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Excited state isomerization and effect of viscosity- and temperature-dependent torsional relaxation on TICT fluorescence of *trans*-2-[4-(dimethylamino) styryl]benzothiazole

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ABSTRACT

Effect of viscosity and temperature on twisted intramolecular charge transfer (TICT) fluorescence of *trans*-2-[4-(dimethylamino)styryl]benzothiazole (DMASBT) have been studied. TICT fluorescence quantum yield in glycerol solution is found to be ~23-fold greater than that in ethylacetate as a non-viscous solvent. For high-viscosity solvent, the fluorescence quantum yield increases at low temperature due to the decrease in free-volume of the solvent, which favors the decrease in torsional relaxation of the molecule that induces the radiationless decay. However, the free-volume concept is not meaningful at a temperature much above the glass transition temperature when the free-volume is highly abundant. The temperature-dependent phenomenon provides a more accurate description compared to the viscosity-dependent study in a series of solvents of varying viscosity at a given temperature. The stabilization of the TICT state, as a consequence of the restricted motion of the $-N(CH_3)_2$ group in DMASBT, results in a large Stokes shifted fluorescence band. The TICT fluorescence characteristics of DMASBT are found to be different from that of molecular rotors. In solvents of low polarity, where TICT is practically zero, the molecule exhibits excited state temperature-induced *cis–trans* isomerization showing fluorescence emissions from both the isomers. However, temperature-induced TICT fluorescence quenching is observed in polar viscous medium without any isomerization. Results indicate that DMASBT can be a potential microsensor for biomimicking as well as real biological systems.

1. Introduction

Multichromophoric molecules displaying characteristic features of charge separation are well-suited model systems demonstrating charge transfer [1,2]. These molecules having intramolecular charge transfer (ICT) and twisted intramolecular charge transfer (TICT) characteristics show highly Stokes shifted fluorescence bands that depend on the chemical and physical properties of the medium [3]. Highly Stokes shifted fluorescence that appears in addition to the normal fluorescence as a result of twisting of the donor part around the single bond connector in a donor–acceptor system is called twisted intramolecular charge transfer (TICT) fluorescence as first suggested by Grabowski et al. [3].

Effect of viscosity and temperature on TICT fluorescence has been an interesting subject of research from long before [2,4]. There are recent reports on effect of solvent polarity and viscosity on TICT properties of various molecular systems such as coumarins, *N*,*N*′-dimethylaminobenzonitrile (DMABN), a group of molecules called fluorescent molecular rotors [4], 9-(*N*,*N*-dimethylamino)anthracene (9-DMA) [5], etc. Although solvent polarity causes a bathochromic shift of the emission band in all these compounds, this shift is smallest in the case of molecular rotors. Peak intensity is influenced strongly by solvent viscosity in DMABN and the molecular rotors, but polarity and viscosity influences cannot be separated with DMABN [4]. Coumarins, on the other hand are not sensitive to viscosity. The dependence of TICT fluorescence on viscosity of the medium has been widely reported and is thought to occur by a time-dependent intramolecular reorientation process [2,6,7]. Dey and Warner [5] have noticed strong dependence of fluorescence peak intensity of 9-DMA on solvent polarity and viscosity similar to DMABN. Mielniczak et al. [2] have studied on a solvent viscosity and polarity sensitive fluorescent

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sensor, 4-(4-dimethylaminostyryl)pyridinium (DMASP) derivative. They explain that the enhancement of the TICT fluorescence intensity with the increase in the viscosity of the medium surrounding the fluorophores is due to the reduction of the non-radiative transitions induced by intramolecular reorientation and diffusional collisions with the solvent molecules and justified by the Debye–Stokes–Einstein (DSE) hydrodynamic model [7]. Haidekker et al. [4] have explained the dependency of the emission intensity of molecular rotors by studying viscosity-dependent phenomena in the solvent of varying viscosities at a constant temperature through a power law relation proposed by Förster and Hoffmann [8] suggesting that the relaxation process is much deviated from the DSE diffusion in the high-viscosity region. In the present work on DMASBT it has been observed that although DMASBT is structurally similar to DMASP but the relaxation process is dependent on the temperature- and viscosity-dependent free-volume of the solvent proposed by Loutfy and Arnold [7] rather than the DSE hydrodynamic model. Moreover, temperature-dependent phenomena provides better description of the torsional relaxation rather than viscosity-dependent study at a constant temperature which is in contrary to the study on some molecular rotors by Haidekker et al. [4]. The free-volume concept [7] rather than the DSE hydrodynamic model gives more satisfactory result to explain the high quantum yield in the highly viscous solvents at low temperature. Recently, Dey and Warner [5] have ruled out the association of large Stokes shifted fluorescence with the TICT process in the viscous medium without any specific reason. But this work suggests that TICT fluorescence can be highly Stokes shifted in viscous medium and the probable reason for the same has been discussed. It has also been observed in this study that not only the fluorescence quantum yield of DMASBT is dependent on viscosity but also the fluorescence band is highly red shifted in a polar solvent [9], which is in contrary to the molecular rotors. The present report on DMASBT shows that unlike coumarins and molecular rotors, both solvent polarity and viscosity have their own effects on emission intensity as well as on emission wavelength. It has been demonstrated that in case of DMASBT this effect is not as complex as DMABN [4]. It can be mentioned that many cellular and organismal functions are related to the viscosity of their environment, and the alteration in cell membrane viscosity is well known to be responsible for several diseases, e.g. cell malignancy, hypercholesterolemia, atherosclerosis and diabetes [4].

In view of the development of a method that could be applicable for the measurements of viscosity in biological systems, and in particular real-time measurements of viscosity changes on a microscopic scale, the present study aims to get a fluorescent molecule that can act as a microsensor for biomimicking as well as real biological systems. We chose DMASBT because of high sensitivity of its fluorescence properties on polarity and pH [9] of the environment. The present work is mainly focused on to see the sensitivity of TICT fluorescence of DMASBT on the viscosity of the medium. Although, some success to measure the viscosity in biological systems has been accomplished through the use of molecular rotors by means of their viscosity-dependent fluorescence quantum yields, but the micropolarity of the biological systems can also get changed due to the intercalation of water molecules. The molecular rotors cannot sense this too effectively. It is also worth mentioning here that conventional mechanical viscometers are cumbersome to use and are incapable to perform real-time viscosity measurements.

Another important excited state process is photoisomerization. A system of excited state charge transfer coupled with isomerization under a certain condition can be an interesting model for important natural photoprocesses like photosynthesis and vision. It is now quite well known that the molecular properties get changed remarkably on photoisomerization [10–15]. In the present work, temperature-dependent excited state isomerization process has also been studied to see how excited state isomerization affects the charge transfer phenomenon. DMASBT has been reported to have non-genotoxicity [16].

2. Experimental

2.1. Materials and methods

DMASBT was procured from Aldrich Chemical Company, WI, USA. The methods of recrystallization and purity check are mentioned elsewhere [9]. All the solvents used are of spectroscopic grade and procured from Spectrochem Chemical Company, India. Triple distilled water was used for the preparation of the aqueous solutions. To record the UV–vis absorption and fluorescence spectra of DMASBT in pure solvents, a stock solution of DMASBT (1.001×10^{-3} M) was prepared in pure methanol, 0.1 mL of which was poured in a 10 mL volumetric flask and left for a few hours for complete evaporation of methanol and then the compound was dissolved in respective solvents to make the final volume to 10 mL. For the preparation of solutions in different percentages of the glycerol–water mixtures, 0.05 mL (2.002×10^{-3} M) of DMASBT solution in methanol was added to a mixture of glycerol and water and the final volume of it was adjusted to 10 mL. Methanol was added due to low solubility of DMASBT in water as well as glycerol–water mixture. The percentage of methanol present in glycerol–water mixture is only 0.5. The concentration of DMASBT in all the experimental solutions used for spectroscopic measurements was 1.001×10^{-5} M. The fluorescence quantum yields were determined with respect to that of quinine sulfate in 0.1N H_2SO_4 as 0.55.

The absorption spectra were recorded using a Jasco V570 UV–vis spectrophotometer. Fluorescence measurements were performed using a Shimadzu RF-5301PC scanning spectrofluorimeter. Fluorescence lifetimes were determined from time-resolved intensity decay by the method of time-correlated single-photon counting using a picosecond diode laser at 370 nm (IBH, U.K., nanoLED-07) as light source. The typical response time of this laser system was 50 ps. The fluorescence decays were deconvoluted using IBH DAS6 software. Mean (average) fluorescence lifetimes for biexponential iterative fittings were calculated from the pre-exponential factors and the decay times using the following equation [17]:

$$< \tau >= a_1\tau_1 + a_2\tau_2 \quad (1)$$

The steady-state fluorescence anisotropy measurements were performed with the same steady state spectrofluorimeter fitted with a polarizer attachment. The excitation and emission bandwidths used for the anisotropy measurements were 5 nm each. The steady state anisotropy, r can be represented as [18]

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad (2)$$

where I_{VH} and I_{VV} are the intensities obtained from the excitation polarizer oriented vertically and the emission polarizer oriented in horizontal and vertical positions, respectively. The factor G is defined as

$$G = \frac{I_{HV}}{I_{HH}} \quad (3)$$

where I terms refer to parameters same as mentioned above for the horizontal position of the excitation polarizer. All measurements other than the study of temperature effect were done at room temperature (25 °C).

To study the temperature dependence of the fluorescence of DMASBT, a simple on–off temperature controller was designed. A suitable heater made of aluminium block was fabricated for holding

the cuvette. The temperature controller is based on a Wheatstone bridge balance. Adjustment of the resistance of the potentiometer (R_{set}) can provide the desired temperature. The bridge was balanced when the sample attained the desired temperature. A thermistor, which forms a part of the bridge, is placed in the heater. The temperature dependence of the non-linear resistance was calibrated with the help of a thermocouple. A digital display was connected to the heater to monitor the temperature. The accuracy of the temperature measurement was within 0.1°. The temperature of the sample was kept fixed during the fluorescence scan.

Hyperchem package version 6.01 (Hypercube Inc., Canada) has been used for the theoretical calculations. The ground state (S_0 geometry) of the compound was optimized by AM1 method. AM1-SCI (singly excited configuration interaction) was performed to get the energy (E_g) and dipole moment in the ground state and the transition energies ($\Delta E_{i\to j}$), and dipole moments of different excited states. We have taken care of all the singly excited configurations within an energy window of 11 eV from the ground state. $\Delta E_{i\to j}$ corresponds to the excitation of an electron from the orbital ϕ_i (occupied in the ground state) to the orbital ϕ_j (unoccupied in the ground state). The total energy of the excited state (E_j) was then calculated as $E_j = E_g + \Delta E_{i\to j}$. The CI wavefunction has been used to generate g orbitals and one-electron density matrices, which were used to calculate the dipole moments of the excited states of the compounds.

3. Results and discussion

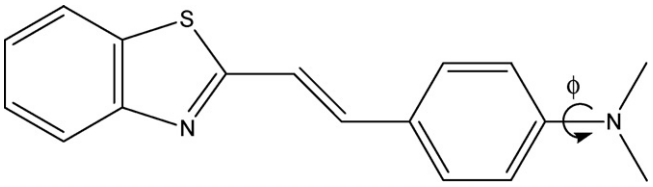
3.1. Absorption and emission characteristics of DMASBT in different solvents

Table 1 summarizes the spectral properties of DMASBT in a series of solvents studied earlier [9]. Both absorption and emission band maxima are red shifted with increasing the polarity of the solvents. Structured absorption and fluorescence bands in non-polar and/or less polar solvents progressively become structureless with the increase in the polarity of the solvent media. The mirror image relationship between absorption and emission bands in non-polar solvents confirmed similar geometries of absorbing and emitting

Table 1
Absorption peak position (λ^{ab}_{max} (nm)), fluorescence peak position (λ^{fl}_{max} (nm))[a], fluorescence quantum yields (φ_f) and Stokes shift (ν_{ss}) of DMASBT[b] in different solvents along with their $E_T(30)$ (kcal mol^{-1}) values

Solvents	$E_T(30)$	λ^{ab}_{max} (nm)	λ^{fl}_{max} (nm)	ν_{ss} (cm^{-1})	φ_f (±0.0001)[c]
Cyclohexane	30.9	384	447	3671	0.0010
Heptane	31.1	387	446	3418	0.0014
Hexane	32.4	384	445	3604	0.0018
Diethyl ether	34.5	390	460	3902	0.0020
1,4-Dioxane	36.0	393	467	4032	0.0022
THF[d]	37.4	396	482	4506	0.0030
Ethylacetate	38.1	391	477	4611	0.0053
DMF[e]	43.6	402	504	5035	0.0070
DMSO[f]	44.8	405	513	5198	0.0078
Acetonitrile	45.6	394	504	5540	0.0066
Isopropanol	48.4	394	493	5097	0.0048
Ethanol	51.9	396	498	5173	0.0045
Methanol	55.4	397	507	5465	0.0030
Glycerol	56.7	409	530	5582	0.1210
Water[g]	63.1	402	516	5496	0.0012

[a] λ_{ex} = 395 nm.
[b] [DMASBT] = 1.001 × 10^{-5} M.
[c] Fluorescence quantum yields at λ_{ex} = 395 nm.
[d] Tetrahydrofuran.
[e] Dimethylformamide.
[f] Dimethylsulfoxide.
[g] At pH 7.2.



Scheme 1. Structure *trans*-2-[4-(dimethylamino)styryl]benzothiazole (DMASBT).

species. The appearance of broad structureless Stokes shifted bands in polar solvents and progressive increase in fluorescence quantum yields with increasing the polarity of the solvents suggested the charge transfer (CT) character of the emitting state in polar media [9]. Emissions in non-polar solvents are described to be originated from the locally excited (LE) state. Upon excitation, transfer of an electronic charge from the donor (D) part to the acceptor (A) site of the molecule leads to a charge separation. According to quantum chemical AM1 calculations [9] on DMASBT, the dimethylamino group (—N(CH_3)$_2$) acts as the donor part and the acceptor part is the 2-styrylbenzothiazole moiety (Scheme 1). The donor and acceptor moieties are flexibly linked by a C–N bond. The torsional angle (ϕ) (Scheme 1) related to the rotation of donor with respect to acceptor around C—N bond is equal to zero when the amino lone pair orbital is perpendicular to the molecular plane of the acceptor subsystem. A twisting with torsional angle, $\phi = 90°$ leads to full intramolecular charge transfer (ICT) in the polar medium at the excited state known as TICT state. Theoretical calculations [9] show that the S_3 state is responsible for the polarity-induced TICT emission. The calculated dipole moments of S_3^{TICT} state and the ground singlet TICT state (S_0^{TICT}) are 12.5 and 0.8 Debye, respectively. Therefore, greater stabilization of S_3^{TICT} state compared to the S_0^{TICT} state by the influence of polar medium results into a decrease in the energy gap, $\Delta E\left(S_3^{TICT} - S_0^{TICT}\right)$ with a consequent red shift in the TICT emission. As a result of that the rate of internal conversion from S_3^{TICT} to S_0^{TICT} is higher in water than that in less polar solvents. This fact is in favor of lower TICT fluorescence quantum yield in the aqueous medium as compared to that in non-polar medium [19]. Moreover, TICT emissions in less polar solvents are also increased due the decrease in the rate of intersystem crossing from the S_3^{TICT} state to triplet TICT state [20]. The detailed explanations of the results obtained from the absorption and fluorescence study of DMASBT in pure solvents of different polarities have been given elsewhere [9]. Fluorescence quantum yields and Stokes shifts of DMASBT in the solvents of different polarities are listed in Table 1. The variation of fluorescence quantum yields and Stokes shift with the solvent polarity parameter, $E_T(30)$ have been shown in Fig. 1 and its inset, respectively. Fig. 1 shows that the fluorescence quantum yield increases, reaches maximum and then decreases with increasing the polarity of the solvents. This can be explained by the fact that initially fluorescence from charge transfer state is increased due to the stability of this state with respect to the LE state, but later it is decreased in highly polar solvents due to the high possibility of non-radiative processes, internal conversion and inter system crossing [9]. As a matter of fact Stokes shifts are also substantially changed with increasing the polarity of the medium (Fig. 1(inset)). Although fluorescence quantum yields are low in most of the solvents, but both quantum yield and Stokes shift of the molecule are highest in glycerol as solvent.

3.2. Fluorescence study in glycerol–water mixture

As mentioned above, both fluorescence quantum yield and Stokes shift of DMASBT are highest in glycerol (Table 1). As depicted in Fig. 2(a), the fluorescence intensity increases with increasing the percentage of glycerol in glycerol–water mixture with a sub-

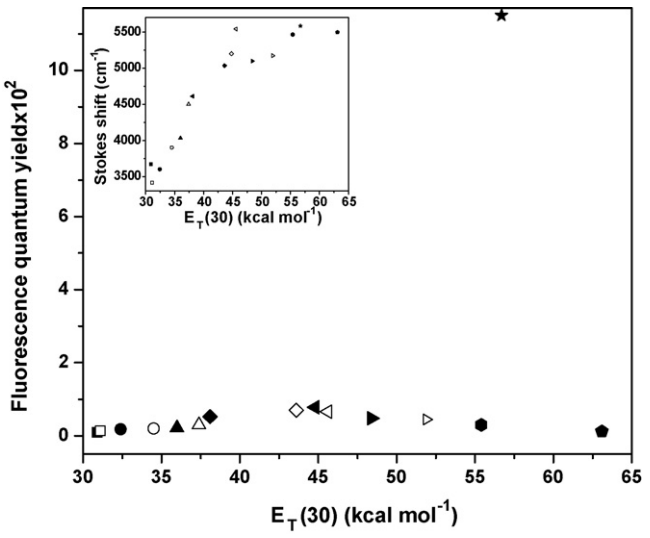


Fig. 1. Plot of fluorescence quantum yields of DMASBT vs. $E_T(30)$ of pure solvents. (Inset) Plot of Stokes shift (cm^{-1}) of DMASBT vs. $E_T(30)$ ($kcal\,mol^{-1}$) of pure solvents: (■) cyclohexane; (□) heptane; (●) hexane; (○) diethyl ether; (▲) 1,4-dioxane; (△) THF; (◆) ethylacetate; (◊) DMF; (◀) DMSO; (◁) acetonitrile; (▶) isopropanol; (▷) ethanol; (⬢) methanol; (★) glycerol; (⬟) water. λ_{ex} = 395 nm [DMASBT] = 1.001×10^{-5} M.

stantial bathochromic shift of the fluorescence band up to 60% glycerol–water mixture. Above this, although increase in intensity continues but the shift of peak maxima plateaus. Increased energy gap between S_3^{TICT} and S_0^{TICT} states in glycerol due to lower polarity compared to water may be a reason for the increased TICT fluorescence but in that case it demands a hypsochromic shift with increasing percentage of glycerol in the mixture. The red shifted band cannot be due to exciplex, because in that case fluorescence bands for monomer as well as exciplex are expected [18]. The fluorescence quantum yield in glycerol is much greater than that in non-viscous solvent. As for example, it is almost 23-fold greater in glycerol than that in ethylacetate as a non-viscous solvent. Fluorescence quantum yield in pure glycerol solution is almost 15 times greater than that in 20% glycerol–water mixture, whereas the same in case of 4-(4-dimethylaminostyryl)pyridinium derivative, reported by Mielniczak et al. [2] got increased by six times only. The presence of shoulder at lower wavelength for the fluorescence band of DMASBT in solution of low glycerol percentages could be due to the contribution of LE fluorescence as a result of introduction of non-polarity by the addition of glycerol in water. This disappears at high percentage of glycerol due to the higher population and stabilization of TICT state as discussed later.

The extent of restriction imposed by the medium in which the fluorophore is dissolved can be described by the study of fluorescence anisotropy of DMASBT in glycerol. The plot for variation of fluorescence anisotropy as a function of weight percentage of glycerol in glycerol–water mixture is shown in Fig. 2(b). Fluorescence anisotropy (λ_{em} = 550 nm) of DMASBT increases with increasing the viscosity of the medium to a very high anisotropy value (~0.38) in pure glycerol solution. This can be attributed to the significant restriction of rotational diffusion of the molecule in a medium of high viscosity and can be correlated with the increase in fluorescence intensity. Loutfy et al. [7] studied the effect of viscosity and temperature on fluorescence quantum yields of molecular rotors extensively. To compare the characteristics of DMASBT with those of molecular rotors, the effect of temperature on fluorescence quantum yields of DMASBT in dioxane (Fig. 3(a)) and in glycerol (Fig. 3(b)) has been studied. It is observed that in case of dioxane fluorescence quantum yield decreases with increasing temperature

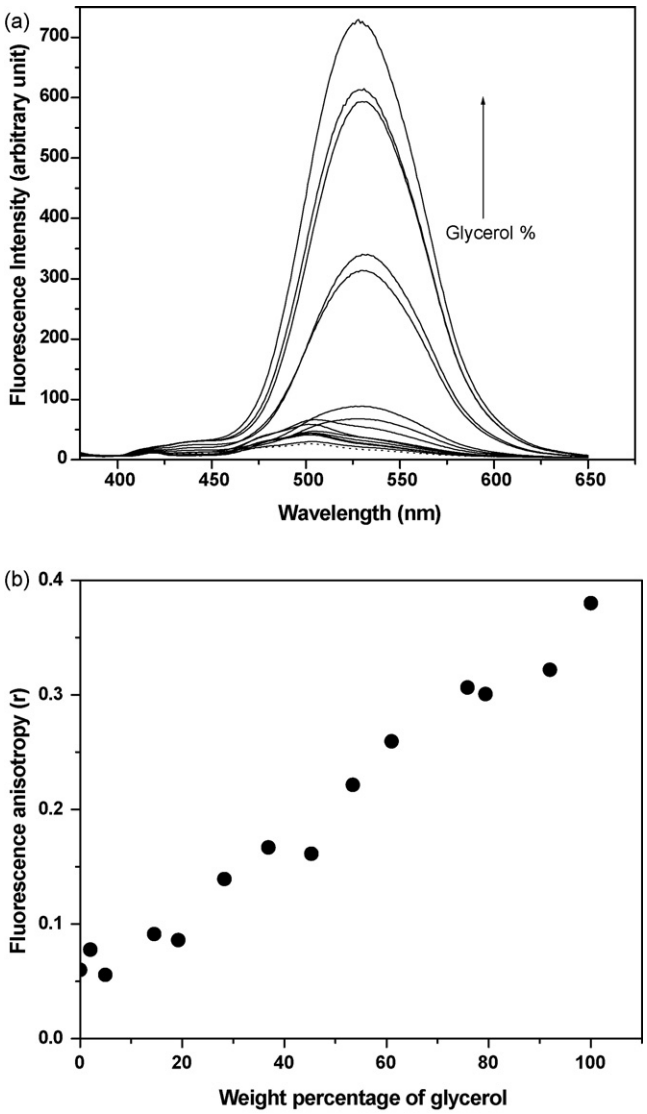


Fig. 2. (a) Plot of variation of fluorescence spectra of DMASBT with the variation of percentage of glycerol in glycerol–water mixture. The broken line represents 0% glycerol, i.e., pure aqueous solvent (λ_{ex} = 367 nm). (b) Plot of variation of fluorescence anisotropy (λ_{em} = 550 nm) of DMASBT as function of the composition of the glycerol–water mixture (λ_{ex} = 367 nm). [DMASBT] = 1.001×10^{-5} M.

accompanied by a substantial blue shift of the wavelength maxima at a temperature higher than 45 °C, whereas in case of glycerol fluorescence quantum yields are decreased without any change in wavelength maxima. Effect on fluorescence quantum yields and wavelength maxima are explained separately in the following sections.

The DSE hydrodynamic model and the free-volume-dependent torsional relaxation dynamics of probe proposed by Loutfy et al. [7] have been tested to describe the relaxation processes. Förster and Hoffmann [8] derived an equation, $\phi_f = B\eta^x$ which was found to hold good for various dyes. In this equation, ϕ_f and η are fluorescence quantum yield and viscosity coefficient, respectively, B is a constant and x lies between 1/3 and 2/3. The fluorescence quantum yields of DMASBT in different composition of glycerol–water mixtures are measured at 25 °C and are listed in Table 2 along with the values of viscosities of the mixtures. Fig. 4(a) shows the plot of $\log\phi_f$ against $\log\eta$. A reasonably good straight line (regression coefficient = 0.94) is obtained with a slope much less than unity (0.38). This suggests that DSE diffusion in high-viscosity region is

not a suitable model for relaxation process. To see the effect of temperature in high-viscosity solvent, fluorescence quantum yields of DMASBT in glycerol have been determined at different temperatures. The values of fluorescence quantum yields in glycerol along with the viscosity values of glycerol at different temperatures are also listed in Table 2. Fig. 4(b) shows the variation of fluorescence quantum yields on η/T. The data displayed in Fig. 4(b) show that fluorescence quantum yield (ϕ_f) is proportional to $(\eta/T)^x$, where x is equal to 0.49 for temperatures between 24 and 80 °C. The much better regression coefficient (0.996) of this plot compared to the plot of variation of quantum yields with viscosity at a given temperature, suggests that rather than the viscosity-dependent phenomenon in a series of solvents at a given temperature, the temperature-dependent studies give more consistent data and provide less ambiguous tests of hydrodynamic predictions. To test the importance of the temperature- and viscosity-dependent free-volume of the viscous solvent on the molecular relaxation process of the excited state (in case of DMASBT it is S_3) the following equations have been used [7]. The free-volume of a solvent is dependent on the temperature as follows:

$$f = f_g + \alpha(T - T_g) \tag{4}$$

where α is the thermal expansion coefficient, f and f_g are the free-volume fractions at given temperatures T and T_g, respectively. The constants $\alpha = 4.4 \times 10^{-4}$ deg^{-1}, $f_g = 0.025$ and $T_g = 187$ K for glycerol [7].

The viscosity of liquid is dependent on the free-volume fraction as follows [21]:

$$\eta = A \exp\left(\frac{f_o}{f}\right) \tag{5}$$

where f and f_o are free- and occupied-volume fractions, respectively. This dependence is controlled by the value of A of liquid. This relationship between viscosity and free-volume fractions has shown more accuracy in the determination of viscosity of *n*-heptadecane at different temperatures compared to that obtained by using Andrade temperature equation [21]. The torsional motion induced non-radiative decay rate constant, k_{nr} can be related to the free-volume fraction of solvent, f according to the equation:

$$k_{nr} = k_{nr}^o \exp\left(\frac{-xf_o}{f}\right) \tag{6}$$

where x is a constant for the particular molecule and k_{nr}^o is the rate of orientation of the free-rotor. The non-radiative decay rate constant, k_{nr} is related to the fluorescence quantum yield and radiative rate constant as follows:

$$k_{nr} = k_r\left(\frac{1}{\phi_f - 1}\right) \tag{7}$$

Therefore, from Eqs. (6) and (7), the following equation is obtained which relates fluorescence quantum yield with the free-volume fraction:

$$\phi_f = \left(\frac{k_r}{k_{nr}^o}\right) \exp\left(\frac{xf_o}{f}\right) \tag{8}$$

Therefore,

$$\ln \phi_f = \ln\left(\frac{k_r}{k_{nr}^o}\right) + \left(\frac{xf_o}{f}\right) \tag{9}$$

The free-volume fractions (f) of glycerol at different temperatures are calculated and tabulated in Table 2. Fig. 5 represents the plot of $\ln \phi_f$ against $1/f$. This plot shows the linear dependence with regression coefficient, 0.99. The slope of this plot (=xf_o, Eq. (9)) =0.598. Fig. 5 (inset) is the plot of $\ln \eta$ against $1/f$. The slope of this plot is found to be 1.18 (regression coefficient = 0.99), which is the value of f_o Eq. (5). From the values of these last two slopes x is calculated to be 0.51. The slight deviation from the linear dependence of $\ln \phi_f$ on $1/f$ at high temperature as explained by Loutfy et al. [7] is due to the high abundance of free-volume available for the rotation dependent relaxation of the molecule.

Combining Eqs. (5) and (8) the relation found is as follows:

$$\phi_f = B\left(\frac{\eta}{T}\right)^x \tag{10}$$

where $B = (k_r/k_{nr}^o)(T/A)^x$. The variation of $\log \phi_f$ with $\log(\eta/T)$ has already been shown in Fig. 4(b). The slope value, i.e., the value of exponent x (0.49) agrees well with those calculated above (0.51). This result is consistent with the fact that the rotation dependent non-radiative decay rate increases with increasing the free-volume and decreasing the viscosity of the solvent. Therefore, the viscosity-dependent fluorescence quantum yield of DMASBT can be explained on the basis of the dependence of viscosity coefficient (η) on the free-volume of solvents whose free-volume is very small to cause high viscosity. At temperature much above the glass transition temperature, the fraction of free-volume is so large that the free-volume dependence of fluorescence quantum yield becomes inappropriate [7].

To provide additional information on the effect of viscosity on the non-radiative decay rate, the fluorescence decay profiles

Table 2
Fluorescence quantum yields (ϕ_f) of DMASBT in different percentage of glycerol–water mixture at constant temperature (25 °C), viscosities (η) of glycerol–water mixtures at 25 °C, fluorescence quantum yields (ϕ_f') in pure glycerol at different temperatures, viscosities (η) and free-volume fractions (f) of pure glycerol at different temperatures

Constant temperature study with glycerol–water mixture			Temperature-dependent study with pure glycerol			
% of glycerol in mixtures (v/v)	η[a] (cP)	Quantum yield (ϕ_f)[b,c]	Temperature (K)	η[d] (cP)	Quantum yield (ϕ_f')[b,c,d]	$f \times 10^2$
24	1.80	0.013	298.15	934.00	0.121	7.391
32	2.38	0.016	303.15	637.28	0.115	7.611
40	3.18	0.017	308.15	449.95	0.098	7.831
48	4.97	0.019	313.15	317.96	0.079	8.051
56	7.59	0.029	318.15	220.03	0.062	8.271
64	15.46	0.039	323.15	156.17	0.050	8.491
71	27.91	0.055	328.15	109.33	0.044	8.711
75	36.43	0.062	333.15	75.27	0.035	8.931
90	267.00	0.112	338.15	49.73	0.030	9.151
100	934.00	0.121	343.15	36.95	0.025	9.371
			348.15	28.44	0.021	9.591
			353.15	15.67	0.018	9.811

[a] Ref. [2] (viscosity data were obtained by extrapolation of dependence of viscosity on (v/v) % of glycerol–water mixture).
[b] $\lambda_{ex} = 395$ nm.
[c] Standard deviation ±0.0001.
[d] Ref. [7] (viscosity data were obtained by extrapolation of dependence of viscosity on temperature in Kelvin scale) [DMASBT] = 1.001×10^{-5} M.

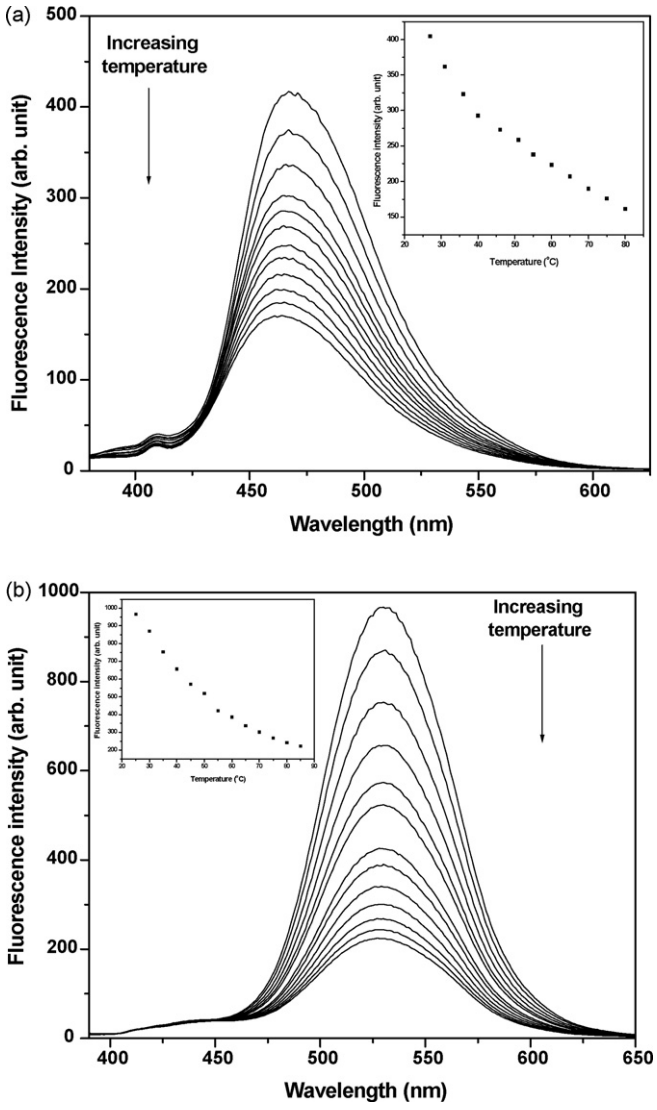


Fig. 3. (a) Variation of fluorescence spectra of DMASBT in dioxane as a function of temperature of the solution. Inset: plot of the fluorescence intensities against temperature. (b) Variation of fluorescence spectra of DMASBT in glycerol as a function of temperature of the solution. Inset: plot of the fluorescence intensities against temperature. λ_{ex} = 395 nm, and [DMASBT] = 1.001 × 10^{-5} M.

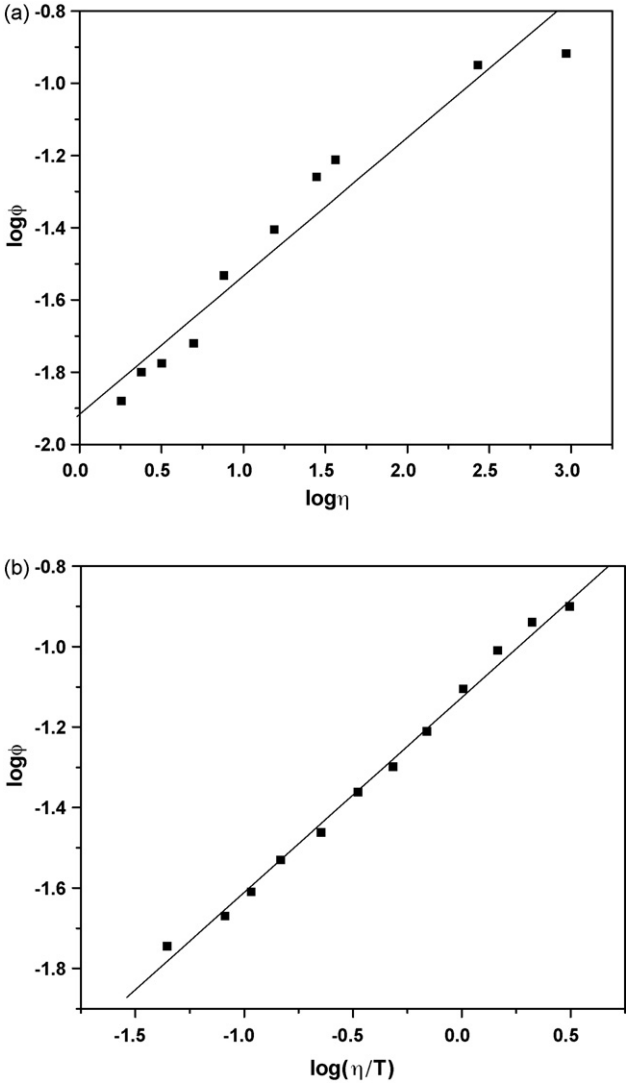


Fig. 4. (a) Plot of fluorescence quantum yields (ϕ_f) of DMASBT against viscosities of different composition of glycerol–water mixtures at 25 °C. (b) Plot of fluorescence quantum yields (ϕ_f) of DMASBT in glycerol against η/T. λ_{ex} = 395 nm, and [DMASBT] = 1.001 × 10^{-5} M.

of DMASBT have been measured in 20% glycerol–water and pure glycerol solutions and are shown in Fig. 6(a) and (b), respectively. The calculated lifetimes are listed in Table 3. Since the lifetime of DMASBT in water is very low (<50 ps), instrumental limitation did not allow us to calculate it. The raw experimental data are suitably fitted by double exponential fitting equation. The contributions of different species in terms of pre-exponential factors, χ^2 and standard deviations are also mentioned in Table 3. The slow decay of fluorescence of both the species with increasing the viscosity of the medium supports the restricted orientational reorganization of solvent molecules in viscous environments. Better value of χ^2

Table 3
Lifetimes (τ) and pre-exponential factors (a) of DMASBT in different percentages of glycerol in glycerol–water mixtures

% of glycerol	a_1	τ_1 (ps)	a_2	τ_2 (ps)	χ^2
20	0.76	130 ± 3	0.24	706 ± 5	1.47
100	0.32	257 ± 3	0.68	1100 ± 6	0.99

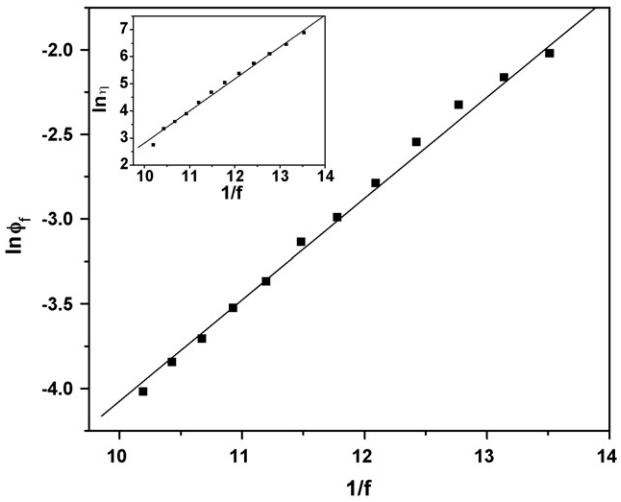


Fig. 5. Plot of ln ϕ_f against 1/f. (Inset) Plot of ln η against 1/f.

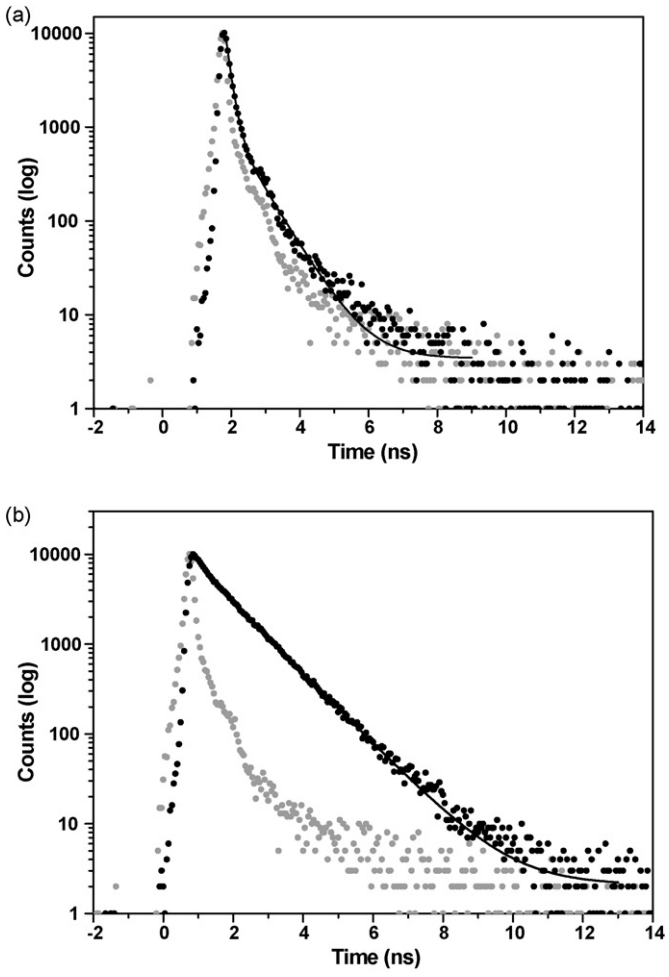


Fig. 6. Lifetime decay profiles of DMASBT in (a) 20% glycerol, and (b) pure glycerol solutions. The gray dots represent the signal for the prompt and the black ones represent that for DMASBT. The solid line is the fit to the raw data. λ_{ex} = 370 nm, λ_{em} = 500 nm for 20% glycerol, and λ_{em} = 550 nm for pure glycerol. [DMASBT] = 1.001 × 10^{-5} M. Instead of the residuals of the fits, the χ^2 values have been mentioned in Table 3 that carries the same significance.

in high percentage of glycerol is in favor of relatively low probability of formation of multiple species (more than 2) in a medium of high viscosity. The restricted orientational reorganization of solvent molecules in viscous environments is consistent with the high values of fluorescence anisotropy and fluorescence quantum yield. The large Stokes shift or the bathochromic shift of the fluorescence band in viscous medium can be due the restricted rotational motion of $-N(CH_3)_2$ group attached to DMASBT. The decrease in free-volume with increasing the viscosity of the solvent causes the restricted motion of $-N(CH_3)_2$ group thereby increasing the TICT fluorescence quantum yield as well as the bathochromic shift of the fluorescence band because the proper orientation of $-N(CH_3)_2$ group is related not only to the population of the TICT molecules but also to the stabilization of the TICT state. The plateau in the shift of band maxima above 60% glycerol–water mixture could be due to the fact that stabilization of TICT state as a result of proper orientation of $-N(CH_3)_2$ group is complete at this viscosity. The continuity in increase in fluorescence quantum yields for solutions above 60% glycerol is as a result of decrease in the free-volume with increasing viscosity. The biexponential decay could be due to two different populations of excited species where one experiences smaller fraction of free-volume giving slower decay, whereas other gets larger fraction of free-volume resulting in faster decay. This is the reason

Table 4
Fluorescence quantum yields (φ_f), average lifetimes ($\langle\tau\rangle$), radiative (k_r) and non-radiative (k_{nr}) rate constants of DMASBT in different percentages of glycerol in glycerol–water mixtures

% of glycerol	φ_f	$\langle\tau\rangle$ (ps)	k_r (s^{-1})	k_{nr} (s^{-1})
20	0.008	268	2.99×10^7	3.70×10^9
100	0.121	830	1.46×10^8	1.06×10^9

that contributions of different fractions are reversed when percentage of glycerol increases (Table 3). The average lifetime values of DMASBT in different percentages of glycerol–water mixtures calculated using Eq. (1) are tabulated in Table 4. The decay parameters are affected by the increased percentage of glycerol, with the average lifetime, $\langle\tau\rangle$ changing from 268 ps in 20% glycerol to 830 ps in pure glycerol. The increased viscosity of medium is reflected in the increase in excited state lifetime. From calculated values of φ_f and $\langle\tau\rangle$, we calculated the radiative and non-radiative rate constants for the TICT process using the following equations [22]:

$$k_r = \frac{\varphi_f}{\langle\tau\rangle} \tag{11}$$

$$k_{nr} = \frac{1}{\langle\tau\rangle} - k_r \tag{12}$$

It is apparent from Table 4 that radiative rate constant (k_r) is increased by 4.9 times whereas non-radiative rate constant (k_{nr}) is decreased by 3.5 times with increasing the concentration of glycerol in glycerol–water mixture. The decrease in non-radiative decay rate as explained above are due to the decrease in free-volume and increase of viscosity of the solvent that causes rotational restriction in the molecule.

It is well known that fluorescence anisotropy may change due to rotational diffusion of the molecule as well as the fluorescence lifetime. To make sure that the observed change in steady state anisotropy of DMASBT in different percentages of glycerol–water mixture is not due to any change in the fluorescence lifetime, Perrin's equation Eq. (13) [18] has been used to calculate the approximate values of the average rotational correlation times:

$$\tau_c = \frac{\langle\tau\rangle r}{r_o - r} \tag{13}$$

where r, r_o and $\langle\tau\rangle$ are the steady-state anisotropy, limiting anisotropy and mean lifetime of DMASBT, respectively. As mentioned above, the calculated values are approximate; there are a few reasons for that. Although Perrin's equation is not strictly valid for the present case yet the equation is considered to be valid due to the use of mean values of fluorescence lifetime. Other reasons are the unavailability of exact value of r_o as the time resolved anisotropy could not be done and the lifetime of species less than 50 ps could not be measured. In the present calculation, the value of r_o is taken as 0.38 in case of glycerol–water mixture, where 0.38 is the anisotropy value of DMASBT in pure glycerol and r_o = 0.18 in case of aqueous medium (taken from the literature [17]). The lifetime value in aqueous medium is considered to be 50 ps. With this lifetime value, although the exact value of τ_c is not found, but at least the correct trend of τ_c values in the medium of different viscosities could be predicted. The values of τ_c calculated using Eq. (13) are found to be ~25 and ~71 ps in aqueous and 20% glycerol–water mixtures, respectively taking experimentally determined r values as 0.06 and 0.08 for water and 20% glycerol–water mixtures, respectively. If the lifetime of DMASBT in aqueous medium was lower than 50 ps (greater value is not expected because then we could have calculated lifetime with our instrument), τ_c value could be further lower than 25 ps according to Eq. (13). The increase of τ_c with increasing viscosity of environments definitely suggests that

the observed changes in steady state fluorescence anisotropy values (Fig. 2(b)) are not due to lifetime-induced phenomena but as predicted earlier that rotational restriction imposed on molecule increases with increasing the viscosity of the medium.

Contrary to the argument made by Dey and Warner [5] regarding the absence of TICT fluorescence of 9-DMA in viscous medium due to the restricted motion of $-N(CH_3)_2$ group, many groups have reported the high yield of TICT fluorescence in viscous medium [2,4]. Compared to these, the simpler and greater dependence of TICT fluorescence quantum yields of DMASBT on viscosity is observed. Unlike coumarin derivatives and molecular rotors (e.g., 9-(dicyanovinyl)-julolidine, DCVJ) [4], fluorescence parameters of DMASBT are dependent on both viscosities and polarities of the solvents. When emission wavelengths of coumarin derivatives are only influenced by solvent polarities without any effect of solvent viscosities on fluorescence parameters, the TICT fluorescence intensities of molecular rotors are sensitive only to viscosities with low sensitivity towards solvent polarities [4]. The fluorescence intensity of DMASBT is increased by 15-fold as compared to the fivefold increase in DCVJ with increasing the percentage of glycerol from 20 to 100. Mielniczak et al. [2] explained the dynamics of reorientation relaxations of the 4-(4-dimethylaminostryryl)pyridinium (DMASP) derivatives in solution with high friction between the rotating probe and solvent molecules, which is already proven for many compounds exhibiting ICT properties including DMABN [6,7,23–25]. This is in support to the argument that dependency of TICT fluorescence intensity on viscosity of medium is a well-known phenomenon. Moderately high fluorescence quantum yields with substantial bathochromic shifts of fluorescence bands in DMF and DMSO as solvents can also be explained by their restricted orientational reorganizations because of viscosity.

3.3. Temperature-dependent fluorescence study of DMASBT

The effects of temperature on the fluorescence properties of DMASBT in dioxane and glycerol as solvents are represented by Fig. 3(a) and (b), respectively. On increasing the environment temperature of the system the fluorescence quantum yield decreases, which is a quite common observation that happens due to the enhanced movement of the molecule and thus opening more and more non-radiative decay routes [26,27]. Interestingly, in dioxane as solvent there is no wavelength shift of fluorescence band maxima up to a temperature of ~45 °C, but once this temperature is crossed there is a clear hypsochromic shift of ~4 nm followed by freezing of any further shift with a gradual quenching of the fluorescence. This appears to be a probable configurational change of the molecular system. It is also pertinent to note that unlike many cases [13], the changed isomer is also fluorescent. Since the molecule was initially in the *trans* form, therefore at a temperature of ≥45 °C the most probable change in configuration is due to its conversion to the *cis* form. However, no such hypsochromic shift is observed in case of glycerol as solvent, although fluorescence is quenched as usual with increasing the temperature. The inset of Fig. 3(a) shows the nature of the decrease in the fluorescence intensity of DMASBT in dioxane

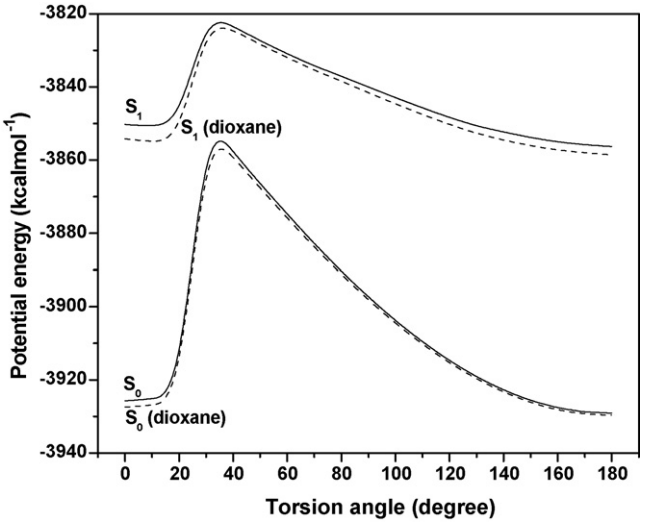
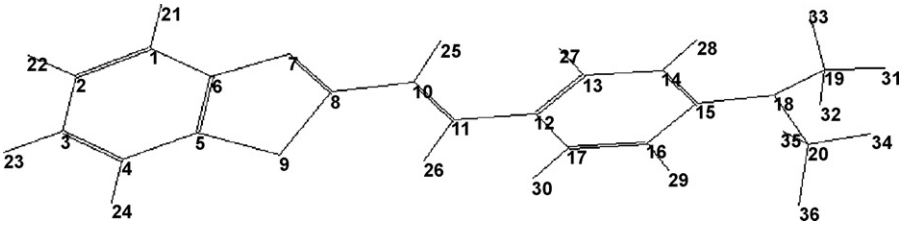


Fig. 7. Configuration interaction calculations using AM1 method showing the potential energy surface (PES) diagram at different twist angles (25-10-11-26, vide Scheme 2).

with increasing temperature. The decline follows a segmented linearity with two different slopes. On the other hand, the same in the case of glycerol involves an exponential decay (inset of Fig. 3(b)). This may be attributed to the fact that in case of glycerol the quenching is simply due to the increase in the rate of non-radiative decay at moderately high temperature; however, at very high temperatures much above the glass transition temperature of the solvent when the free-volume is plentiful and free-volume concept becomes useless, the decrease in fluorescence quantum yield is probably due to the increase in singlet–triplet intersystem crossing [28]. On the other hand in case of dioxane, the quenching is definitely due to the increase in the rate of non-radiative processes but in addition to that there is a proposed configurational change after a certain temperature (45 °C). Configuration interaction calculations using AM1 method can be efficiently used to get the potential energy surface (PES) diagrams (Fig. 7) at different torsion angles (25-10-11-26, vide Scheme 2) [29,30]. Solid lines indicate the PES for DMASBT in vacuum and the broken lines constitute those for the solvated molecule in dioxane (relative permittivity = 2.22). The figure clearly shows the change in the magnitude of the energy barrier in the two electronic states, viz., S_0 (ground state) and S_1 (first excited singlet state). In both the states, *trans* isomer of DMASBT is the most stable one. The potential energy surface constructed by rotating the aromatic moieties on both sides of the double bond connecting C10 and C11 (vide Scheme 2) clearly shows that the *trans* geometry is more stable than the *cis* form in the ground state. An energy barrier of 77 kcal mol^{-1} separates the two isomers in the ground state.

The next question that arises is whether the molecule retains its *trans* geometry even after excitation. AM1-SCI calculations show that the energy barrier between the *cis* and *trans* geometries



Scheme 2. Optimized geometry of *trans* isomer of DMASBT in the ground state.

decreases remarkably to 36 kcal mol^{-1}. The structure of DMASBT is quite similar to that of stilbene derivatives those are well known for their photoisomerization [13]. The ground state configuration changes on excitation. The peculiarity in the present case is that unlike stilbene, DMASBT retains its *trans* geometry and shows solvent polarity-dependent TICT in the excited state. Therefore, temperature-induced quenching study of the fluorescence of DMASBT in a solvent of low polarity (dioxane in the present study) so as to rule out the interference of any TICT seemed to be a very appropriate method of investigation.

4. Conclusion

DMASBT has been found to be highly sensitive to solvent polarity. TICT fluorescence characteristics also depend on the viscosity of the environment profusely. In high viscous solvent such as glycerol, the free-volume concept proposed by Loufty et al. has been found to provide the accurate description of the solvent's viscosity and temperature-dependent torsional relaxation of DMASBT. The torsional motion in the molecule responsible for inducing the non-radiative decay processes is hindered by the viscous drag of the solvent that results in an increase in fluorescence quantum yield in highly viscous solvents. The rotation dependent radiationless decay rate gets reduced thereby increasing the fluorescence quantum yield due to the decrease in the free-volume and increase of viscosity of the solvent. The free-volume of the solvent decreases with an increase in viscosity and decrease in temperature of the solvent, which in turn reduces the torsional relaxation in the molecule resulting into an increase in the fluorescence quantum yield. At temperature much above the glass transition temperature, this dependence is meaningless as it is known that the temperature-dependent viscosity of the solvent will then be related to the specific details of the molecular structure of the solvent rather than the free-volume. Results also show that the temperature-dependent studies provide more accurate description than the viscosity-dependent phenomena in the medium of varying viscosity at a constant temperature. Therefore, DMASBT can be used as a potential microscopic probe molecule that can provide valuable information about the hydrodynamic interaction in various solvents. The restricted rotation of $-N(CH_3)_2$ group causes greater extent of donation of charge towards acceptor thereby stabilizing the TICT state responsible for large Stokes shifted fluorescence in highly viscous medium at low temperature. The temperature-dependent *cis–trans* isomerism has also been observed in solvents of low polarity where there is no TICT. Once the molecule gets the suitable environment for TICT, it prefers to undergo that said process. The characteristic of DMASBT is therefore, found to be highly polarity and viscosity dependent. The remarkably high fluorescence quantum yield in polar viscous medium compared to the non-viscous medium can make the molecule a useful microviscosity sensor to study the biological systems where many cellular and organismal functions are dependent on the viscosity of their environments. There are a very few fluorescent probes available for real-time measurements of viscosity changes on a microscopic scale. In this regard DMASBT can be a useful candidate not only due to its high sensitivity towards viscosity change, but also because of its high sensitivity towards polarity and pH [9]. Therefore, the present study provides a molecular probe which can behave as a microsensor to study biological functions as well as biomimicking systems. Moderately high fluorescence quantum yields in polar aprotic and viscous solvents like DMF and DMSO can be explained on the basis of restricted molecular rotation.

Acknowledgements

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