Photostability of Uranine Via Crossed-Beam Thermal Lens Technique

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Abstract. Uranine is a diagnostic aid in ophthalmology and used as immuno-histological stain. Photostability study on such important compound using crossed-beam thermal lens (TL) technique was carried out. The study is based on the photodegradation (PD) behavior and rate regarding some parameters such as the incident laser power, wavelength, modulation frequency and sample concentration. The effects of such parameters on the TL signal and PD rate are discussed in details. The rate of PD is found to be proportional to the power of the pumping laser and concentration of the sample within the investigated range. The modulation frequency is found not to influence the PD rate. The photochemical quantum yield has been measured using potassium ferrioxalate actinometry and it was found to be very low.

INTRODUCTION

Importance of knowledge about drug photostability is a loss of potency of the drug product, which leads to a therapeutically inactive drug, not yet, but also, can lead to adverse effects due to the formation of minor degradation products during storage and administration [1]. In addition, the drug can cause light-induced side effects after administration to the patient by interaction with endogenous substances.

Fluorescein sodium, resorcinol phthalein sodium or uranine, is a highly fluorescent chemical compound. It absorbs visible light in the blue range with peak absorption and excitation band occurring at wavelengths between 465-490nm. The fluorescence emission occurs in the yellow-green range from 520 – 530 nm. These fluorescent properties have made fluorescein useful in a variety of medical applications such as ophthalmologic diagnostic (corneal trauma indicator, ophthalmic angiography, contact lens fitting) in the form of strips and intravenous injection to determine circulation time [2,3].
The Photothermal lens (PTL) effect was discovered by Gordon et al., [4] when they observed transient power and beam divergence changes in the output of a He-Ne laser. Because of its high sensitivity and versatility this technique has been widely used in spectroscopy, micro volume, trace analysis and photodegradation, as well as, thermo-optical characterization of solid, liquid and gas samples [5.7].
The PTL results from optical absorption and heating of the sample in region localized to the extent of the excitation source. This lens-like element arises from the temperature dependence of the sample refractive index. Moreover, because of the fact that most materials expand upon heating and the refractive index is proportional to the density, this lens usually has a negative focal length. This negative lens causes beam divergence and the signal is detected as a time-dependent decrease in power at the center of the beam [8].

The present study aimed to investigate of the photostability based on photodegradation (photobleaching) behavior of sodium Fluorescein in its aqueous solution using a crossed-beam TL set up.

CP888, *Modern Trends in Physics Research,*
Second International Conference on Modern Trends in Physics Research—MTPR-06,
edited by L. El Nadi

MATERIALS AND METHOD

10^{-3} mol/lt stock solutions of sodium Fluorescein (Uranine) puriss (Fluka) were prepared in double distilled water. Different concentrations were prepared to study the photodegradation. The photochemical quantum yield was determined by using potassium ferrioxalate, 1, 10-phenanthroline and sodium acetate buffer solution, puriss (Fluka). Photochemical quantum yield was determined by the conventional ferrioxalate actinometry as described by Hatchard, Parker, and Murov [9,10]. UV-Visible absorption measurements were carried out using spectrophotometer (Perkin Elmer Lambda 40). Laser beam of 488 nm wavelength from an argon-ion laser (Coherent Innova 400) was used to irradiate the sample within a (3 x 1 x 1 cm) quartz cuvette.

The experimental set up of the crossed-beam thermal lens employed in the photostability study is shown in figure (1). The 488 and 514 nm excitation wavelengths from an argon-ion laser (Coherent Innova 400) were used as pump sources to generate the TL in the medium. Radiation of wavelength 632.8 nm from a He-Ne laser source (Melles Griot, 10.0 mW) was used as the probe beam. The pump beam is intensity modulated at different frequencies using a mechanical chopper (SR-540). The two laser beams are focused into the sample cell and strike it perpendicularly, an interference filter was placed in the path of probe beam which allows only the 632.8 nm wavelength to reach the photo-diode detector and the signal was then processed using a dual-phase Lock-in amplifier (Stanford Research Systems SR-530).

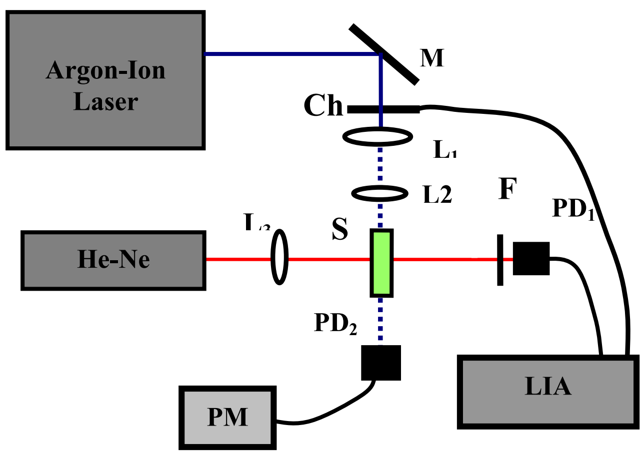


FIGURE 1: Schematic diagram of Crossed-beam TL spectrometer. Where, L2, L3 are convex lenses & L1 is a cylindrical lens, SC is the sample cell, F is the filter, PD1, 2 is the photodiode, PM is the power meter, Ch is a chopper, M is a mirror, and LIA is a lock-in amplifier.

RESULTS AND DISCUSSION

It is interesting to discuss the reasons for choosing an aqueous solution of Uranine substance although its small change in the temperature dependent refractive index (*dn/dT*) and high thermal conductivity (K). A consequence is a relatively low TL signal with respect to other solvents. The major reason is that to simulate the same conditions of medical use of such substance where water is the most encountered solvent in medical diagnostics. Another reason, water belongs to the non-absorbing liquids. These liquids are well transparent in visible region and their thermal properties are well known. Therefore, the absorbed light energy is then due to the dissolved substance only.

Figure (2) shows the absorption spectra of 133×10^{-6} mol/lt of sodium fluorescein irradiated by 488 nm of argon laser. The figure demonstrates significant decreases in the optical density with increasing the exposure time without any changes in the maximum absorption band (λmax.= 481 nm). The decrease in the optical density at the maximum wavelength may be due to the photodegradation of the uranine dye.

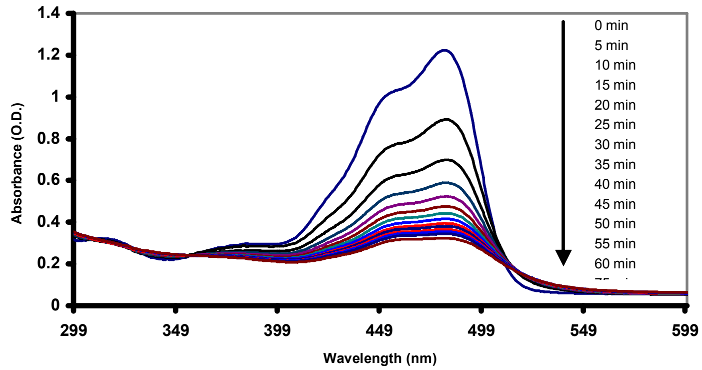


FIGURE 2: The absorption spectra of irradiated sodium fluorescein by argon laser of 488 nm at different exposure time intervals and sample concentration of 133x10^{-6} mol/lt.

The photochemical quantum yield (φ_c) was measured using potassium ferrioxalate actinometry method [9, 10] in case of the sample irradiated by argon laser of 488 nm. Some constants used as: the photochemical quantum yield (φ) of actinometer is 1.08 (at 488 nm, .0816 mol/lt, 23^{o}C) and the excitation coefficient of ferrous 1,10-phenanthroline complex at 510 nm is (~1.11 x 10^4 l/mol.cm). The result photochemical quantum yield was 2.2 x 10^{-5}. The photostability based on the photodegradation (PD) behavior and the rate regarding on some parameters will be discussed according to the following:

Effect of the concentration

Figure (3) shows the dependence of photodegradation (PD) rate with the different concentrations. On contrary, the earlier reported Photothermal studies concerning with PD measurement on laser dye doped polymer such as poly (methyl methacrylate PMMA)[7] show inverse proportionality of PD rate with concentration. This discrepancy can be ascribed mainly to the host of the dye whether liquid or solid. This may be due to that in case of the dye doped polymer i.e. solid matrix, the number density of absorbing species (undegraded) of low concentration levels is less than that of higher levels. Moreover, the absorbing species in laser-exposed area will not be replaced after degradation due to the static nature of such species in a solid matrix. Consequently, the low concentration levels are logically faster in PD than the high levels. On the other hand, in case of solutions, the species in the exposed area are dynamic in laminar flow due to laser-induced heating i.e. undegraded molecules replace those degraded. So that at higher concentrations, the released heat will be greater than that in case of lower concentrations which can be observed evidently in the irregular decay of the lower concentration (16×10^{-6} mol/lt), figure 4. Therefore, the convection current will be increased leading to increasing the vertical laminar flow of solution within the sample cell, this in turn increases the PD rate of molecules. In this study the levels of concentrations are in the micromoles scale.

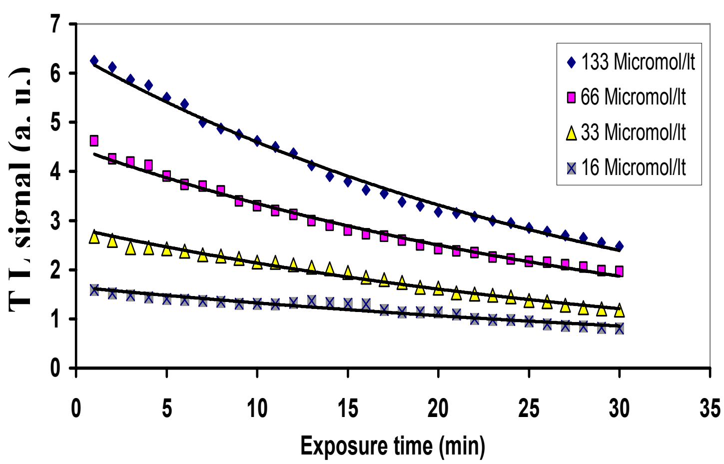


FIGURE 3: PD behavior of different concentrations represented in TL signal.

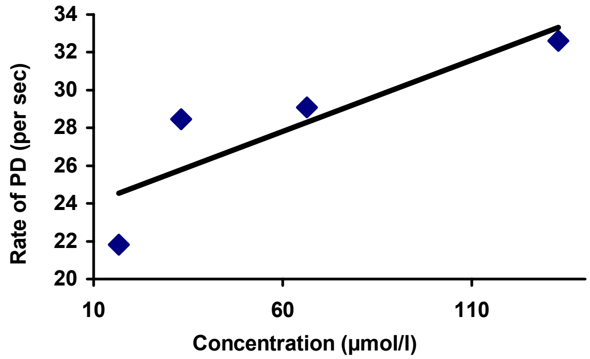
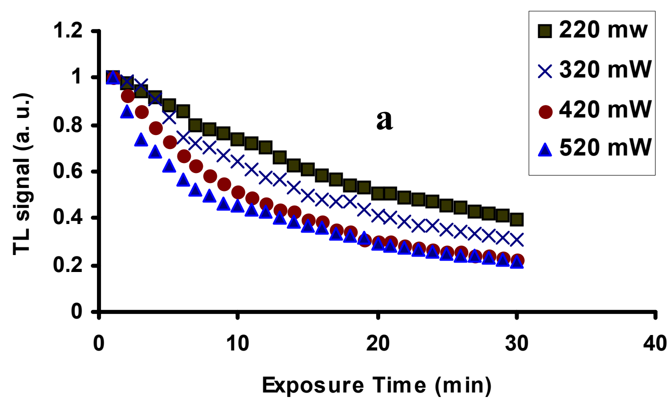


FIGURE 4. Variation of the rate of PD of Na-Fluorescein with concentration.

So, there are slight differences between the rates of PD over the selected levels of concentration. However, they can be clearly observed as shown in figure (4), where, the PD rate at a concentration 133×10^{-6} mol/lt is found to be 1.12 times greater than that at a concentration of 66×10^{-6} mol/lt, 1.15 times greater than that at a concentration of 33×10^{-6} mol/lt and 1.5 times greater than that at a concentration of 16×10^{-6} mol/lt.

Effect of the pumping laser power

The variation of PD rate with the power of the pumping laser is investigated at four levels namely 220, 320, 420 and 520 mW, constant conditions of power are carried out at conditions of 133 $x10^{-6}$ mol/lt of sample concentration, 1.0 kHz of modulation frequency and 488 nm of laser wavelength. The observed variations with time for such power levels are shown in figure (5-a , b).



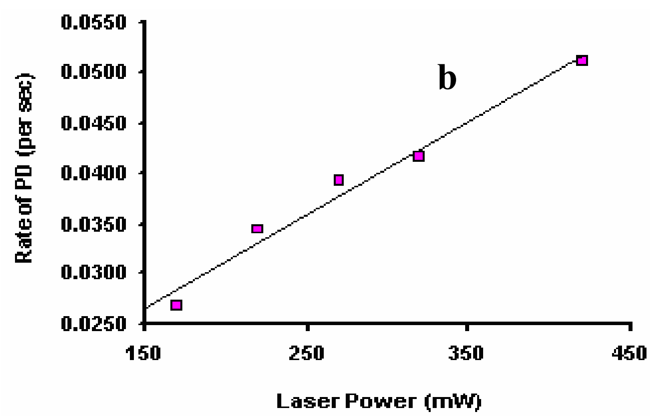


FIGURE 5: Variation of the rate of photodegradation with incident laser power over 30 minutes exposure time (a) the PD behavior and (b) is the absolute values of PD rate.

This revealed a clear proportionality between the incident power and PD rate. Furthermore, the increase in laser power is accompanied with an enhancement in the TL signal. These results are in a good agreement with that of Achamma et al. [7].

Effect of the pumping laser wavelengths

The effect of the pumping wavelength on PD rate and TL signal is studied adopting two different lines of argon ion laser at 488 and 514 nm in order to investigate whether these distinct lines have a major effect on the PD rate and TL signal of Na-Fluorescein. The measurements are carried out while the other parameters are kept constant at 220 mW of pump laser, 133 x 10^{-6} mol/lt of sample concentration and 1.0 kHz of chopper frequency.

From the absorption spectra of Na-Fluorescein figure (2), one can observe that its absorption band falls in the blue-green region peaking at 489 nm, which is much closed to the blue line (488 nm) of argon laser and away from the 514 nm line.

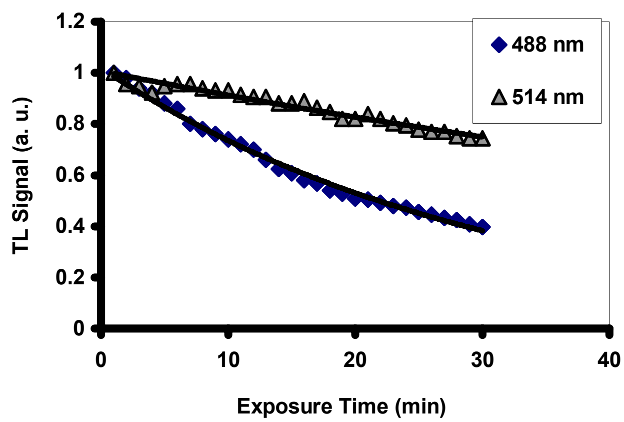


FIGURE 6. Variation of PD behavior of Na- Fluorescein with wavelength.

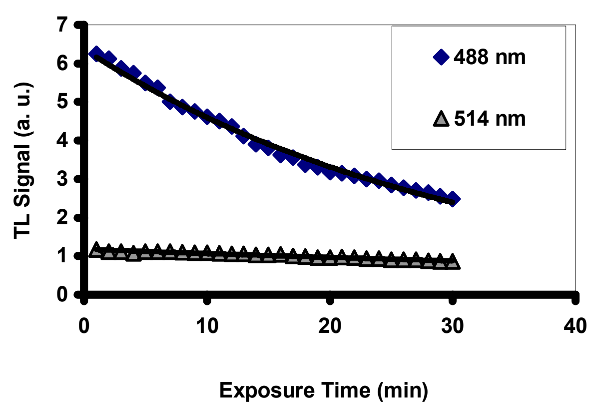


FIGURE 7. Variation of TL absolute signal of Na- Fluorescein with wavelength of pump laser.

So, the PD rate at 488 nm of pump laser is higher than that at 514 nm as shown in figure (6). Where, the PD rate at 488 nm is found to be nearly 3.3 times greater than that at 514 nm. In addition, the extreme higher signal of 488 nm than of 514 nm is due to relative higher absorbance of Na-Fluorescein at 488 nm than at 514 nm as shown in figure (7). Consequently, the line of 488 nm of argon laser has a major effect on PD rate and TL signal of Na-Fluorescein substance.

Effect of the chopper frequency

The effect of modulation frequency of the pumping laser beam on the PD rate and TL signal of Na-Fluorescein is investigated at five distinct chopping frequencies, 500 Hz, 750 Hz, 1.0 kHz, 1.25 kHz and 1.5 kHz. Such measurements are carried out at the same conditions of 488 nm, 220 mW of the pumping laser and 133 x 10^{-6} mol/lt. Figure (8) illustrates that within the range selected, the modulation frequency of pump laser has no obvious effect on the PD rate of sample molecules. This denotes that the PD process of Na-Fluorescein depends only on the total incident energy per unit time on the sample, which is the same for all the chopping frequencies [11]. On the other hand, as predicted, TL signal has inverse proportionality with the modulation frequency as shown in figure (9).

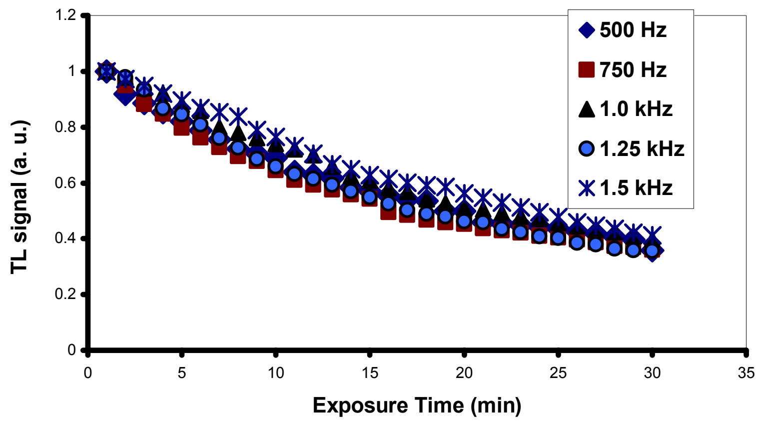


FIGURE 8. Effect of modulation frequency on PD rate of Na-Fluorescein.

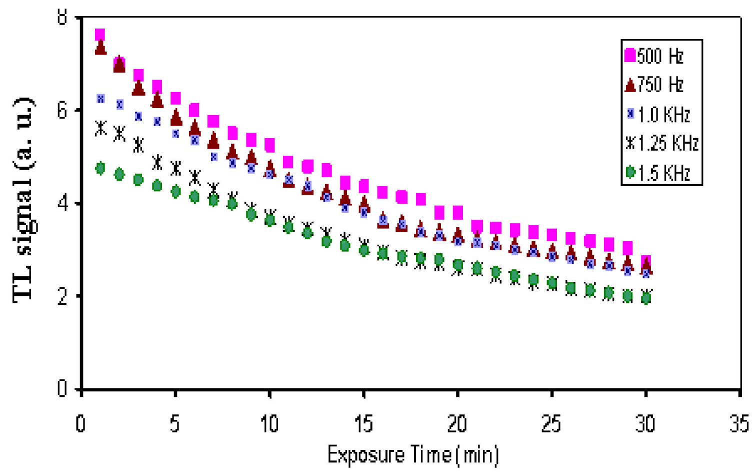


FIGURE 9. Variation of TL absolute signal of Na- Fluorescein with modulation frequency.

CONCLUSION

The dual-beam thermal lens technique has been found to be useful to study the photostability of sodium fluorescein in aqueous solution using crossed-beam set up. The results clearly show that the rate of PD and TL signal amplitude of Na-Fluorescein are found to increase with increasing of pump laser power and the concentrations of sample. In the case of modulation frequency, PD rate is found to be independent on it. While TL signals amplitude is found to be inversely proportional to the modulation frequency. With respect to wavelength effect, the PD rate and TL signal amplitude are found to be higher using 488 nm than 514 nm.

The photochemical quantum yield (φ_c) was measured using potassium ferrioxalate actinometry method for sample irradiated by argon laser of 488 nm wavelength. The resultant photochemical quantum yield was 2.2 x 10^{-5}.

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