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Photobleaching Influence in Different Phases for Coumarin 307 and Acriflavine Laser Dyes

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Abstract: Under high-excitation irradiance conditions to induce fluorescence, the dependence of photobleaching of Coumarin 307 (C307) and acriflavine (ACF) laser dyes in liquid and solid phases have been studied. A cw LD laser source of 1 mW and 407 nm wavelength was used as an exciting source. For one hour exposure time, it was found that the solid dye samples suffer photobleaching more than the liquid dye samples. This is because in liquid solutions the dye molecules can circulate during the irradiation, while the photobleaching is a serious problem when the dye is incorporated into solid matrix and cannot circulate.

Key words: Photobleaching, organic laser dyes, dye-doped PMMA.

1. Introduction

Laser dyes are organic compounds relax radiatively after optical excitation and emit in the visible or IR range. Fluorescent molecules, also called fluorophores, respond distinctly to light compared to other molecules. A photon of the exciting light is absorbed by an electron of the fluorescent particle, which is raised to an energy level of an excited state then losses energy nonradiatively till it reaches the lowest vibrational state of the excited state [1, 2]. During this short excitation period, some of energy is dissipated by molecular collision or transfer to a proximal molecule, and then the remaining energy is emitted as a photon after the electron is relaxed again to the ground state. The emitted photon always has a longer wavelength and less energy than the excitation photon. The absorption and emission of photon from a fluorophore is a cyclical process, and until the fluorophore is irreversibly damaged (photobleached) it

can be repeatedly excited. The various energy levels involved in the absorption and emission of light by a fluorophore are represented by Jablonski [3]; diagram as shown in Fig. 1.

The issue of photostability has been addressed in related with fields such as laser dye investigations, fluorescence microscopy, and DNA sequencing. Each fluorophore has different photobleaching-characteristic, so its stability can be characterized by the average number of absorption-emission cycles that the molecules of this fluorophore undergo before they are irreversible photobleached. The time measurement in single molecule experiments, however, is limited by irreversible photobleaching process [4, 5], where the photobleaching is caused by the irreversible destruction of fluorophore due to either the prolonged exposure to the excitation source or exposure to high-intensity excitation light, hence dye molecules are not able to be excited again after undergoing repetitive excitation and emission cycles [6]. While for weak illumination the probability of photobleaching is independent of the excitation

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intensity. This means that, irrespective of the excitation, so for each laser dye there is a characteristic number of emitted photons before the dye undergoes photobleaching [7]. All materials are damaged by light exposure if the light intensity is high enough or the exposure time is long enough. It is the photobleaching process that causes the vibrant colors of clothing to fade or the catastrophic laser damage of optical components in a laser cavity [8].

The polymer opens the field when used as a solid matrix that includes laser dye. It is an attractive alternative to the conventional liquid dye solution due to its obvious advantages like rigidity and compactness, lack of solvent evaporation and toxicity, safety operation and low cost etc. Over the last decade, the interest in the development of solid-state dye lasers has been increased. Optical properties and commercial viability of polymer were making it to be as a host material for laser dyes. In addition, they can quite effectively control the chemical composition at the formation stage, also they show much better compatibility with organic laser dyes, and the fabrication procedure are simple and inexpensive [9]. So in order to improve the photostability of a given emitter, it is important to first investigate and identify the pathways leading to photobleaching. Where, fluorescence of a molecule depends on the structure

and environment [10]. One can hope to modify the dye or change the environment in appropriate manner to control this process.

2. Materials and Samples Preparation

In this study, we have used a laser dye acriflavine (3, 6-diamino-10-methylacridinium chloride hydrochloride euflavine ($C_{14}H_{14}C_1N_3$)) of molecular weight 259.73 g/mol, supplied by Sigma Chemicals (USA), and laser dye coumarin 307 (7-ethylamino-6-methyl-4-trifluoromethylcoumarin ($C_{13}H_{12}NO_2F_3$)) of molecular weight 257.21 g/mol, supplied by Lambda Physik. The dyes were used without further purification. The chemical structures of these dyes are shown in Fig. 2. The polymer polymethyl methacrylate (PMMA) was supplied by Aldrich, and spectroscopic grade ethanol and chloroform solvent have been used.

2.1 Liquid Samples Preparation

Coumarin 307 and acriflavine dyes solutions of primary concentration of 10^{-3} M were prepared by dissolving the appropriate amounts of these dyes in spectroscopic grade ethanol. The concentration of each dye was then diluted to get concentrations in the range of 10^{-3} - 5×10^{-6} M.

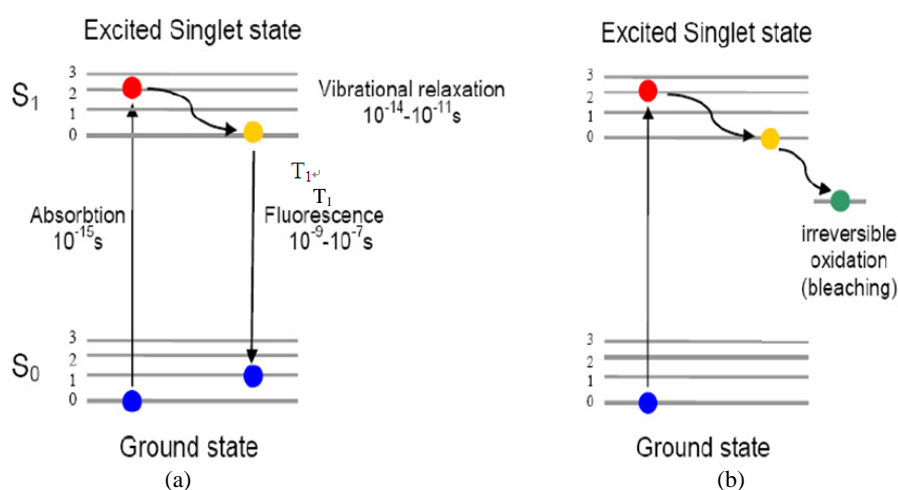


Fig. 1 Jablonski diagram: (a) the absorption-emission cycle during fluorescence process and (b) photobleaching is assumed to be linked to a transition to the excited triplet state T_1 .

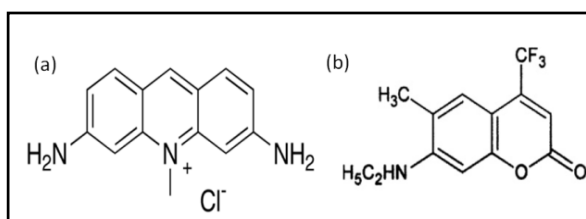


Fig. 2 Chemical structure of (a) acriflavine and (b) coumarin 307 dyes.

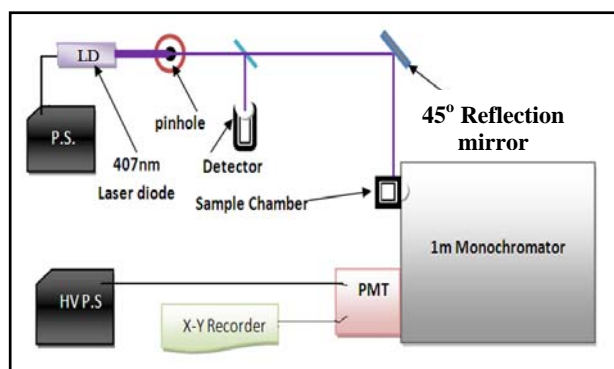


Fig. 3 Schematic diagram for the fluorescence measurement setup.

2.2 Solid Samples Preparation

The PMMA cubic crystals have been washed in distilled water 2-3 times and then dried into an oven at 60 °C for two days. A 7 g of PMMA was dissolved in 100 ml of chloroform (spectroscopic grade). Three different concentrations of C307 and ACF dyes (that represent the most efficient concentrations for fluorescence emission in liquid measurements) have been doped in PMMA in 33:67 ratio, and they were used to produce disk shaped samples for each dye. The mixture of dye and PMMA solutions were filtered into cylindrical molds and kept to dry at room temperature and under atmospheric pressure until they became solid samples. The resulting disc sample molds were of about 1.5 cm diameter and 0.3 cm thickness.

3. Experiments

The fluorescence measurement setup was performed using a continuous wave (cw) 50 mW diode laser (LD) of 407 nm wavelength, pinhole (to reduce the laser power to 1 mW), beam splitter, power

meter, a darkened 90° sample chamber, a 1 m monochromator, mirror, cooled housing photomultiplier tube (508 Hamamatsu) and x-y recorder (Siemens). The schematic diagram for the setup is shown in Fig. 3.

4. Results and Discussion

Photostability study of C307 and ACF dyes samples in each of liquid and solid state phases have been investigated depending on photobleaching effect. Starting with dye solution samples, the absorption spectra for C307 and ACF dyes of the prepared concentrations were recorded using UV/VIS SP8001 (Metertech) spectrophotometer. These spectra are shown in Fig. 4. The fluorescence emission of the samples was measured using the fluorescence measurement setup, and using quartz sample cell of 1 cm path length. The fluorescence spectra were recorded by exciting the samples with the LD laser source after reduced its power to 1 mW by the pinhole. The fluorescence spectra of C307 and ACF dyes of different concentrations are shown in Fig. 5.

Fig. 5 illustrates that the concentrations 5×10^{-5} M and 1×10^{-4} M to be the most efficient concentrations for fluorescence emission of liquid samples for C307 and ACF dyes. Hence, at very low concentration, fluorescence intensity increases with increasing concentration. At higher concentrations fluorescence intensity reaches a limiting value and then decreases with further increase in concentration. This can be related to the phenomenon of re-absorption and re-emission, which ultimately reduces fluorescence emission. With increasing dye concentration the formation of dimers and higher aggregates decreases the fluorescence emission.

The photobleaching measurements have been done for the three efficient concentrations (5×10^{-4} , 1×10^{-4} and 5×10^{-5} M) for C307 and ACF dyes, where the monochromator was fixed on the wavelength of peak fluorescence intensity. Fig. 6 shows that photobleaching process did not occur after 1 h of

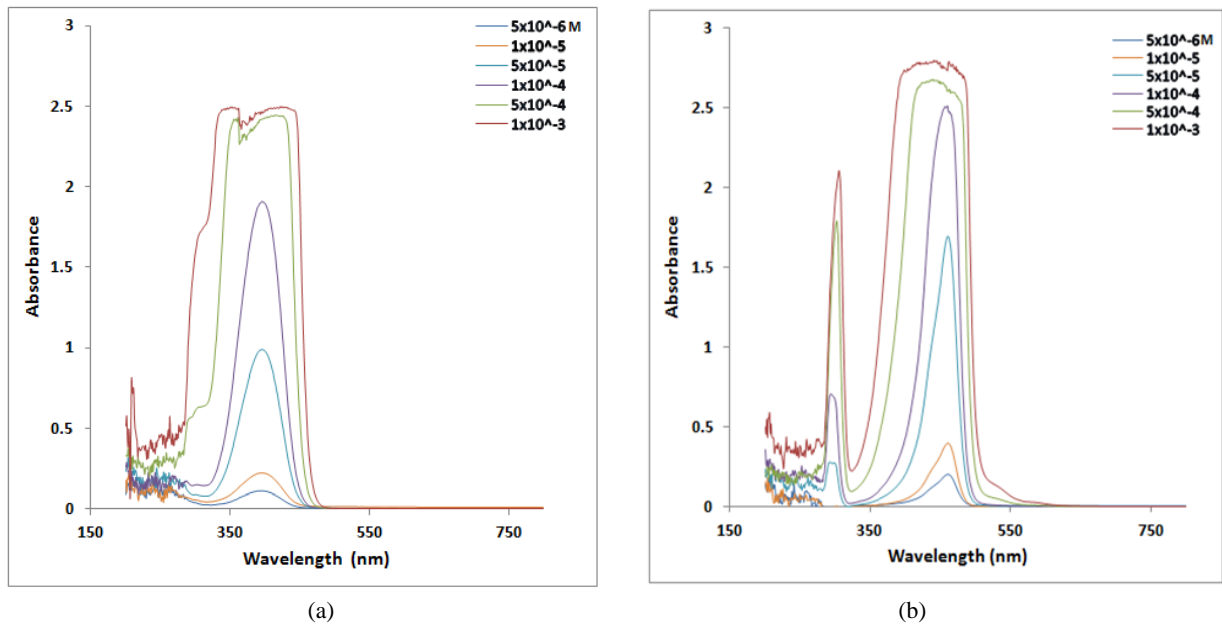


Fig. 4 Absorption spectra of (a) C307 and (b) ACF of different concentrations.

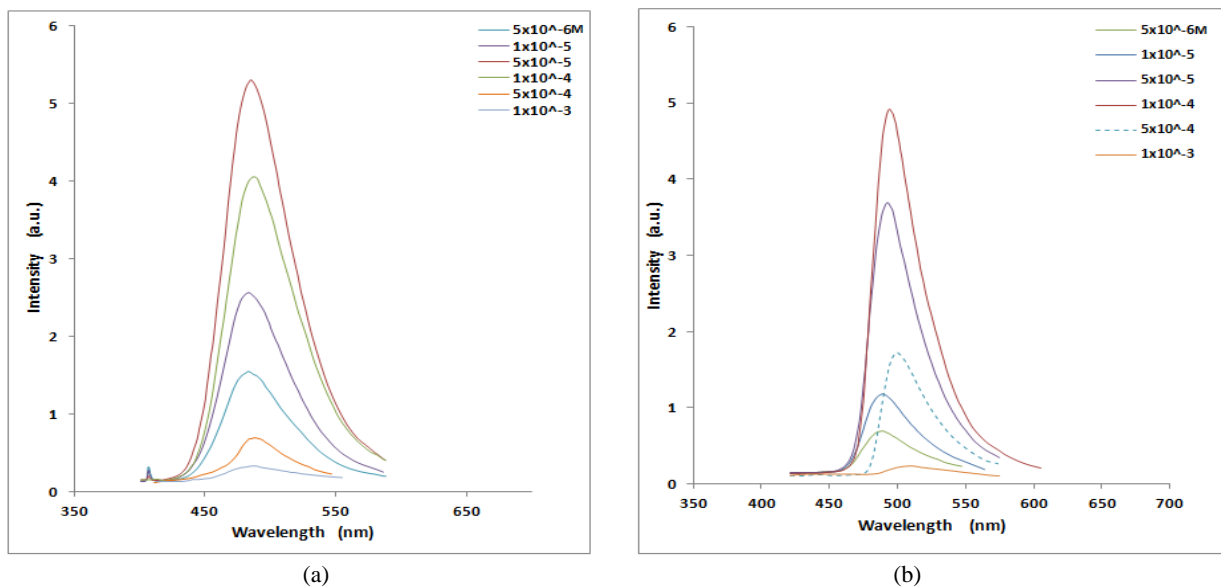


Fig. 5 Fluorescence spectra of (a) C307 and (b) ACF of different concentrations.

exposure time. This is because for liquid samples the dye molecules can move and circulate freely during the exposure period, hence avoiding continuous irradiation and interaction with laser beam.

For dye doped PMMA disk samples, the fluorescence spectra were recorded for C307 and ACF dyes of different concentrations. This is shown in Fig. 7. The x-y recorder sensitivity and the chart speed were adjusted to record the best spectra.

It has been found that the fluorescence intensity of solid samples were about ten times higher than that of liquid samples. This is because of the increase of the concentration during the solidification process. Fig. 7 illustrates that the concentration 5×10^{-4} M to be the most efficient concentration for both C307 and ACF dyes doped in PMMA solid samples .

The photobleaching measurements had been done for the three efficient concentrations (5×10^{-4} , 1×10^{-4}

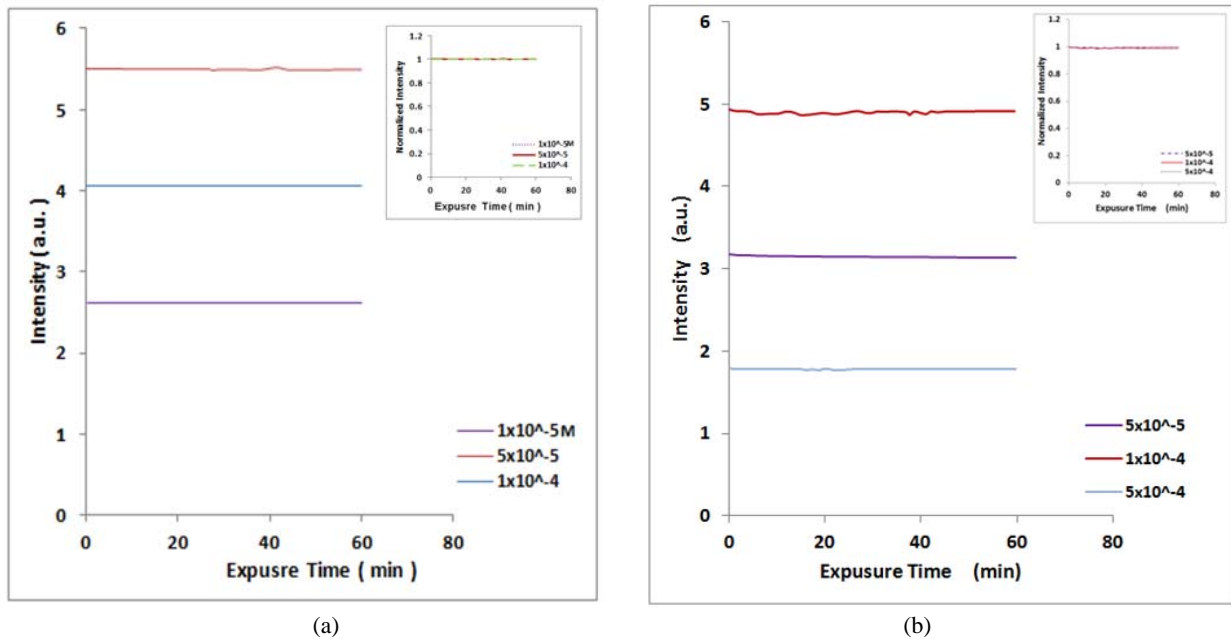


Fig. 6 Peak fluorescence intensity vs. time of exposure for (a) C307 and (b) ACF dyes liquid samples at different concentrations.

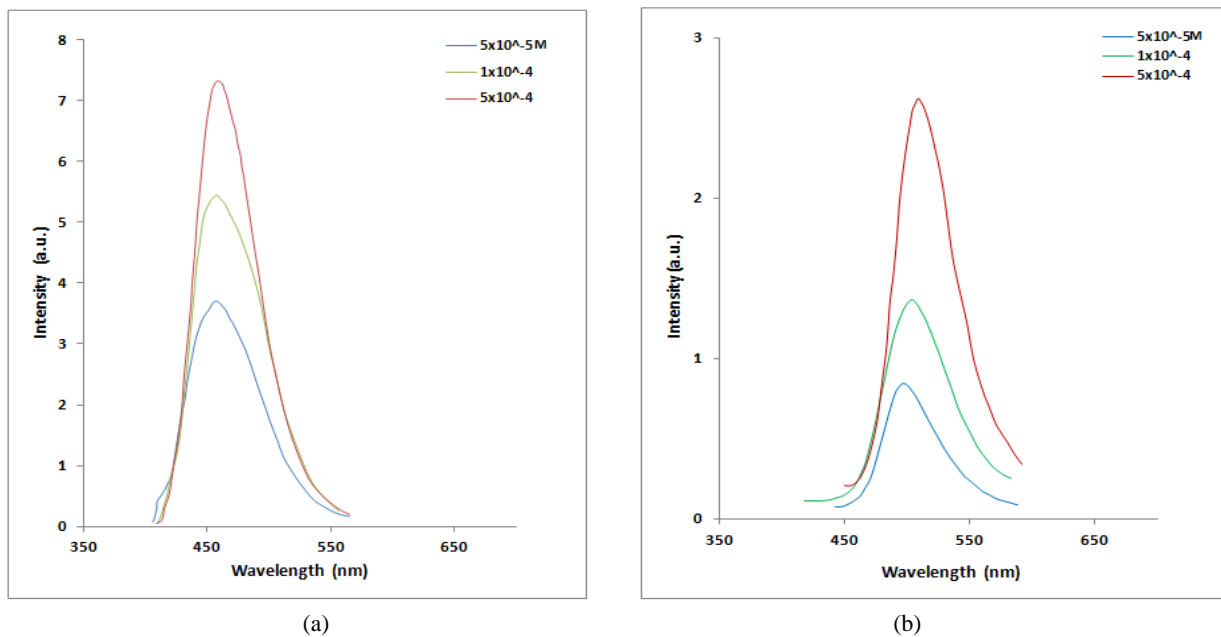


Fig. 7 Fluorescence spectrum for (a) C307, and (b) ACF doped in PMMA solid disks of different concentrations.

and 5×10^{-5} M) for C307 and ACF dyes doped in PMMA. Fig. 8 gives us a strong and clear indication that the photobleaching process had occurred. Hence the solid samples in this study suffered photobleaching. This is because dye molecules were confined and fixed in the polymer host matrix. Hence they were exposed continuously to the laser radiation.

From the obtained results we observed the following differences between liquid and solid samples for C307 and ACF dyes: Firstly, the fluorescence intensity for solid samples were about ten times higher than those of liquid samples; and Secondly, the optimum concentration were different in solid phase than for liquid phase; Finally, a blue shift

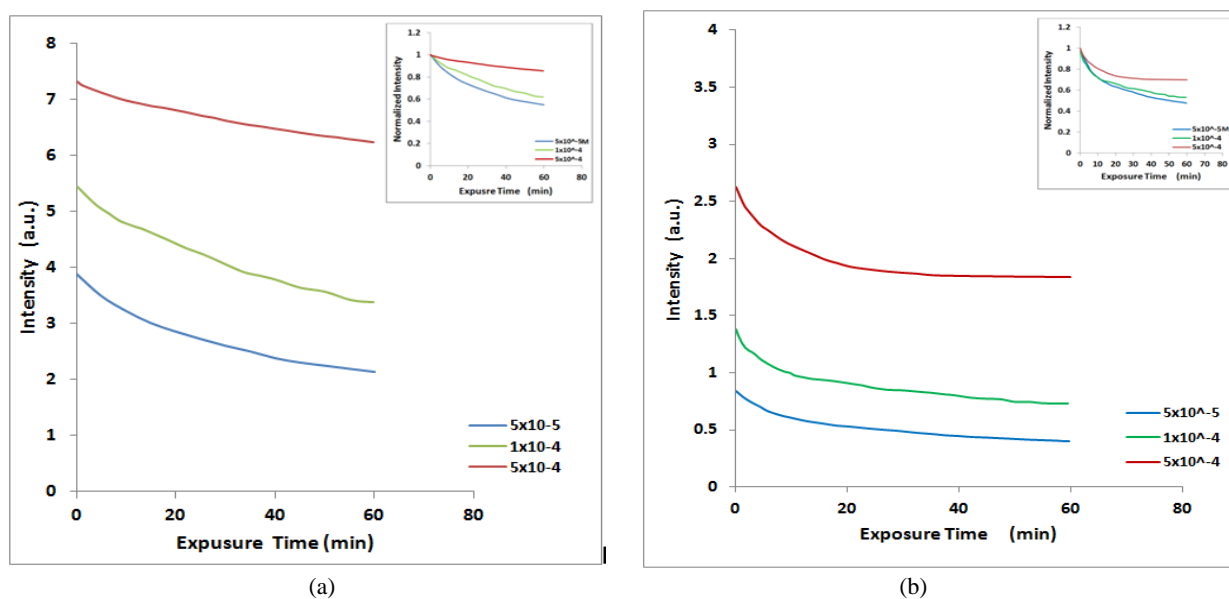


Fig. 8 Peak fluorescence intensity vs. time of exposure for (a) C307 and (b) ACF dyes doped in PMMA at different concentrations.

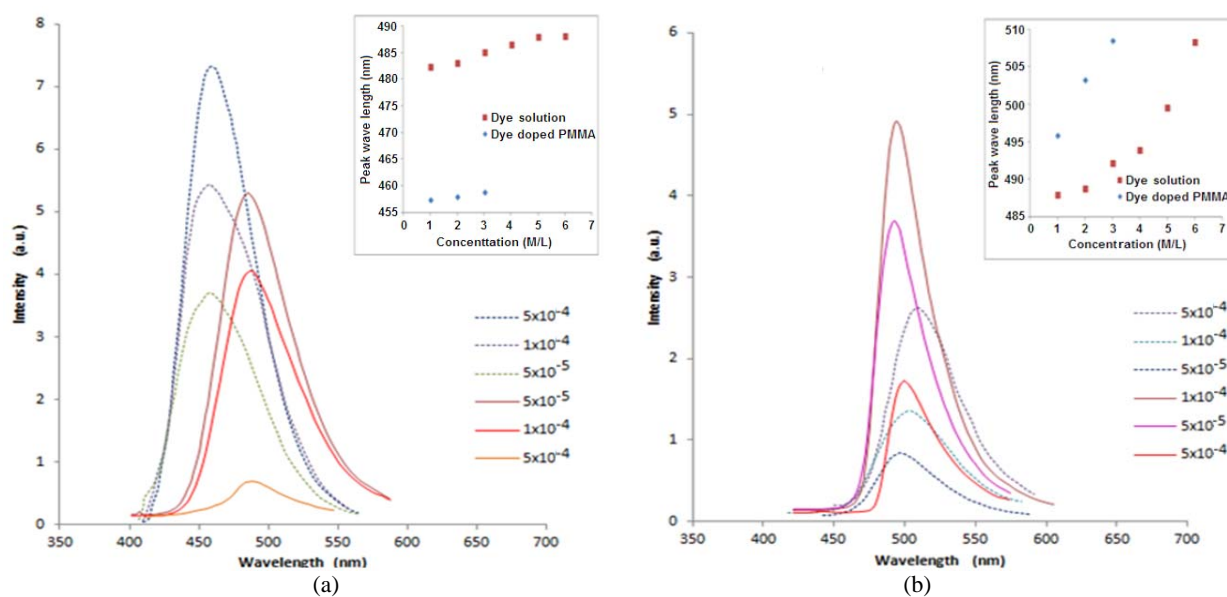


Fig. 9 Fluorescence spectra between liquid (lines) and solid (dots) samples for (a) C307 and (b) ACF dyes at different concentrations. Inset: peak fluorescence intensity shift with concentration.

(about 28 nm) has occurred for peak fluorescence intensity of C307 dye doped in PMMA, while a red shift (about 8 nm) has occurred for that of ACF dye doped in PMMA compared with that of liquid solutions. This is shown in Fig. 9.

5. Conclusions

We have conducted photobleaching measurements

of C307 and ACF dyes in liquid (ethanol) and solid (PMMA) phases. Photobleaching in liquid samples was negligible because the dye molecules can circulate and move through the solvent, where no noticeable change in the fluorescence intensity was observed. The reverse is true for solid samples where a noticeable photobleaching was observed, and the peak fluorescence intensity of the solid samples

dropped down with continuous pumping. These are attributed to the caging effects of the solid host material that confine or freeze the dye molecules. Hence photobleaching occurs because each fluorophore can undergo a limited number of excitation/emission cycles. After progressing through this number of cycles, the fluorophore will no longer release visible photons of light. And from solid samples which indicating that the bleaching effect is a slow process, and this property is an advantage that can be invested in some applications

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