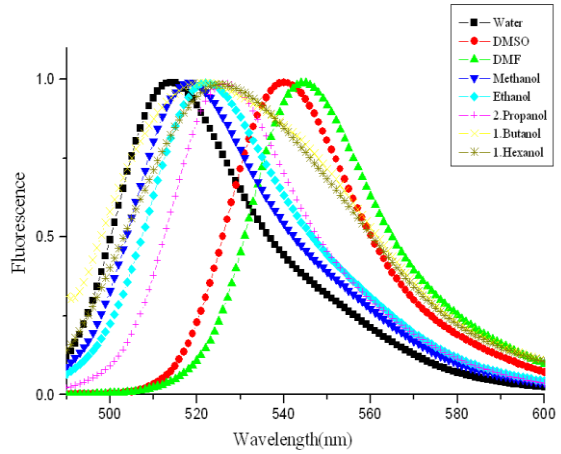
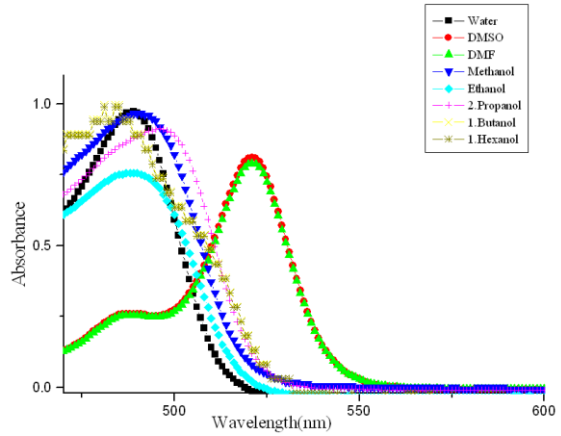


(a)



(b)

Fig. 1. Absorption and fluorescence spectra of fluorescein disodium in selected solvents with different polarity.

Table 2. Maximum absorbance (*a*) and fluorescence (f) data of fluorescein disodium in various solvents.

Solvent	$\lambda_{max}(a)$ (nm)	$\lambda_{max}(f)$ (nm)	ν_a-ν_f (cm^{-1})	ν_a+ν_f (cm^{-1})
Water	489	514	994.64	39905.15
DMSO	521	540	675.33	37712.37
DMF	521	544	811.50	37576.21
Methanol	489	518	1144.87	39754.91
Ethanol	487	522	1376.79	39690.96
2.Propanol	496	527	1185.95	39136.62
1.Butanol	483	521	1510.07	39897.79
1.Hexanol	481	525	1742.40	39837.63

Moreover, the knowledge about absorption and fluorescence characteristics of various groups of materials in different environments plays important role in designing various systems. In general, the observable difference between the position of maximum absorption and emission of used samples in solvent environments leads to stokes shifts. Indeed, the emission process is slow as compared to the absorption process and solvent molecules have an opportunity to reorient around solute molecule in the excited state. So, emission process occurs in a relaxed state. Stokes shift changes (ν_a-ν_b) by increasing the polarity of organic solvents is also due to the reorientation of solvent molecules around the used dye molecules. Under this condition, nature and degree of interactions between understudy molecules and their surrounding molecules in the ground and excited state have significant contributions in the molecular behavior in various systems. In the next section, to investigate the various environmental effects on the molecular linear optical behaviors, multi-linear analysis was used.

B. *Correlation with multi-parameter solvent polarity scale*

For describing solvent contributions into spectral characteristics of fluorescein disodium, multi-parameter solvent polarity scales were used that it is known as Kamlet- Abboud- Taft formulation. In the first step, we used all solvents in multi-parameter analysis. Then according to statistical factors (R2 and significance of F-test) and visual inspection, we selected appropriate solvent collections for obtaining the best result. Multiple-linear analysis of absorption, fluorescence and stokes shift data with multi-parameter polarity scales show a satisfactory correlation for the selected collection of solvents.

The results of the multiple-linear analyses are presented in Table 4. For a better comparison of data in Table 3, we transformed them into contribution percentages of different solvent polarity parameters (Table 4). The description of environment polarity effects on the spectral features of fluorescein disodium is performed using obtained data in Tables 3 and 4. Investigation of the relation between the value of solvent polarity parameters and the spectral behavior of fluorescein disodium dye indicates that solvent hydrogen bond donor ability has a main contribution in the spectral shifts of it in the absorption and emission process. So, the effects of nonspecific interactions between solute and solvent molecules in this situation are lower than specific interactions.

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Table 3. Regression fit to solvatochromic polarity scales for absorbance, fluorescence and stokes shift of used sample.

Multi-parameter scale	v_0 (10^3cm^{-1})	a (10^3cm^{-1})	b (10^3cm^{-1})	s (10^3cm^{-1})
A	18.81±1.88	1.30±0.33	0.86±1.61	-0.27±0.79
F	17.80±0.64	0.94±0.11	0.49±0.52	0.30±0.28
S	-0.32±1.26	0.56±0.22	1.78±1.04	-0.23±0.56

Table 4. Percentage contribution of solvatochromic parameters.

Multiparameter scale	$P\alpha$(%)	$P\beta$(%)	$P\pi^*$(%)	R_2
Absorbance	53	36	11	0.95
Fluorescence	54	28	18	0.97
Stokes shift	22	69	9	0.93

The stability of ground and excited states with change of solvent polarity parameters is another issue that it is investigate by using Eq. 1. In fluorescein disodium, increment of dominant solvent polarity parameter, solvent hydrogen bond donor ability, increases the stability of ground state. In fluorescence process, the increase of dominant solvent polarity parameters in fluorescein disodium decreases the stability of the excited state. The study of linear correlation between stokes shift data and multi-parameter polarity scales also gives useful information about solvent effects on the molecular reorientation of used dye. Stokes shift values of fluorescein disodium increase by enhancement of dominant solvent polarity parameter, hydrogen bond acceptor ability, so this parameter increases molecular reorientation of used dyes in solvent media.

The spectral behavior of fluorescein disodium can be related to different substituents in its chemical structures that are capable to participate in specific and nonspecific interactions with solvent molecules. This description is in agreement with data in Table 4 that demonstrates hydrogen bonding and dipole-dipole interactions between solute and solvent molecules with different contributions play important roles in spectral shifts. However, almost the irregular behaviors of these dyes can be related to different resonance structures (Fig. 2) in the various solvent environments that contribute to the spectral properties of the selected dye differently.

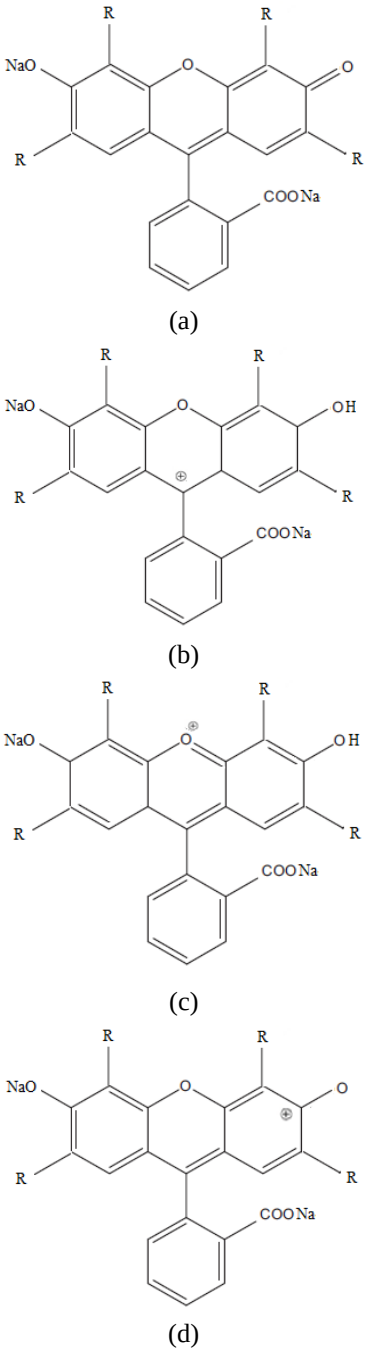

Fig. 2. Different resonance structures of Fluorescein disodium (R=H).

C. *Estimation of ground and excited state dipole moments*

In order to determine ground and excited state dipole moments of used dye, solvent polarity functions, $f(\varepsilon,n)$ and $f(\varepsilon,n)+2g(n)$, were calculate (Table 1). Then linear correlation of spectral shifts v_a-v_f and v_a+v_f of selected sample against solvent polarity functions $f(\varepsilon,n)$ and $f(\varepsilon,n)+2g(n)$, respectively, were used. Therefore, from the slopes of these lines and

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Eqs. (10) and (11) the ground and excited state dipole moments of fluorescein disodium was estimated. The obtained experimental data are summarized in Table 5. The calculated dipole moments of spectral shifts indicate that dipole moment of excited state is higher than ground state. Increases in excited state dipole moments are about 7.15 Debye. This variation could be due to charge transfer during the excitation process of dye in solvent media. In general, solvent polarity and the local environments have important role on observable variations of dipole moments in the ground and excited states.

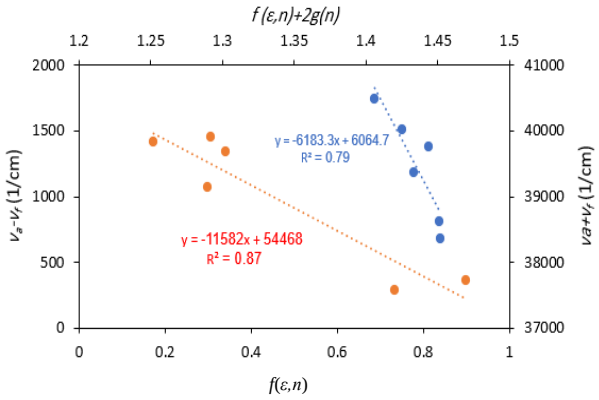

Fig. 3. The variation of ν_a-ν_f with $f(\varepsilon,n)$ and ν_a+ν_f with $f(\varepsilon,n)$+2$g(n)$ for Fluorescein disodium.

Table 5. Dipole moments values.

M (g/mol)	Radius (A°)	μ_g (D)	μ_e (D)	$\mu\Delta$
367.27	4.53	3.12	10.27	7.15

During the last years, different dyes with different various molecular structure were synthesis in various laboratories for application in various physical, chemical and biological systems. Different dye molecules with different molecular structure and substituents in their chemical structure behave differently in various systems. Fluoresceine disodium is an organic dye of xanthene groups. Hence, a comparison between the results of this research with the results of other xanthene dyes will give valuable information. According to studies by Zakerhamidi *et al.* rhodamine 6G and rhodamine B as xanthene dyes indicate different behaviors in comparison to fluoresceine disodium dye in different environments [34]. In this case, fluoresceine disodium dye with different active groups in its chemical structure indicate high dipole moment in ground and excited state. Fluoresceine disodium indicate high polar structure in ground and excited state as compared to rhodamine dyes. Moreover, the studies on fluoresceine dye properties in mixed water-alcoholic solvents confirm the important roles of substituent effects on the molecular spectral behavior. A comparison between calculated dipole moments by Dimitriu *et al.* for a fluoresceine dye indicate that presence of Na atom in the molecular structure of fluoresceine disodium molecular structure led to a small decreasing of $\Delta\mu$ [35-36]. Therefore, this dye with high dipole moment and controllable spectral behaviors in different environments can be considered as a good candidate for designing various systems.

IV. Conclusion

Our results indicate the interesting properties of fluorescein disodium dye. This dye with unique structure can be used as a good candidate for designing optical systems. In this case, molecular surrounding environment can modify its function in various phenomena. Solvents with different polarity properties can be used for investigation of different environment effects. Our results indicate, solvents with different polarities can change spectral, molecular structure, and dipole moments of under study molecules. These changes can be related to different resonance forms of used dye dependence on polarity or hydrogen bonding nature of the used solvents. The significant differences between the ground and excited states' dipole moments are also due to different operations of these molecules in ground and excited states. The large variations between dipole moments of ground and excited states show that charge transfer process can occur during the excitation process. Therefore, by selection of a proper solvent or other environments with similar polarity characteristics, it is possible to control the function of fluoresceine disodium in various systems.

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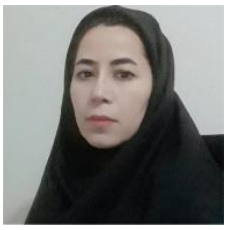

Mahsa Khadem Sadigh completed her PhD in Photonics in 2018 at University of Tabriz, Iran. Then, she started her research in postdoctoral position at University of Tabriz. She is currently an assistant professor at Department of Photonics, Faculty of Basic Sciences, University of Bonab. Her main interests are in optics, photonics, bio-photonics, optical and electro-optical devices, material science, and

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molecular medicine. Her research team works in the laboratory on smart materials, optical and electro-optical systems, important biological molecules, and molecular medicine.

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