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ORIGINAL PAPER

Influence of Substituent and Solvent on the Radiative Process of Singlet Excited States of Novel Cyclic Azacyanine Derivatives

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Abstract The photophysical properties of novel cyclic azacyanine derivatives have been investigated in acetonitrile, N-butyronitrile, methanol, ethanol, DMF and water. Introduction of electron donating or accepting groups on the cyclic azacyanine has a direct impact on the spectroscopic and photophysical properties. Irrespective of the nature of the substitution, azacyanine shows a general solvent relaxation in accordance with Lippert-Mataga's prediction; however, in protic solvent, specific interactions are encountered. Fluorescence lifetime decay suggests a relaxation in the nanosecond time scale with monoexponential decay in polar solvents and biexponential decay in non polar solvents. The fluorescence lifetime of azacyanines are found to be longer than popular cy3 dyes. An electron donating substituent increases the fluorescence lifetime and influences the radiative process, whereas an electron withdrawing group marginally increases the excited state lifetime but remarkably enhances the radiative process. The fluorescence quantum yield of substituted cyclic azacyanine in water is noted to be at least five fold higher than the popular cy3 dye.

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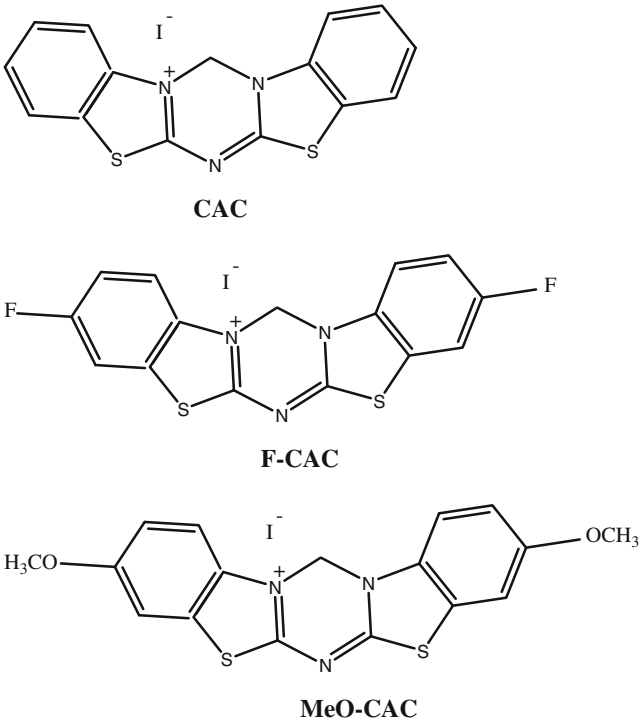
Introduction

Solvents exert a strong influence on chemical equilibria, reaction rates, and electronic spectral position and intensity as revealed from physicochemical studies performed on many chemical processes [1–4]. The solvent environment influences the spectral properties of a chemical species, thus there has been a great deal of interest in understanding both static and dynamic solvent effects. One way to understand such effects is to study the same solute in different solvents and attribute the resulting difference as being due to polar/nonpolar or protic/aprotic solvent properties [5–7]. A blue/red shift, negative/positive solvatochromism, in absorption and emission spectral properties of a molecule with increase in solvent polarity depends on the difference in dipole moment between the ground and excited states of the chromophore. When there is no specific molecular interaction, the overall solvent dependence of absorption and emission spectra can normally be predicted based on continuum electrostatics and Franck-Condon ideas [8, 9]. The underlying continuum electrostatic theory of a self-consistent solute-solvent "reaction field" for a polar molecule was explained by Onsager [10]. This idea was expanded with slightly different assumptions, and found functions of the dielectric constant and refractive index to describe both polar and nonpolar solvent dependence of electronic spectra [11–14]. These theories suppose that the chemical state of the molecule is the same in different solvents and indicate that a polar solvent response requires both a dipole moment and a change in dipole upon electronic

excitation. Furthermore, substituents can greatly influence the photophysical and photochemical properties of molecules, particularly those having closely located excited states of different character [15–18]. The effect can be described in terms of low-lying excited states caused by a change in solvent polarity or by electron-donating or withdrawing substituents.

The cyanine dyes are of importance because of their use to enhance the sensitivity range of photographic emulsions to form an image on film [19–21]. These dyes have also found application in optical recording media in laser disks, laser dyes and optical sensitizers in various other fields as well as in molecular electronics. However, much interest has recently focused on application of these dyes as fluorescent probes for biomedical imaging [22–24] and single molecule studies [25–29]. Among cyanine dyes, merocyanine dyes have been broadly investigated to establish relationships between solvent polarity and photophysical behavior in the probe molecules and it was observed that these dyes possess large solvatochromic shifts, second-order hyperpolarizability, and are usefulness in diagnostics and therapeutics [30–32]. West and Geddes studied the steady-state solvation of several small carbocyanine dyes and found nonpolar solvation [33]. Several groups have used various tricarbocyanine dyes in nonlinear optical experiments to characterize solvation time scales and reached different conclusions about the role of intramolecular interactions and solvent relaxation in the observed signals [34, 35]. The impact of solvent polarity on the electronic spectral characteristic of cyanine dyes has been extensively investigated [36].

Although intensive study of the solvatochromism and relaxation dynamics of cyanine and merocyanine dyes has been undertaken and some of them are widely used in fluorescence probing and single molecule study/manipulation, we find little or no evidence of solvatochromic investigations on cyclic azacyanine [37–50]. Azacyanines are another important class of cyanines dyes and the present study relates to the use of cationic azacyanine dyes for coloring keratin fibers; for example, wool, silk, furs and, in particular, human hair. In the past decades, much attention has been given to the synthesis of novel cyclic azacyanies [37–48] for various applications including in optical recording [40] and biomedical use [44]. Recently, azacyanine dye-based fluorescent indicators have been made with high affinity for nucleic acid complex [49]. The goal of the present work is to study the photophysical and spectroscopic properties of a new class of cyclic azacyanine (CAC) shown in Scheme 1 with respect to mirror symmetry, solvent sensitivity and radiative processes. The purpose of the present study is to differentiate the steady-state and excited-state properties of CAC molecules in various solvents, and to understand how solvent affects the optical dynamics and



Scheme 1 Chemical structure of azacyanine (CAC), fluorine substituted azacyanine (F-CAC) and methoxy substituted azacyanine (MeO-CAC)

energy states. The experimental results of the steady-state and the time-resolved fluorescence measurements of CAC in various solvents are presented. In addition, explicit photophysical parameters are also reported to recognize the effect of electron donating and withdrawing substituents on the cyclic azacyanine.

Materials and Methods

Synthesis

The syntheses of cyclic azacyanine and its derivatives, fluorine-substituted azacyanine (F-CAC) and methoxy-substituted azacyanine (MeO-CAC), (see Scheme 1), were carried out in a one step reaction of an amino-substituted heterocycle with diiodomethane and the detailed synthetic procedure and characterization were reported elsewhere [37, 38].

Materials

To prepare the stock solution, cyclic azacyanines were dissolved in spectroscopic grade dichloromethane (Acros Organics). A desired amount of the stock sample was taken in a vial and the solvent, dichloromethane, was evaporated by gentle heating. The final sample solution was prepared

by adding the required amount of the desired solvent into the same vial. Cyclohexane, ethanol, methanol, acetonitrile, n-butyronitrile (nBN), and N,N-dimethylformamide (DMF) were of spectroscopy grade (Acros Organics). The solvents were used without further purification.

Spectroscopic Measurements

The absorption spectra in various solvents were recorded at room temperature using a JASCO V-570 UV–VIS-NIR Spectrophotometer. Fluorescence measurements were done on a JOBIN YVON Horiba Fluorolog 3 spectrofluorometer. The excitation source was a 100 W Xenon lamp. The detector used was R-928 operating at a voltage of 950 V. The excitation and emission slits width were 5 nm. The spectral data were collected using Fluoroescence software and data analysis was made using OrginPro 6.0 software.

Determination of Fluorescence Quantum Yield

The fluorescence quantum yield was determined in dilute conditions (≤1 μM) to avoid self-quenching of fluorophores and inner filter effects. Perylene was used as a reference standard sample to determine the fluorescence quantum yield of the unknown samples. The excitation wavelength used for the reference and standard samples was 380 nm and the fluorescence quantum yields of cyclic azacyanines in various solvents were determined using the following equation [51]:

$$\Phi_{unk} = \Phi_{Ant} \times \frac{F_{unk} \times A_{Ant} \times {n_{unk}}^2}{F_{Ant} \times A_{unk} \times {n_{Ant}}^2}$$

where Φ_{unk} and Φ_{Ant} are the quantum yield of the cyclic azacyanines samples, and that of the reference sample, respectively. The quantum yield of perylene in cyclohexane was taken from the literature as 0.94 [52]. F_{unk} and F_{Ant} are the integrated intensities of the emission spectra of the cyclic azacyanines and that of the reference sample, respectively. A corresponds to the optical density of cyclic azacyanines and the reference taken at the excitation wavelength (380 nm); finally, n is the refractive index of the solvents used. The dependence of radiative rate on the square of the refractive index is related by the Strickler-Berg equation [53].

Lifetime Measurements

Fluorescence lifetime measurements were done using a Jobin-Yvon-Horiba Fluorolog III time correlated single photon counting fluorometer fitted with a pulsed diode laser. A 405 nm diode laser was used as the excitation source. The detector used was an R-928 operating at a voltage of 950 V. The fluorescence decay was acquired with a peak preset of 10,000 counts. The decay data were analyzed using Data Analysis Software.

Results and Discussion

The UV-visible absorption, fluorescence excitation and emission spectra of cyclic azacyanine in acetonitrile are depicted in Fig. 1. In general, broad absorption spectra were observed in the wavelength regions 350–450 nm and two vibronic bands were observed at ~384 and 397 nm. The fluorescence emission spectrum revealed a maximum at 529 nm and a vibronic band at ~492 nm. The excitation spectrum of CAC exhibited similar vibrational structured transitions. The longest wavelength transition at ~400 nm has a close mirror image symmetry relation with the fluorescence emission and there is no overlap of the longest wavelength vibronic excitation and emission bands. The differences between absorption and fluorescence excitation spectra could be interpreted as being due to different species present in the ground and excited states respectively. Stokes' shift was estimated to be 45 nm or 7,138 cm^{-1} (see Table 1). Cyanine based dyes usually exhibit sensitive solvatochromic behaviour in which the relative intensity of emission bands is dependent on solvent polarity [32–36]. The absorption maxima, emission maxima, and Stokes' shifts of CAC are summarized in Table 1. CAC demonstrated a strong and intense absorption band at 384 nm to 397 nm in all solvents used in this study. A loss of absorbance was observed going from methanol to the more polar solvent water. The excitation spectra, however, shifted in various solvents. In these solvents, the lowest energy 0–0 absorption band is clearly seen around 400 nm and absorption

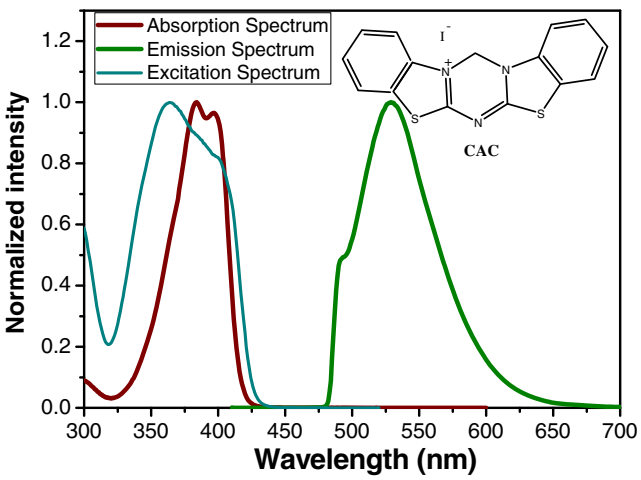


Fig. 1 UV-Visible absorption, fluorescence excitation and emission spectra of CAC in acetonitrile

Table 1 Absorption Maxima (λ_{abs}), Emission Maxima (λ_{em}), Stokes' shift of some cyclic azacyanines in various solvents along with their solvent parameters such as π* values, dielectric constant (ε), hydrogen-bond donating ability (α), hydrogen bond acceptor ability (β) and dipole moment (μ)

Compound	Solvent	Spectroscopic Parameters			Solvent Parameters				
		λ_{abs} (max)/nm	λ_{em} (max)/nm	Stokes' shift (cm^{-1})	π*	ε	α	β	μ
CAC	Acetonitrile	384	529	7138	0.713	37.5	0.19	0.40	3.92
	Cyclohexane	349	426	5179	0	2.02	0	0	0
	Water	382	528	7239	1.09	80	1.17	0.47	1.85
	DMF	386	524	6823	0.875	38.3	0	0.69	3.82
	Ethanol	385	528	7035	0.54	24.3	0.86	0.75	1.66
	Methanol	384	528	7102	0.586	32.7	0.98	0.66	2.87
	n-Butryronitrile	385	527	6999	0.71	–	–	–	3.6
F-CAC	Acetonitrile	360	492	7453	0.713	37.5	0.19	0.40	3.92
	Cyclohexane	362	440	4897	0	2.02	0	0	0
	Water	384	439	3263	1.09	80	1.17	0.47	1.85
	DMF	374	465	5233	0.875	38.3	0	0.69	3.82
	Ethanol	358	459	6146	0.54	24.3	0.86	0.75	1.66
	Methanol	374	493	6454	0.586	32.7	0.98	0.66	2.87
	N-Butyronitrile	374	493	6454	0.71	–	–	–	3.6
MeO-CAC	Acetonitrile	405	534	5965	0.713	37.5	0.19	0.40	3.92
	Cyclohexane	387	426	2366	0	2.02	0	0	0
	Water	404	470	3476	1.09	80	1.17	0.47	1.85
	DMF	406	536	5974	0.875	38.3	0	0.69	3.82
	Ethanol	405	527	5716	0.54	24.3	0.86	0.75	1.66
	Methanol	405	532	5894	0.586	32.7	0.98	0.66	2.87
	N-Butyronitrile	406	529	5727	0.71	–	–	–	3.6

maximum at around 380 nm. There is appreciable change in the energy of transitions in the different solvents (349 nm in cyclohexane to 382 nm in water and 384 nm in acetonitrile) suggesting that solvent stabilization of the ground state species is significant. The fluorescence emission spectra also demonstrated significant solvent dependent shifts in emission maxima. The fluorescence maximum of CAC broadly shifted to longer wavelength going from nonpolar cyclohexane at 426 nm to polar solvents (water 528 nm and acetonitrile 529 nm). The fluorescence quantum yield summarized in Table 2 was found to be relatively low in all solvents. The quaternary nitrogen centre can act as an acceptor for intramolecular charge transfer as observed in various other classes of cyanine dyes [21].

To realize the polarity effect of CAC in various solvents, solvent dependent spectral shifts were considered. The theory of general solvent effects considers fluorophore as a dipole in a continuous medium of uniform dielectric constant without containing any other kinds of interaction that affect fluorescence emission properties. The Lippert-Mataga equation [54, 55] shows that the solvent dependence of the Stokes' shift for a compound depends on the change in dipole moment of the fluorescence moiety upon excitation, the dielectric constant, and the refractive index of the solvents being used [13, 14, 54, 55]:

$$\overline{\nu_A} - \overline{\nu_F} = \frac{2}{hc}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right)\frac{(\mu_E - \mu_G)^2}{a^3} + Constant$$

where $\overline{\nu_A}$ and $\overline{\nu_F}$ are the wave numbers (cm^{-1}) of the absorbance and fluorescence emission, respectively, h is Planck's constant, c is the speed of light in vacuum, *a* is the radius of the cavity in which the fluorophore resides, μ_E and μ_G are the dipole moments in the excited and ground states, respectively, and ε and *n* are the dielectric constant and the index of refraction of the solvents, respectively [14]. The Lippert-Mataga plot can be obtained by plotting the Stokes' shift versus the term in the brackets in the above equation, referred to as the orientation polarizability (Δf) of the solvent, which is the result of both the mobility of the electrons in the solvent and the dipole moment of the solvent.

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$$

Figure 2 represents the Lippert-Mataga plot of CAC in different solvents. There is a linear correlation between the

Table 2 Photophysical parameters estimated for some cyclic azacyanines in various solvents

Compounds	Solvent	τ_1 (B1)/ns	τ_2 (B2)/ns	χ^2	ϕ_f	k_r/ (10^8 s^{-1})	k_{nr}/ (10^8 s^{-1})
CAC	Acetonitrile	3.70 [100.00]	–	1.27	0.03	0.08	2.62
	Cyclohexane	0.48 [44.64]	2.43 [55.36]	3.76	0.07	0.43	5.82
	Water	3.68 [100.00]	–	0.98	0.03	0.08	2.64
	DMF	2.47 [100.00]	–	1.09	0.04	0.16	3.89
	Ethanol	3.44 [100.00]	–	1.37	0.03	0.09	2.82
	Methanol	3.68 [100.00]	–	1.45	0.03	0.08	2.64
	n-Butryronitrile	3.50 [100.00]	–	1.35	0.03	0.09	2.77
F-CAC	Acetonitrile	4.01 [100.00]	–	1.31	0.63	1.57	0.92
	Cyclohexane	2.10 [56.11]	5.25 [43.89]	1.13	0.51	1.39	1.34
	Water	3.99 [100.00]	–	1.31	0.97	2.43	0.08
	DMF	2.46 [100.00]	–	1.31	0.44	1.79	2.28
	Ethanol	3.72 [100.00]	–	1.59	0.42	0.57	0.79
	Methanol	4.03 [100.00]	–	1.21	1.00	2.69	0.00
	N-Butyronitrile	3.81 [100.00]	–	1.33	0.47	1.17	1.32
MeO-CAC	Acetonitrile	4.22 [100.00]	–	1.21	0.29	0.69	1.68
	Cyclohexane	0.77 [48.24]	3.24 [51.76]	2.76	0.35	1.75	3.25
	Water	3.97 [100.00]	–	1.34	0.96	2.42	0.10
	DMF	4.02 [100.00]	–	1.23	0.30	0.75	1.74
	Ethanol	4.15 [100.00]	–	1.49	0.34	0.82	1.59
	Methanol	4.23 [100.00]	–	1.32	0.29	0.69	1.68
	N-Butyronitrile	4.08 [100.00]	–	1.19	0.34	0.83	1.62

orientation polarizability of the solvent and Stokes's shift, as estimated by the difference between absorption and emission maxima obtained from the corrected spectra on the wavenumber scale, of CAC. An enhancement in refractive index (part of Δf) instantaneously stabilizes the ground and excites states by movement of electrons within the solvent molecules. This redistribution of electrons within the solvent molecules results in a reduction in the energy difference between the ground and excited states. Increase in dielectric constant also stabilizes the ground and excited states, but the energy decrease of the excited state because of dielectric constant occurs only after reorientation of the solvent dipoles, as this process necessitates movement of the entire solvent molecules and not just its electrons. Thus, stabilization of the ground and excited states of CAC depends on the dielectric constant and is time dependent. In this case, in a polar solvent like methanol/acetonitrile, a large Stokes' shift is expected compared to non-polar solvent like cyclohexane. Generally it is found with the increase in solvent polarity leading to a positive solvatochromism (bathochromic shift) in the fluorescence spectra of CAC implies that CAC in the excited state is better stabilized relative to the ground state (see Scheme 2) which means dipole moment increases during the electronic transition ($\mu_E > \mu_G$). Since the time required for electronic excitation (in femtosecond scale) is much shorter than that required time to execute vibrations to rotations (picosecond time scale), the nuclei of the absorbing entity (absorbing molecule plus solvation shell) do not appreciably change their position during electronic transition [14]. Hence, the excited state of a molecule in solution has the same solvation pattern as the corresponding ground state, whereas the ground state corresponds to an equilibrium ground state. If the lifetime of the excited molecule is large enough, then reorientation of the solvent molecules, as per the new excited state situation takes place, which results in a relaxed excited state with a solvent shell in equilibrium with this state. From this equilibrium state (excited), fluorescence occurs.

Beside a change in dipole moment upon excitation, the ability of solute to donate or accept a hydrogen bond to/from the surrounding solvent molecules in its ground and Frank-Condon excited state determines further the extent and sign (red or blue shift) of solvatochromism. In the case of charge transfer in a molecule, the gross solvent polarity indicator scale, such as E_T30, is more applicable [56, 57]. E_T (30) is defined as the molar electronic transition energies (E_T) of dissolved pyridinium N-phenolate betaine dye in kilocalories per mole at room temperature (25 °C) and normal pressure [57]. A plot of Stokes' shift versus the E_T30 values of the solvents is given in Fig. 2. Correlation of solvent-dependent properties with the E_T30 scale normally gives two

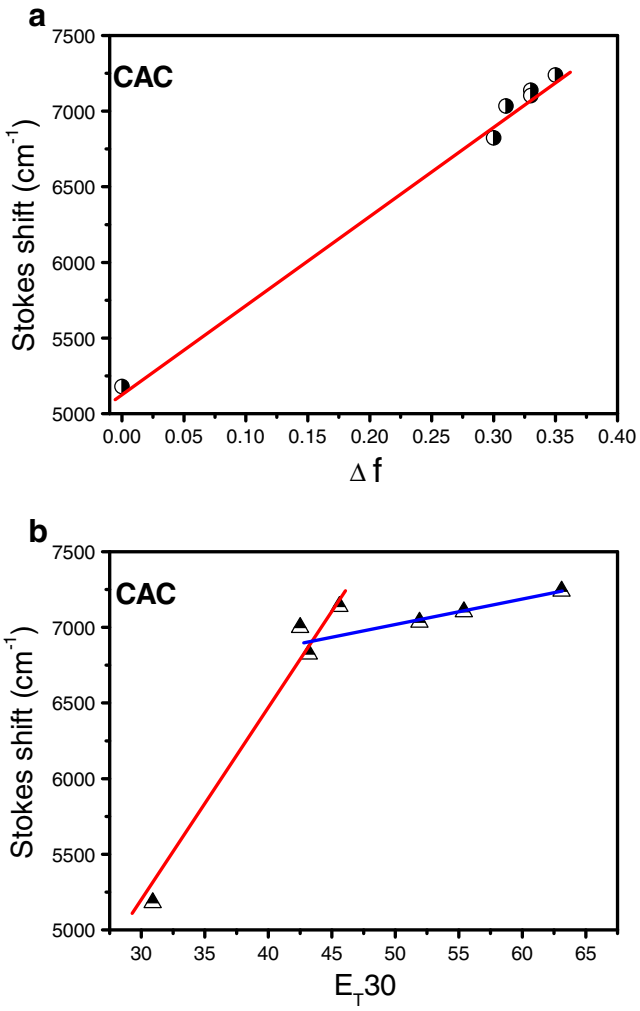
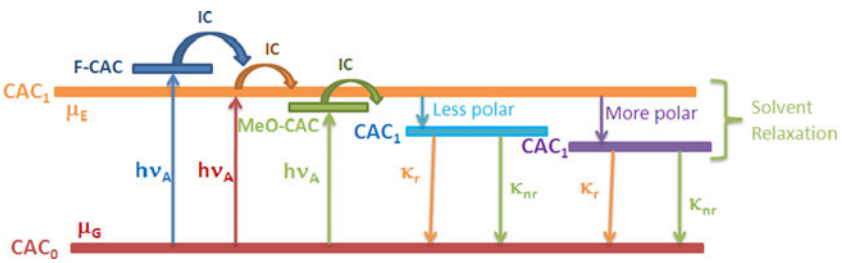


Fig. 2 (**a**) Lippert Mataga plot for CAC in various solvents showing the variation of Stokes' shift as a function of orientation polarizability of the solvents. (**b**) Correlation of Stokes shift of CAC with $E_T(30)$ parameter in various solvents. E_T (30) is defined as the molar electronic transition energies (E_T) of dissolved pyridinium N-phenolate betaine dye in kilocalories per mole at room temperature (25 °C) and normal pressure [57]

distinct lines, one for protic solvents and another for the non-protic solvents. CAC also provided two different lines. It is expected that the N bearing ring would interact specifically with protic solvents by forming H-bonds. Thus, CAC has a strong ability to form H-bonding with solvents like water, methanol and ethanol. The dipole moment (μ), dielectric constant (ε), hydrogen-bond donating ability (α), hydrogen bond acceptor ability (β) and π* values of the solvent with the corresponding absorption maxima and emission maxima are given in Table 1. The π* scale of solvent polarity accounts for both non-specific as well as specific interaction between solute and solvent [58–60]. When the π* scale is used to quantify solvent polarity (polarizability effects), it is correlated with either reaction rate or equilibrium constant or position/intensity of the spectral absorption [58]. π* scale has also been correlated with the position of emission maximum in wavenumber scale [57]. To correlate the solvatochormic effect of CAC for specific and non-specific interactions, π* scale of solvent polarity vs. absorption maximum (λ_{Abs}^{max}) and emission maximum (λ_{Em}^{max}) were plotted as in Supplement 1.

The fluorescence lifetime of CAC in various solvents showed monoexponential decay having nanosecond fluorescence lifetime except for cyclohexane where a biexponential decays could be fitted with a short component of 480 ps (refer to Table 2). The fluorescence lifetime is found to be longer than as reported for some other merocyanine dyes [61] and other popular cyanine dyes extensively used in fluorescence probing and single molecule studies such as Cy3 [62] and Cy5 [63], but shorter than polymethine dyes [64]. The fluorescence lifetime values were highest in polar solvents like acetonitrile, water and methanol as polar solvents stabilize the excited-states thus increasing the fluorescence lifetimes. On the other hand, the relatively short lifetimes of these compounds in cyclohexane can be attributed to the less stable excited state in a non-polar solvent. Biexponential decay behaviour in non-polar solvent presumes multiple structures in the ground and/or excited states, such as the possibility of intramolecular charge transfer, which cannot be ruled out. The fluorescence quantum yield was determined as stated in the experimental section using perylene as a standard reference. The fluorescence quantum yield of CAC was found to be low in most solvents studied. Although fluorescence quantum yield depends on the solvent



Scheme 2 Effect of substitutes and solvent on excited states of cyclic azacyanine (CAC). CAC_0: Azacyanine singlet ground state; CAC_1: Azacyanine singlet first excited state; F-CAC: Fluorine substituted cyclic azacyanine; MeO-CAC: Methoxy substituted cyclic azacyanine; IC: International Conversion

polarity, the nature of this dependence shows the opposite trend in this study. The fluorescence quantum yield was found to be relatively high in cyclohexane (non-polar solvent) and low in polar solvents. However, this trend is similar to earlier reported values on substituted pyrene [58]. If the intrinsic fluorescence lifetime of the fluorophore in the absence of any non-radiative processes and fluorescence quantum yield are known, then the radiative rate constant can be calculated by the equation [14]

$$k_r = \phi_f / \tau_f$$

where k_r is the radiative rate constant, ϕ_f is the fluorescence quantum yield, and τ_f is the fluorescence lifetime. The non radiative constant k_{nr} is evaluated as [14]

$$k_{nr} = \left(1/\tau_f\right) - k_r$$

The calculated radiative rate constants and nonradiative rate constants for CAC (see Table 2) are of the order of 10^8 s^{-1} and are much greater than the corresponding values found for pyrene (approximately 10^6 s^{-1}) [65]. In non-polar solvents, the radiative rate constants and non-radiative rate constants of CAC were found to be higher compared to polar solvents.

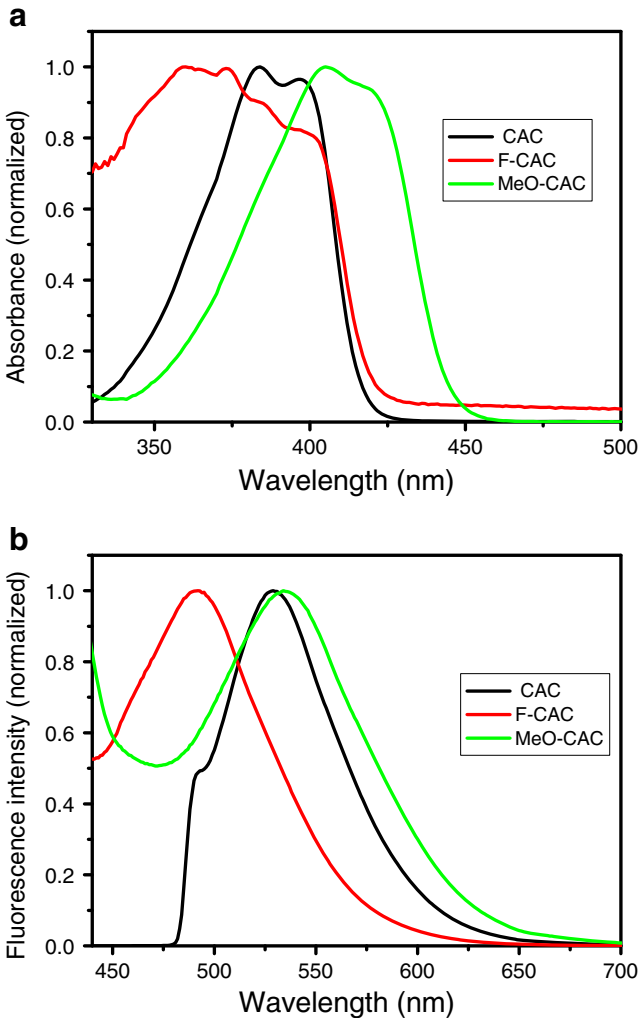


Fig. 3 UV-Visible absorption (**a**) and fluorescence (**b**) spectra of CAC, F-CAC and MeO-CAC in acetonitrile

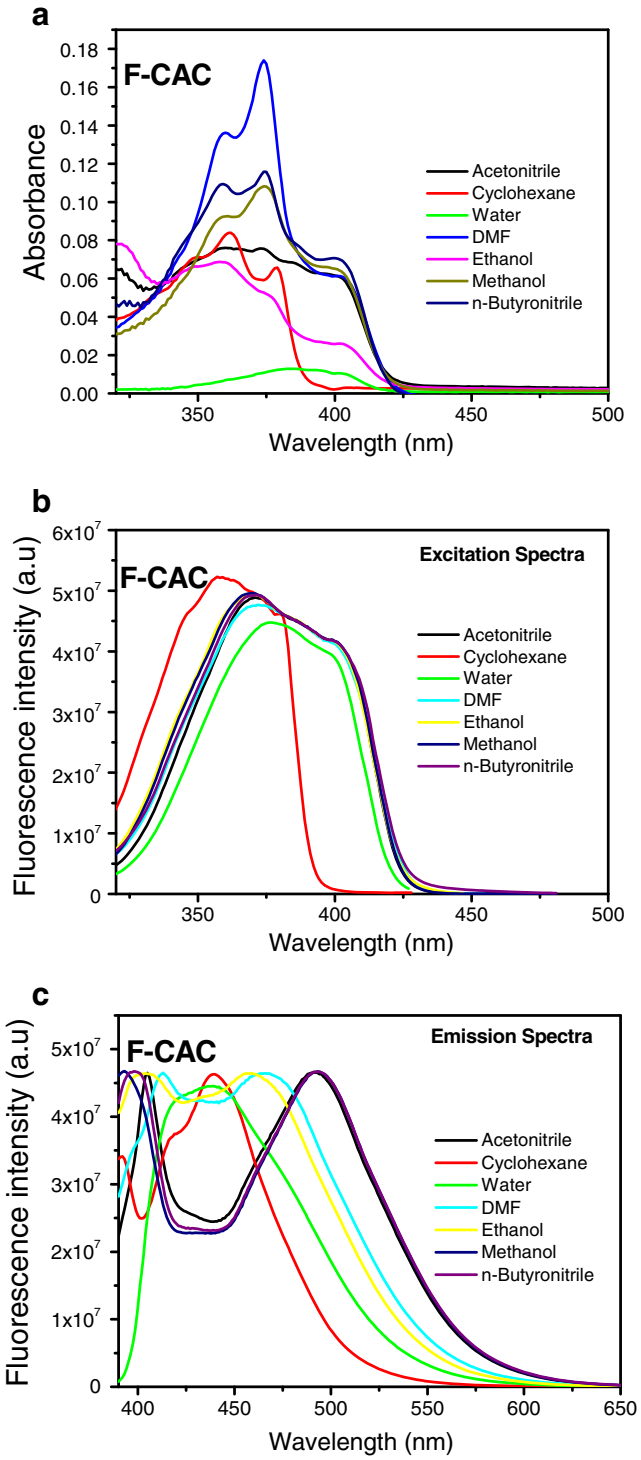


Fig. 4 UV-Visible absorption (**a**), fluorescence excitation (**b**) and emission (**c**) spectra of F-CAC in various solvents of different polarity

The normalized absorption and fluorescence spectra of CAC, F-CAC and MeO-CAC in acetonitrile are shown in Fig. 3. Like the parent compound, broad absorption spectra were generally observed, for the substituted compounds, in the wavelength regions 350–450 nm. When an electron withdrawing group such as fluorine was introduced as a substitutent in the parent system (CAC), a much broader absorption spectrum was obtained for F-CAC, with a blue shift in the absorption maxima by around 24 nm. However, when the fluorine was replaced by an electron donating group such as methoxy (MeO-CAC), the spectrum resembled more the parent compound with a red shift in the absorption spectrum and the absorption maximum was shifted about 21 nm compared to the parent CAC. Although a similar trend was observed in the fluorescence spectrum, the blue shift was 37 nm (in case of electron withdrawing F group for F-CAC) and the red shift was 5 nm (in case of electron donating methoxy group for MeO-CAC) with respect to the parent CAC. The spectral shift in the absorption and fluorescence measurements suggest that an electron withdrawing F group

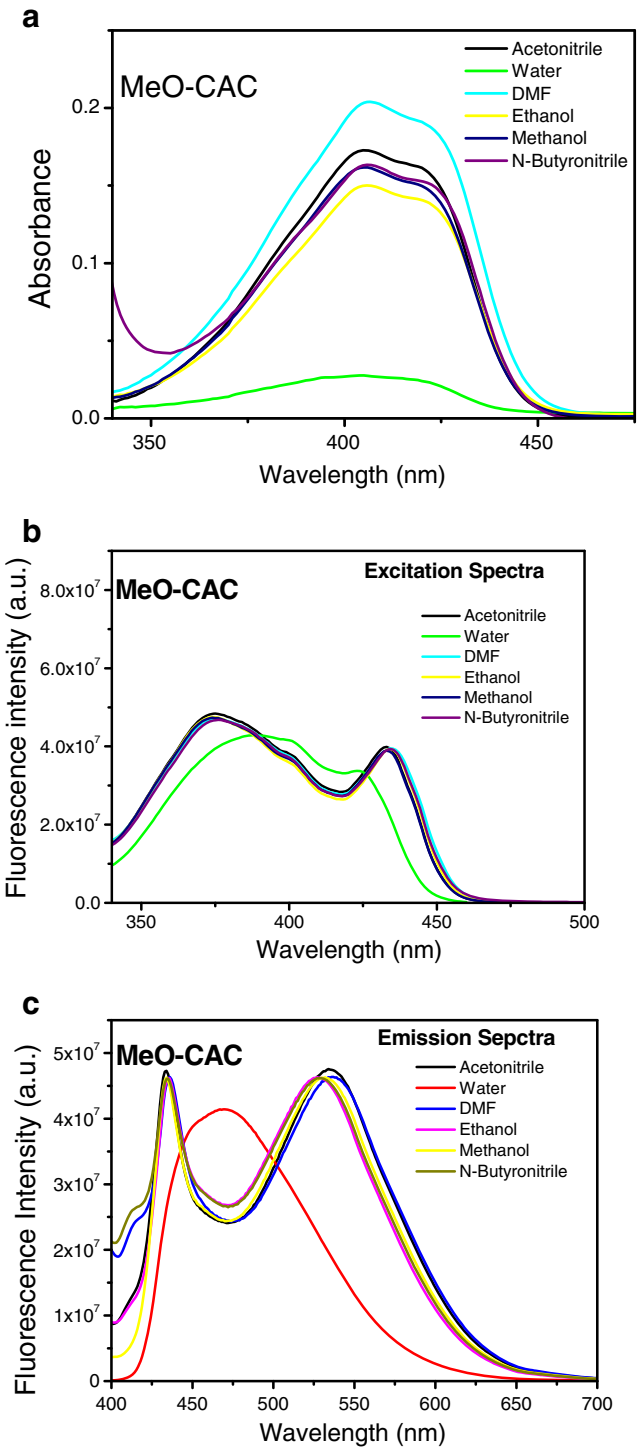


Fig. 5 UV-Visible absorption (**a**), fluorescence excitation (**b**) and emission (**c**) spectra of MeO-CAC in various solvents of different polarity

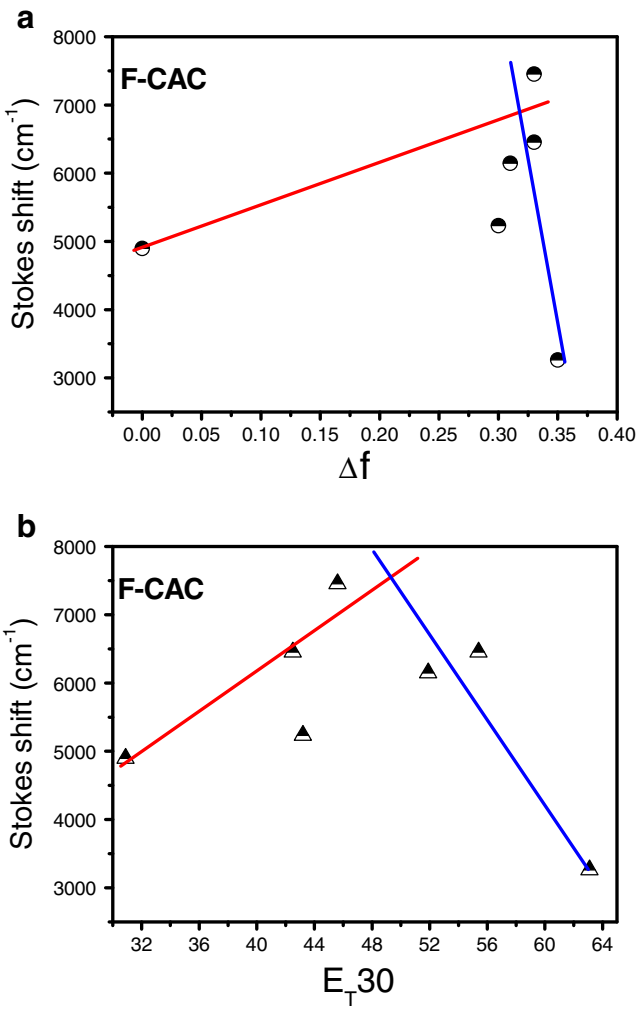


Fig. 6 (**a**) Lippert Mataga plot for F-CAC in various solvents showing the variation of Stokes' shift as a function of orientation polarizability of the solvents. (**b**) Correlation of Stokes shift of F-CAC with $E_T(30)$ parameter in various solvents. E_T (30) is defined as the molar electronic transition energies (E_T) of dissolved pyridinium N-phenolate betaine dye in kilocalories per mole at room temperature (25 °C) and normal pressure [57]

destabilizes the positive charge present on the N-atom in the parent structure by effectively discouraging conjugation and increasing the π-π* or n-π* energy gap, whereas the electron donating OCH_3 group stabilizes the positive charge present on the N-atom in the parent structure by favoring conjugation and decreasing the π-π* or n-π* energy gap (see Scheme 2). The electronic absorption, fluorescence excitation and emission spectra in various solvents of F-CAC and MeO-CAC are, respectively, given in Figs. 4 and 5. The Stokes's shift for the fluorine substituted compound marginally increased from 7,138 cm^{-1} (for parent CAC) to 7,453 cm^{-1} (for F-CAC) whereas it decreased for the methoxy substituted compound to 5,965 cm^{-1} (see Table 1). The Lippert-Mataga plot, as depicted in Figs. 6 and 7, respectively, for F-CAC and MeO-CAC could best be explained by two linear changes, as expected, based on protic and aprotic solvents. The E_T30 plot of F-CAC and MeO-CAC showed a similar behaviour like parent CAC confirming a general solvent effect accompanied by a special solvent effect. Beside conjugation and specific solvent-fluorophore interaction, many molecules can undergo internal charge transfer (ICT) when a fluorophore contains both an electron donating and an electron accepting group. The amino as electron donating and the carbonyl as electron accepting groups have been widely investigated. This may happen due to an increase in charge separation within the fluorophore. Since the parent chromophore, CAC, has a positive charge distributed on the aromatic ring due to resonance, substitution of an electron donating/withdrawing group may increase the charge separation as observed in the fluorescence band in the blue region for compounds F-CAC and MeO-CAC.

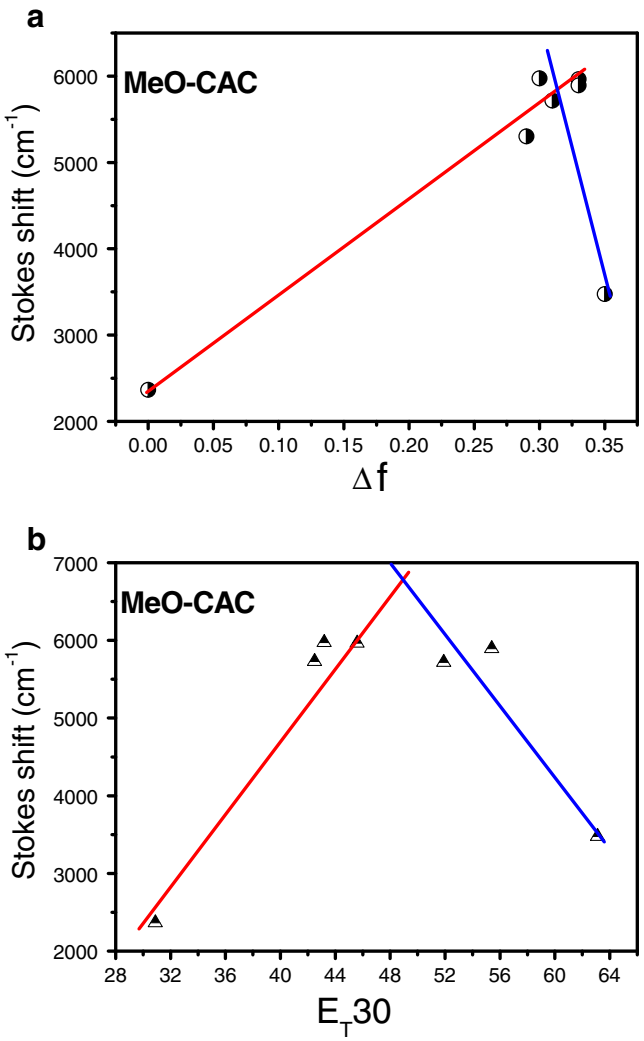


Fig. 7 (**a**) Lippert Mataga plot for MeO-CAC in various solvents showing the variation of Stokes' shift as a function of orientation polarizability of the solvents. (**b**) Correlation of Stokes shift of MeO-CAC with E_T (30) parameter in various solvents. E_T (30) is defined as the molar electronic transition energies (E_T) of dissolved pyridinium N-phenolate betaine dye in kilocalories per mole at room temperature (25 °C) and normal pressure [57]

Although absorption and emission maxima showed a large variation in polar solvents compared to non-polar solvents for CAC and MeO-CAC, the change among polar solvents was remarkable for F-CAC. The quaternary nitrogen centre as acceptor and the methoxy group as a donor can facilitate intramolecular charge transfer in MeO-CAC, whereas such charge transfer is discouraged in the case of F-CAC compared to CAC and MeO-CAC.

A representative fluorescence decay curve for F-CAC in various solvent environments is presented in Fig. 8. In general in polar solvents like acetonitrile, ethanol, methanol, DMF, and N-butyrinitrile, the fluorescence lifetime of MeO-CAC is higher than for F-CAC and than for unsubstituted CAC. However, in the case of water, the fluorescence lifetime of MeO-CAC and F-CAC remained similar whereas, in non-polar solvent like cyclohexane, fluorescence lifetime F-CAC is higher than for MeO-CAC and than for unsubstituted CAC. The fluorescence quantum yield of substituted

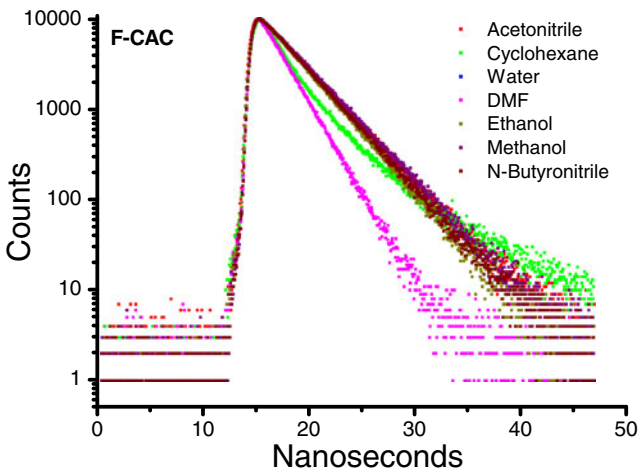


Fig. 8 Fluorescence life time decay profile of F-CAC in various solvents of different polarity

cyclic azacyanines, F-CAC and MeO-CAC, is found to be high and at least five fold higher than cy3 dye [62]. The fluorescence quantum yield could not be systematically correlated with solvent polarity for F-CAC and MeO-CAC, however, the fluorescence quantum yield and radiative rate constant of F-CAC was in general found to be higher than for MeO-CAC and than for unsubstituted CAC. On the other hand, the non-radiative rate constant of MeO-CAC was in general found to be higher than for F-CAC and than for unsubstituted CAC. The non-radiative rate constant in non-polar solvent was found to be higher than in polar solvents for these cyclic azacyanines. This confirms that the electron donating group remarkably increases the excited state lifetime but influences the radiative process, whereas an electron withdrawing group marginally increased the excited state lifetime but remarkably enhances the radiative process.

Conclusion

Photophysical behavior of the excited singlet of a novel cyclic azacyanine and its derivative has been studied in various solvents. Absorption, steady-state and time-resolved fluorescence studies indicate that the major photophysical properties of cyclic azocyanines dictate a general solvent relaxation; however, in protic solvents specific interactions of solvent molecules are encountered. The fluorescence quantum yields are very low in all the solvents used here; however, quantum yield remarkably increases in substituted cyclic azocyanines - higher than the popular cy3 dye. The internal conversion process is the major relaxation pathway for the excited singlet state. The fluorescence lifetime data suggest a relaxation in the nanosecond time scale with monoexponential decay in polar solvents and biexponential decay in non polar solvents. An electron donating group increases the fluorescence lifetime and influences the radiative process, whereas an electron withdrawing group marginally increases the excited state lifetime and remarkably enhances the radiative process of cyclic azacyanine. The fluorescence lifetime of azacyanines are found to be longer than popular cy3 dyes, which are extensively used for biomedical imaging. The fluorescence quantum yield of substituted cyclic azacyanine in water is also noted to be at least five fold higher than the popular cy3 dye, which may potentially draw more attention towards biomedical applications of CAC.

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References

1. Amos AT, Burrows BL (1973) Adv Quantum Chem 7:289–313
2. Rossky PJ, Simon JD (1994) Nature 370:263–269
3. Maroncelli M, MacInnis J, Fleming GR (1989) Science 243:1674–1681
4. McHale JL (2001) Acc Chem Res 34:265–272
5. Horng ML, Gardecki JA, Papazyan A, Maroncelli M (1995) J Phys Chem 99:17311–17337
6. Joo T, Jia Y, Yu J-Y, Lang MJ, Fleming GR (1996) J Chem Phys 104:6089–6108
7. Vöhringer P, Westervelt RA, Arnett DC, Feldstein MJ, Scherer NF (1995) J Raman Spectrosc 26:535–551
8. Bayliss NS (1950) J Chem Phys 18:292–296
9. Bayliss NS, McRae EG (1954) J Phys Chem 58:1002–1006
10. Onsager L (1936) J Am Chem Soc 58:1486–1493
11. Mataga N, Kubota T (1970) Molecular Interactions of Electronic Spectra. Marcel Dekker, New York
12. Liptay W (1974) Excited States. In: Lim EC (Ed) Academic Press: New York; Vol. I, pp 129–229
13. Patra D, Barakat C (2011) Spectrochim Acta Part A 79:1034
14. Lakowicz JR (1999) Principles of Fluorescence Spectroscopy. Kluwer Academic, Plenum Publishers, New York
15. Tolbert LM, Nesselroth SM, Netzel TL, Raya N, Stapletons M (1992) J Phys Chem 96:4492–4496
16. Biczok W, Berces T, Marta F (1993) J Phys Chem 97:8895–8899
17. Turro N (1978) Modern Molecular Photochemistry. Benjamin Cummings, New York
18. Wagner PJ, Siebert EJ (1981) J Am Chem Soc 103:7329–7335
19. Behera GB, Behera PK, Mishra BK (2007) J Surface Sci Tech 23:1–31
20. Doja MQ (1932) Chem Rev 11(3):273–321
21. Mishra A, Behera RK, Behera PK, Mishra BK, Behera GB (2000) Chem Rev 100:1973–2011
22. Mujumdar RB, Ernst LA, Mujumdar SR, Lewis CJ, Waggoner AS (1993) Bioconjug Chem 4:105–111
23. Ernst LA, Gupta RK, Mujumdar RB, Waggoner AS (1989) Cytometry 10:3–10
24. Blower MD, Feric E, Weis K, Heald R (2007) J Cell Biol 179:1365–1373
25. Patra D, Gregor I, Enderlein J (2004) J Phys Chem A 108:6836–6841
26. Patra D (2008) Appl Spectrosc Rev 43:389–415
27. Patra D (2008) Curr Chem Biol 2:267–277
28. Singh MK (2009) Phys Chem Chem Phys 11:7225–7230
29. Deniz AA, Laurence TA, Beligere GS, Dahan M, Martin AB, Chemla DS, Dawson PE, Schultz PG, Weiss S (2000) Proc Nat Acad Sci USA 97:5179–5184
30. Brooker LGC, Keyes GH, Sprague RH, Van Dyke RH, VanLare E, Vanzandt G, White FL, Cressman HWJ, Dent SG (1951) J Am Chem Soc 73:5326–5332
31. Valenzeno DP (1987) Photochem Photobiol 47:147–160
32. Panigrahi M, Dash S, Patel S, Mishra BK (2011) J Phys Chem B 115:99–108
33. West W, Geddes AL (1964) J Phys Chem 68:837–847
34. Cong P, Yan YJ, Deuel HP, Simon JD (1994) J Chem Phys 100:7855–7866
35. Yang T-S, Vöhringer P, Arnett DC, Scherer NF (1995) J Chem Phys 103:8346–8359
36. Yu A, Tolbert CA, Farrow DA, Jonas DM (2002) J Phys Chem A 106:9407–9419
37. Haddadin MJ, Kurth MJ, Olmstead MM (2000) Tetrahedron Lett 41:5613–5616
38. Huang KS, Haddadin MJ, Olmstead MM, Kurth MJ (2001) J Org Chem 66:1310–1315

39. Verbeeck A, Verrept P, Callant P, Caron K (1999) Eur Pat Appl EP 890873 A1 19990113
40. Kurose Y, Miyazawa T, Kubo H, Uchida N, Furomoto S, Satake K, Shoda H, Aizawa Y, Dan-Oh Y, Toki M (2007) PCT Int Appl WO 2007074861 A1 20070705
41. Rout MK, Patnaik LN, Bhuyan B (1977) Zeitschrift Physikalis. Chem (Leipzig) 258:601–604
42. Ozawa T, Minakami J (1998) Jpn. Kokai Tokkyo Koho JP10086533 A 19980407
43. Albert IDL, Das PK, Ramasesha S (1993) J Opt Soc Am B Opt Phys 10:1365–1371
44. Griffiths J, Li Z (1993) Dyes Pigmen 21:205–216
45. Nourmohammadian F, Gholami MD, Davoudzadeh M (2009) Synthetic Comm 39:1981–1991
46. Plater MJ, Barnes P, McDonald LK, Wallace S, Archer N, Gelbrich T, Horton PN, Hursthouse MB (2009) Org Biomol Chem 7:1633–1641
47. Cetinkol OP, Engelhart AE, Nanjunda RK, Wilson WD, Hud NV (2008) ChemBio Chem 9:1889–1892
48. Nakamura T, Takeuchi K (2003) Jpn Kokai Tokkyo Koho JP 2003064083 A 20030305
49. Nakamura T (2003) Jpn Kokai Tokkyo Koho JP 2003052400 A 20030225
50. Viaene K, Callant P (2002) U.S. Pat Appl Pub US 20020004185 A1 20020110
51. Berlman IB (1965) Handbook of fluorescence spectra of aromatic molecules, Academic Press
52. Parker CA (1968) Photoluminescence of Solutions. Elsevier Publishing Company, Amsterdam
53. Strickler SJ, Berg RA (1962) J Chem Phys 37:814
54. Lippert EZ (1957) Elektrochem 61:962–975
55. Mataga N, Kaifu Y, Koizumi M (1956) Bull Chem Soc 29:465–470
56. Reichardt C (1994) Chem Rev 94:2319–2348
57. Reichardt C (1988) Solvents and Solvent Effects in Organic Chemistry, 2nd edn. VCH Verlagsgesellschaft, Weinheim
58. Subuddhi U, Haldar S, Sankararaman S, Mishra AK (2006) Photochem Photobiol Sci 5:459–566
59. Kamlet MJ, Abboud JL, Taft RW (1977) J Am Chem Soc 99:6027–6038
60. Kamlet MJ, Abboud JL, Abraham MH, Taft RW (1983) J Org Chem 48:2877–2887
61. Mishra A, Behera GB, Krishna MMG, Periasamy N (2001) J Luminescence 92:175–188
62. Sanborn ME, Connolly BK, Gurunathan K, Levitus M (2007) J Phys Chem B 111:11064–11074
63. Huang Z, Ji D, Xia A (2005) Colloid Surface Physicochem Eng 257–258:203–209
64. Demchuk MI, Ishchenko AA, Krasnaya ZhA, Mikhailov VP (1990) Chem Phys Lett 167:170–174
65. Karpovich DS, Blanchard GJ (1995) J Phys Chem B 99:3951–3958