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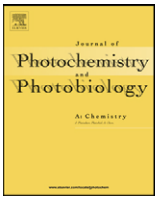

Dual fluorescence of Methylene Blue encapsulated in silica matrix

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ABSTRACT

Dynamics related to the optical effect of Methylene Blue (MB) dye molecules in solid host has been investigated. MB doped silica samples of varying concentrations are prepared by the sol–gel technique in acidic environment. Absorption and laser induced fluorescence spectra have been recorded for the samples. Interesting result of dual fluorescence at lower concentrations is explained. Attempt is done to study the dynamics of the dual fluorescence. These results may be useful for designing and developing solid-state optoelectronic devices.

1. Introduction

Each dye molecule has a characteristic absorption spectrum that depends upon its structure and excitation mechanism. One of such elegant molecule Methylene Blue (MB) is important to investigate because of its vast uses in various fields viz. physical, biological, chemical, etc. [1–3]. Fluorescence and absorption spectroscopy are important tools that help to investigate the dynamics of the optical properties of the molecules having sufficiently large excited state lifetime. MB exhibits excellent absorbance in the red region and a transition to excited singlet state, with the probability either to return back to ground state or transition to an excited triplet state. This excited triplet state further gives rise to self-quenching, which causes photobleaching effect in the dye. The dynamics of this effect gives rise to phenomena related to great importance in biological and other sciences [1–5]. An attempt is made to investigate the laser induced fluorescence of MB in various environments (solid and liquid). It has been found that photobleaching effect is less prominent in solid (glassy) phase as compared to liquid phase.

MB (a member of thiazine dye group) is a very efficient dye which is used as a stain in bacteriology, as an oxidation–reduction indicator, as an antidote to cyanide and as an antiseptic in veterinary. MB can be activated by light to an excited state which in turn activates oxygen to yield oxidizing radicals [6]. Such radicals can cause cross-linking of amino acid residues on proteins and hence achieve some degree of cross-linking. Investigation on the use of MB to achieve photochemical welding of tissues is also under process [7].

It has been already established that encapsulation of organic dyes in solid host minimizes the photobleaching effects of the dye molecules [8]. Incorporation of perlimide and pyromethene dyes into glasses prepared by the sol–gel method enables the design of new types of visible, stable solid-state lasers [9–11]. During the present work, MB is embedded in silica matrices using sol–gel process as it offers many advantages over the other conventional methods; mainly because of low temperature solution synthesis via chemical route. It avoids degradation of dye molecules that persists at high temperature. Solid-state dye materials produced by the sol–gel method have advantages over liquid dye materials being non-volatile, non-flammable and mechanically stable. Therefore can be used easily as sensors, solid state dye lasers (SSDL) and other useful materials [12]. Solid phase leads to increase in absorption band that directly results in increased fluorescence band width giving access to high tunability of the molecule.

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The present communication discusses the results of absorption and fluorescence spectra of MB doped silica samples at various concentrations. The fluorescence of the samples has been recorded using He–Ne laser having characteristic wavelength of 632 nm. Dual fluorescence of the samples at some optimum conditions is reported.

2. Experimental

2.1. Preparation of samples

Glass encapsulated with the variable concentration of MB has been prepared by employing the alkoxide based chemical route of sol–gel technique. Tetraethoxyorthosilane (TEOS) is used as precursor and hydrolysis is done in the appropriate pH controlled environment. It has been found that the samples are better formed in slightly acidic environment and therefore HCl is used as catalyst. The chemicals are taken in the following proportion:

$[\mathrm{TEOS} + \mathrm{EtOH} + \mathrm{HCl}] : [\mathrm{H_2O}] :: 10 : 03$

A weighed amount of dye is dissolved in a suitable solvent separately and mixed uniformly with precursor TEOS using constant stirring over a magnetic stirrer. The dye, MB, is miscible in alcohol and therefore it is dissolved in ethyl alcohol. The solution is kept under vigorous stirring at room temperature ~29 °C for about 3–4 h to yield clear and stable sol and then to get in gelation form. This is put in glass cuvettes for a week for drying and the glassy matrices are formed. The method further involves hydrolysis followed by condensation. The general reaction of the sol–gel method is shown below where –R is alkyl group and the first reaction shows the hydrolysis and the second and third reaction exhibits the condensation reaction occurring in the sol–gel method.

$$\equiv\mathrm{Si{-}OR} + \mathrm{H_2O}\equiv \rightleftarrows \equiv\mathrm{Si{-}OH} + \mathrm{ROH} \qquad (1)$$

$$\equiv\mathrm{Si{-}OH} + \mathrm{HO{-}Si}\equiv \rightleftarrows \equiv\mathrm{Si{-}O{-}Si}\equiv + \mathrm{H_2O} \qquad (2)$$

$$\equiv\mathrm{Si{-}OH} + \mathrm{RO{-}Si}\equiv \rightleftarrows \equiv\mathrm{Si{-}O{-}Si}\equiv + \mathrm{ROH} \qquad (3)$$

Slow ageing is preferred to avoid cracking in the glass so formed; hence ageing is done at normal temperature. During the time of ageing condensation of the gel continues, thereby raising its viscosity. A surface finished solid rectangular sample (~15 mm × 5 mm × 5 mm) of the silica doped with MB is obtained. The process is repeated to get samples of different MB concentrations in glassy matrix.

2.2. Spectroscopic measurements

The absorption spectra of the samples are recorded by UV–Vis–NIR spectrophotometer (varian made). Fluorescence spectra are recorded by exciting the samples with He–Ne laser of wavelength 632 nm and 10 mW power using transverse geometry arrangement (Fig. 1). The absorption spectra of MB samples show that emission wavelength of the laser lies well within absorption band of MB samples. The laser light is line focused on to the sample along the length through a cylindrical lens of 10 cm focal length. The diagnostic equipment used in this study is compact hand held plug and play Spectrometer (Ocean Optics Inc. USB 2000), connected to PC through optical fibre where spectra are recorded using Tarang software developed by H.S. Vora of RRCAT, Indore.

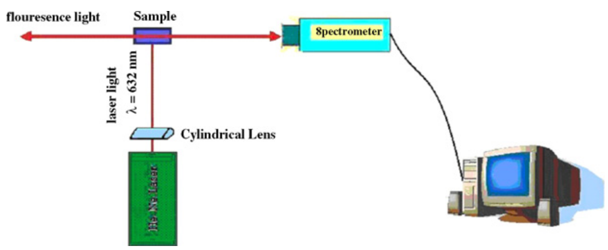


Fig. 1. Schematic of transverse geometry for the fluorescence set-up.

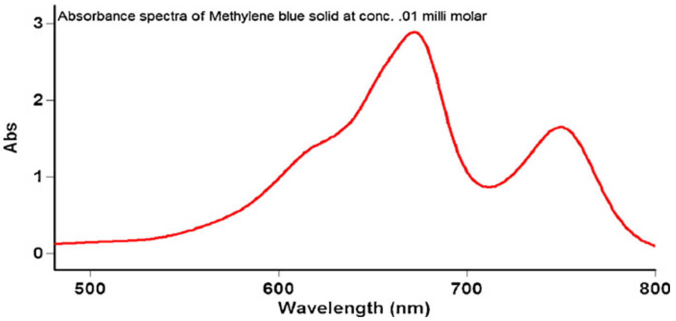


Fig. 2. Absorption spectra of the Methylene Blue in silica matrix at concentration of 0.01 mM.

3. Results and discussion

3.1. Absorption characteristics of MB in TEOS

Absorption spectra for samples of different MB concentrations varying from 0.01 mM to 1.0 mM have been recorded for a complete UV–Vis–NIR range. Fig. 2 represents the absorption spectra for 0.01 mM concentration. All other curves show similar behavior except change in peak absorption wavelength and change in intensity of the second peak (Fig. 2).

Absorption spectra are found to be a function of concentration of the dye present in the glassy material which leads to the less transparency in the highly concentrated samples. Absorbance of the samples increases with increasing MB concentration, as expected. The variation of absorption cross-section with concentration of dye is due to aggregation of dye molecules. Aggregation reduces its surface area and thereby decreasing absorption cross-section. The mutual interaction of dye molecules at higher concentration is responsible for the concentration-dependent absorption cross-section changes [13].

However, the aggregate formation of the dye molecules at higher concentrations is to be minimized by restricting their mobility by entrapping them in solid matrices. Because of the restricted mobility of the dye molecules in solid host the aggregate formation is less in comparison to the liquid phase. Aggregate formation is a phenomenon which is present in various phases and it cannot be completely eliminated [14]. Unfortunately, problem arises because of photo-degradation of the dye molecules and therefore photostable dyes are proposed to be used. Molecules are required to be put into solid-state hosts of high optical quality and low thermal conductivity to maintain better performance.

The first absorption peak lies within the range of 610–670 nm (visible red region) for various concentrations of MB. A linear red shift is observed with the decrease in concentration from 1×10^{-3} mM to 1×10^{-4} mM (Fig. 3(a)). However on dilution beyond 1×10^{-4} mM, a sudden jump in peak wavelength is observed, that is understood due to low threshold energy requirement at lower concentrations of the dye. At low concentration intermolecular distance increases, which further reduces self-quenching and hence lesser energy is required to excite a molecule, causing red shift to appear.

Fig. 3(b) shows the wavelength shift with respect to concentration of dye molecules in the glassy samples. The pattern of the shift observed is similar to Fig. 3(a). This implies that variation of wavelength shift is not linear w.r.t. concentration everywhere.

It is composed of two linear regions one at higher concentration and another at low concentration combined together by a phase change.

The second absorption peak (at wavelength ~750 nm) appears on dilution beyond 1×10^{-4} molar concentration. These results are equally supported by Silvia et al. where they observed the same pattern [15]. The existence of second peak at lower concentrations is due to the presence of monomer and protonated form of MB molecule in acidic environment. It is well known that even at lower concentrations MB is present in solution as a monomer or dimer and a typical value for the dissociation constant is 3970 dm^3/mol [16]. The monomer and dimer absorb light of different wavelength in different environment. Mills and Wang also observed that photobleaching of MB is due to presence of both monomeric and dimeric forms even at very low concentration [17].

To have a close look at the concentration dependence of the absorbance, a theoretical model suggested by Beer has been used in which molar extinction coefficient is defined and calculated [18]. The ideal absorbance of a dilute solute in a transparent solvent follows the Beer's law:

$$A = ecd \tag{4}$$

where e (l/mol cm) is molar extinction coefficient of the solute at the wavelength of measurement, c (moles/l) is the molarity of the solute, and d (cm) is the optical path length. The linear dependence of A on c may not hold if there are concentration-dependent effects that include aggregation or acid–base dissociation of the solute. This is certainly the case for given samples.

The absorption coefficient μ_a is directly proportional to the concentration and molar extinction coefficient and is expressed through the relation:

$$\mu_a = ec \ln 10 = 2.303ec \tag{5}$$

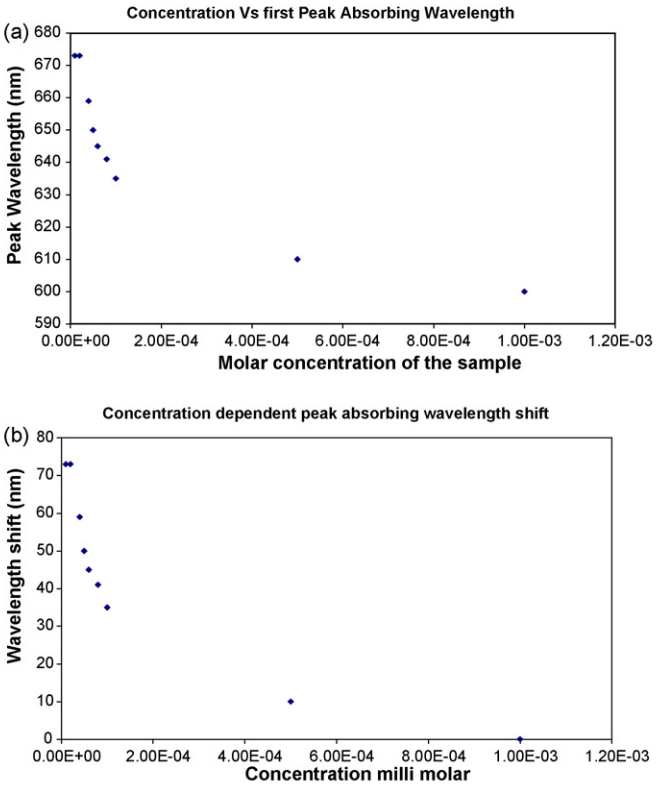
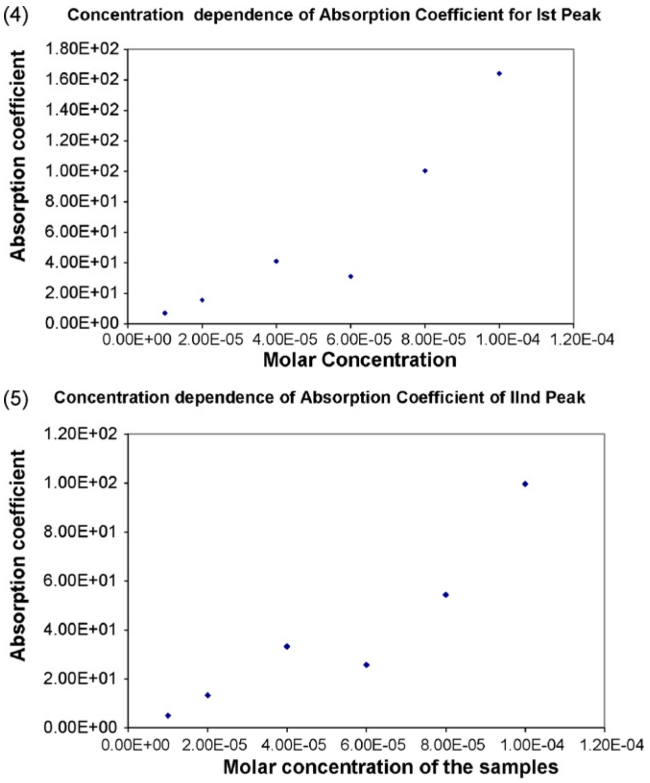


Fig. 3. (a) Concentration vs peak wavelength for the first absorption peak. (b) Concentration-dependent wavelength shift for first absorption peak.



Figs. 4 and 5. Concentration-dependent absorption coefficient for first and second peak, respectively.

Concentration-dependent molar absorption coefficient for both the peaks (Figs. 4 and 5) shows similar behavior. At a critical concentration of $\sim 6 \times 10^{-5}$ M, the molar absorption coefficient decreases as MB does not show significant aggregation due to self-organization of the dye. This self-organization of the dye is because of the various competing forces: dispersion forces due to interaction between the π systems of the dye and hydrophobic effects, since for the formation of stable aggregates, the sum of these forces needs to be larger than the electrostatic repulsion between the positive charges in the dye molecules [19].

3.2. *Fluorescence spectroscopy*

Fluorescence spectra of the samples under reference have been recorded by illuminating with He–Ne laser using transverse geometry arrangement. A decrease in the intensity of the peak fluorescent

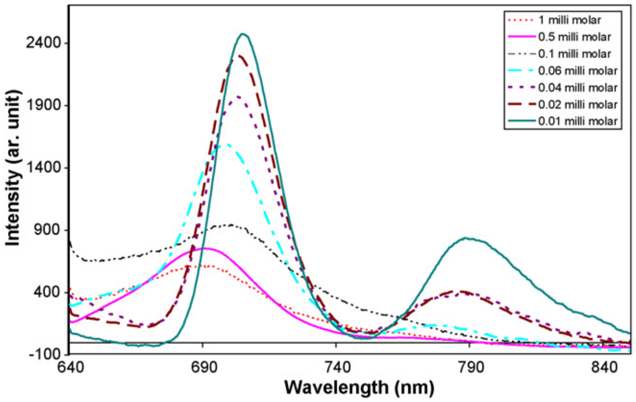


Fig. 6. Fluorescence spectra of the varying concentration of Methylene Blue doped silica glasses.

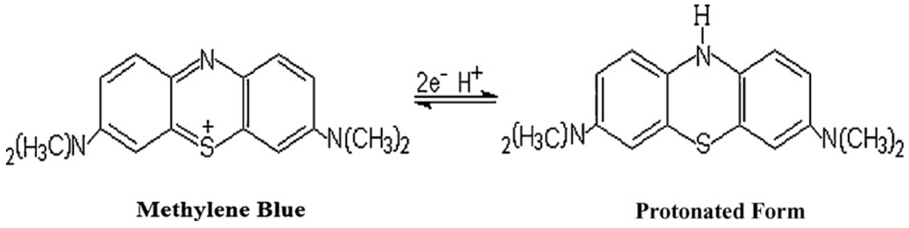


Fig. 7. Conversion of MB monomer to protonated form.

wavelength has been observed (Fig. 6) with increasing concentration of the MB molecules. This is understood due to the fact that dye molecules firstly excite to a singlet state and then there is either a radiative transition to the ground state or there may be a transition to the triplet state. The probability of transition from singlet to triplet state is more at higher concentration, which further leads to non-radiative transition to ground state and accompanied by the emission of higher frequencies photons. The phenomena may be termed as self-absorption in the dye molecules at high concentrations.

Furthermore, the cationic form of MB is the most desirable species for the lasing action [20]. In dye doped silica gels, the cage environment with acidic nature due to HCl is capable of supplying more protons (H^+) to MB molecules. These ions are attached to the nitrogen atom carrying a lone pair of electron in the structure resulting higher protonated form of the dye as shown in Fig. 7. The protonation in acidic solutions of Xanthene dyes is also reported by various researchers [21,22].

Study also reveals that fluorescence intensity of first peak decreases with increasing concentration (Fig. 6), which is due to inner filter effects that cause an apparent decrease in fluorescence quantum yield as a result of re-absorption of emitted radiation or absorption of incident radiation by spices other than those of the intended primary absorber. This is a direct indication that non-radiative processes are significant at higher concentrations and self-quenching becomes more prominent. This perturbation is attributed mainly due to the formation of closely spaced pairs, which have zero or very small fluorescence quantum yield and also depends on the local electric fields generated by the surrounding polar solvent molecules. This solvation effect is a result of intermolecular solute–solvent interaction forces.

Figs. 8(a) and 9(a) shows the variation in the wavelength of the fluorescing peaks I and II, respectively. It is in accordance with the absorption spectra where wavelength of the first peak increases with decreasing concentration. Fig. 8(b) represents wavelength shift of first fluorescence peak with concentration, which clearly indicates that variation in wavelength is no longer linear with concentration it shows a dip at 0.06×10^{-5} M concentration similar to absorption spectra. In Fig. 9(b) wavelength shift of the second peak is plotted against concentration. The second peak appears only at the lower concentration of 0.06×10^{-5} M hence no dip is observed as in the first case. These results lead to a phase transformation of the molecules at this particular concentration.

Another interesting result observed is the presence of dual fluorescence at ~700 nm and ~790 nm on excitation with He–Ne laser

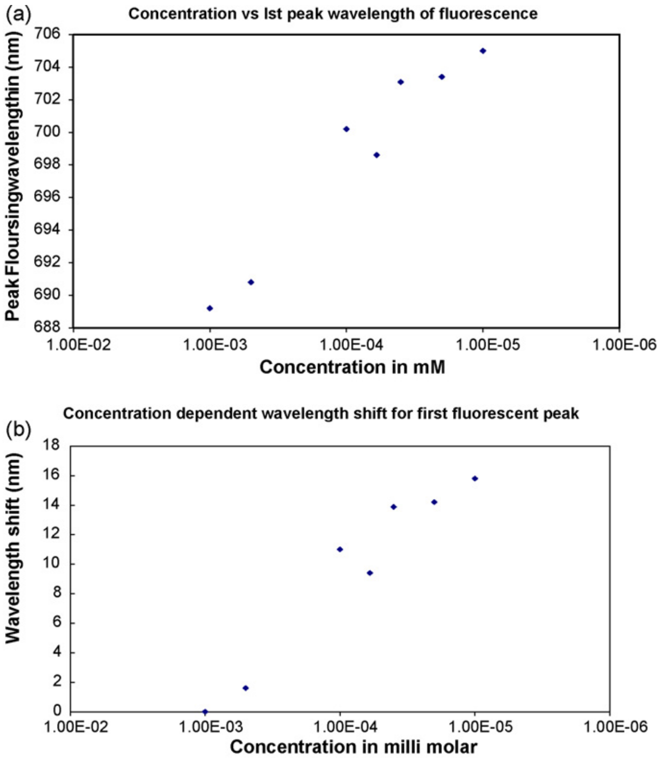


Fig. 8. (a) Concentration dependence of first peak wavelength for fluorescence. (b) Concentration-dependent wavelength shift for first fluorescence peak.

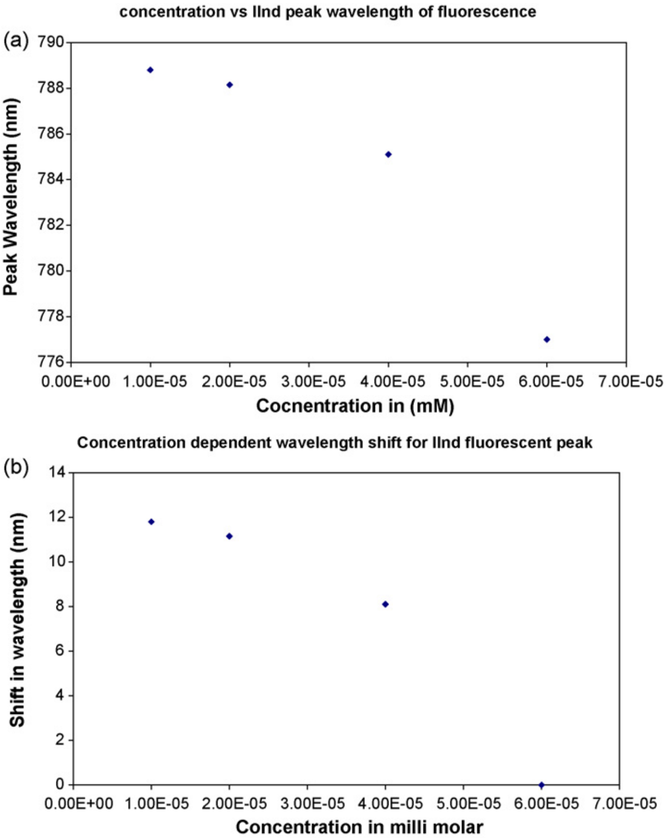


Fig. 9. (a) Concentration dependence of second peak wavelength for fluorescence. (b) Concentration dependence of wavelength shift of second fluorescence peak.

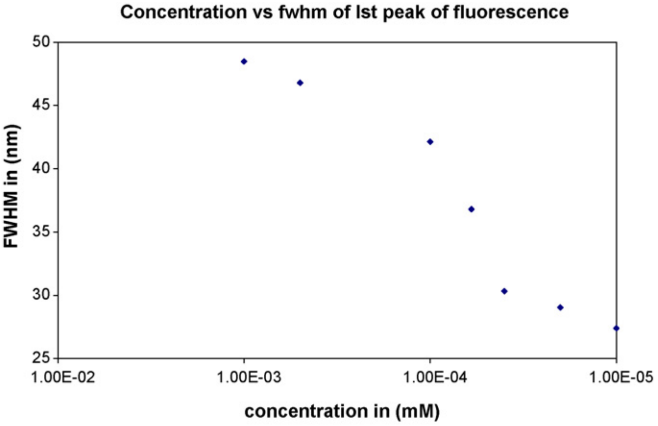


Fig. 10. FWHM for first peak of fluorescence.

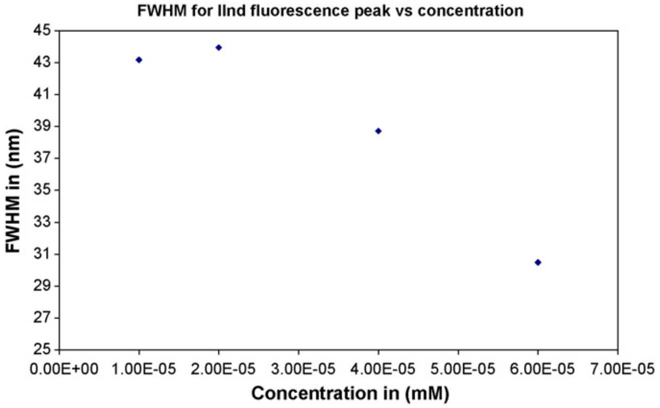


Fig. 11. FWHM for second peak of fluorescence.

at lower concentration of about 0.06 mM. Fluorescence at double wavelength is well supported by the absorption spectra. Molar absorptivity and peak wavelength also shows abnormal behavior at a particular concentration. The organic molecule dissolved in the acidic water and being a hydrophobic tends to coagulate and this aggregation is more at higher concentration of the dye. In spite of the fact, there is separation in the aggregates due to presence of silica caging so sample provides significant flourishing yield at lower concentration as the number of organic molecules in the aggregates decreases giving rise to increase in concentration of more protons. The study reveals that at lower concentration of the dye, monomeric form of the dye gets converted into the more protonated form (MBH^+). After a critical concentration of dye the second fluorescence peak becomes prominent with the more protonated form of MB. All the spectroscopic parameters show abrupt change at that optimum concentration where the dual nature of MB molecule comes into play. Further Figs. 10 and 11 presents the variation in full width at half maxima (FWHM) with varying concentration of MB molecules. Exhibiting inverse trend with the concentration, which is required for the any material to work as a lasing material. It is very well understood and also clear from Fig. 6 that peak intensity is also reciprocal to the FWHM. Thereby low concentration dye system work well for the SSDL.

4. Conclusion

Absorption intensity increases with increasing concentration is due to the aggregation of the molecules. Aggregation effects are being reduced by using silica cages that decreases more at lower concentrations. Fluorescence spectra as well as absorption spectra exhibit the red shift at lower concentration. Increase in the fluorescence intensity of the samples with lowering the concentration is due to presence of more monomeric and protonated form of MB molecules which are bound in the aggregates at higher concentrations. Another important result discovered from the studies is the presence of dual form of the MB molecule (monomeric and protonated) in solid host silica matrix and both forms are behaving independently.

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References

[1] L.Z. Zhang, G.Q. Tang, The binding properties of photosensitizer methylene blue to herring sperm DNA: a spectroscopic study, Journal of Photochemistry and Photobiology B: Biology 74 (2–3) (2004) 119.
[2] A. Ghanadzadeh, M.S. Zakerhamidi, Absorption anisotropy and molecular association of some ionic dyes in liquid crystalline solution, Journal of Molecular Liquids 109 (2004) 149–154.
[3] N. Matsuda, J.H. Santos, A. Takatsu, K. Kato, In situ observation of absorption spectra and adsorbed species of methylene blue on indium-tin-oxide electrode by slab optical waveguide spectroscopy, Thin Solid Films 445 (2) (2003) 313–316.
[4] G.J. Smith, A. Harriman, A.D. Woolhouse, T.G.H. Barnes, Photoinduced electron transfer in dry gelation containing bound methylene blue, Photochemistry and Photobiology 67 (1998) 101.
[5] S. Chakrabarti, B.K. Dutta, On the adsorption and diffusion of Methylene Blue in glass fibers, Journal of Colloid and Interface Science 286 (2) (2005) 807–811.
[6] J.M. Fernandez, M.D. Bilgin, L.I. Grossweiner, Singlet oxygen generation by photodynamic agents, Journal of Photochemistry and Photobiology B: Biology 37 (1–2) (1997) 131–140.
[7] Z.R. Liu, A.M. Wilkie, M.J. Clemens, C.W.J. Smith, Detection of double-stranded RNA–protein interactions by methylene blue-mediated photo-crosslinking, RNA 2 (1996) 611–621.
[8] A.M. Weiss, E. Yariv, R. Reisfeld, Photostability of luminescent dyes in solid-state dye lasers, Optical Materials 24 (2003) 31–34.
[9] S. Singh, V.R. Kanetkar, G. Sridhar, V. Muthuswamy, K. Raja, Solid state polymeric dye lasers, Journal of Luminescence 101 (2003) 285–291.
[10] R.J. Nedumpara, B. Paul, A. Santhi, P. Radhakrishnan, V.P.N. Nampoori, Photoacoustic investigations on the photostability of Coumarin 540-doped PMMA, Spectrochimica Acta Part A 60 (2004) 435–439.
[11] Y. Yang, G. Qian, D. Su, Z. Wang, M. Wang, Luminescence and laser performances of coumarin dyes doped in ORMOSILs, Materials Science and Engineering B 119 (2005) 192–195.
[12] R. Reisfeld, Prospects of sol–gel technology towards luminescent materials, Optical Materials 16 (2001) 1–7.
[13] M. Wittmann, A. Penzkofer, Concentration-dependent absorption and emission behaviour of Sulforhodamine B in ethylene glycol, Chemical Physics 172 (1993) 339–348.
[14] M.D. Rahn, T.A. King, Comparison of laser performance of dye molecules in sol–gel, polycom, ormosil and poly(methyl methacrylate) host media, Applied Optics 24 (36) (1995) 8260–8271.
[15] H. Silvia, A. Nicolai, J.C. Rubim, Surface-enhanced resonance Raman (SERR) spectra of Methylene Blue adsorbed on a silver electrode, Langmuir 19 (2003) 4291–4294.
[16] W. Spencer, J.R. Sutter, Kinetic study of the monomer–dimer equilibrium of Methylene Blue in aqueous solution, The Journal of Physical Chemistry 83 (1979) 1573–1576.
[17] A. Mills, J. Wang, Photobleaching of methylene blue sensitized by TiO_2: an ambiguous system? Journal of Photochemistry and Photobiology A 127 (1999) 123–134.
[18] D. Russell, C. Olson, S. Shadle, M. Schimpf, Spectroscopic, chromatographic and visual investigation of organic dyes, The Chemical Educator 2 (1997) 1–19.
[19] S. Jockusch, N.J. Turro, Aggregation of Methylene Blue adsorbed on Starbust dendrimers, Macromolecules 28 (1995) 7416–7418.
[20] T.L. Chang, H.C. Cheung, Solvent effects on the photoisomerization rates of the zwitterionic and the cationic forms of rhodamine B in protic solvents, Journal of Physical Chemistry 96 (1992) 4874–4878.
[21] A.V. Deshpande, U. Kumar, Effect of method of preparation on photophysical properties of Rh-B impregnated sol–gel hosts, Journal of Non-Crystalline Solids 306 (2002) 149–159.
[22] H. Mohan, R.M. Iyer, Photochemical behaviour of rhodamine 6G in Nafion membrane, Journal of the Chemical Society-Faraday Transactions 88 (1992) 41–45.