Romanian Reports in Physics, Vol. 63, Supplement, P. 1267–1284, 2011

Dedicated to Professor Marin Ivaşcu's 80th Anniversary

SPECTRAL PROPERTIES OF SOME MOLECULAR SOLUTIONS

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Received July 25, 2011

Abstract. There are many reports concerning the occurrence, evolution, detection and removal of some pharmaceutical pollutants from environment. This report is focused on the Phenothiazines, particularly on Thioridazine. It discusses the time evolution of the absorption spectra of Thioridazine HCl (Thr) solutions in ultra-pure water in three temperature/light sets of conditions: 4^0C in dark, 22^0C in dark and 22^0C in daylight, for a concentration's range (10^{-5}–10^{-3})M. Thr solutions are exposed at 266 nm / 355 nm Nd:YAG pulsed laser radiation for different time intervals and at several energy levels. The modified absorption spectra following the irradiation are discussed.

The lasing obtained from pendant droplets containing Rhodamine 6G (R6G) in ultrapure water is reported. The water droplets were seeded with R6G at different concentrations and were irradiated at 532 nm emitted by a laser Nd:YAG – SHG laser system. The droplets were generated using a computer controlled set-up and the pumped liquid volumes were typically 12.5 µl (*i.e.* droplet diameter of around 3 mm). The producing of the lasing effect depends on the: R6G concentration, droplets volume, incidence angle of the pumping laser beam on the droplet. The most intense lasing line of R6G was obtained at 10^{-3}M concentration.

Key words: phenothiazines, thioridazine, absorption, microdroplets, Nd:YAG laser, laser dyes, optical emission, lasing.

1. INTRODUCTION

1.1. Pharmaceutical residues in the environment and their potential toxic effects have been recognized as one of the emerging research area in the last decade. By contrast with regular pollutants, which often have longer environmental half-lifetimes, the continuous introduction in the environment may make pharmaceuticals "pseudo-persistent" [1].

Pharmaceutical residues and/or their metabolites are usually detected in the environment at trace levels, but, even at low concentrations (ng/L or µg/L) they can

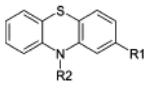
induce toxic effects. Frequent occurrences of cumulated drugs in the environment could increase the resistance of bacteria to drugs, and favour spreading the medicines resistance genes into the environment [2]. It is very important to establish sensitive and selective analytical methods and procedures to identify, detect and monitor such pollutants in the environment. One of the most sensitive methods is based on the measurement of the spectroscopic properties (absorption and emission of radiation) of these substances.

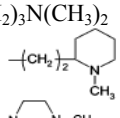
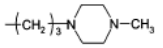
Phenothiazines, $[S(C_6H_4)_2NH]$ are organic compounds utilized in various antipsychotic and antihistaminic treatments. They contain a tricyclic ring with S and N atoms bridging the middle ring and are soluble in water, acetic acid, benzene and ether. The term "phenothiazines" describes the largest of the five main classes of neuroleptic antipsychotic drugs which have antibacterial properties so that they are of interest for applications in the reversal of drug resistance [3].

Some literature data reveal that the photophysics and photochemistry of phenothiazines are influenced by the substituents at positions 1 and 2 (Table 1), the nature of the solvent, and the excitation energy [4, 5].

Table 1

Structure of some Phenothiazine derivatives



Compound	R1	R2
Promazine	H	$-(CH_2)_3N(CH_3)_2$
Chlorpromazine	Cl	$-(CH_2)_3N(CH_3)_2$
Triflupromazine	CF_3	$-(CH_2)_3N(CH_3)_2$
Thioridazine (Thr)	SCH_3	
Trifluoperazine	CF_3	

Thioridazine (10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylthio) phenothiazine) is a piperidine antipsychotic drug (Fig. 1) and was previously used in the treatment of schizophrenia and psychosis. Due to its cardiotoxicity and retinopathy risks at high doses, it is not commonly prescribed.

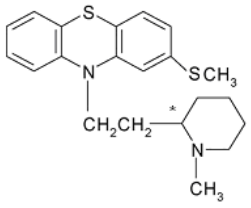


Fig.1 – Molecular structure of thioridazine.

The thioridazine (Thr) is a compound which exists in two mirror image forms. Such enantiomers exhibit chirality due to the lack of symmetry in the molecule usually at a C atom bound to four different substituents. The chiral center in Fig. 1 is marked with an asterix (*). Enantiomers have the same physical properties except for their interaction with polarized light which leads to the rotation of the plane of polarization. This property is used for chiral compounds and the direction of the rotation is given by (+) for right-rotation and (–) for left-rotation. The sum of the degree of polarization of the enantiomers may be zero for racemic mixture. In the Thr mixture the (–) compound has the lowest potency on the brain and all the three chiral compounds (rac, +, –) are equal in antimicrobial activity [6].

The photochemical behaviour of phenothiazines (PNT) has gained interest as the compounds containing a PNT moiety may promote photosensitizing effects in patients under therapy with these drugs [7, 8]. Under irradiation, PNT can attain an excited singlet state (either the first S_1 or another, higher excited, S_n) depending on the energy of excitation and on the solvent [9]. The formation of an excited singlet state S_n can occur by a single photon or biphotonic absorption process. The decay of the singlet excited states can occur via internal conversion ($S_n \rightarrow S_1$ and $S_1 \rightarrow S_0$ plus heat), fluorescence ($S_1 \rightarrow S_0$ plus $h\nu$), and intersystem crossing ($S_1 \rightarrow T_1$ or $S_2 \rightarrow T_2$ and $T_2 \rightarrow T_1$, *via* internal conversion).

The decay of the first excited triplet state can occur *via* phosphorescence. Phenothiazine derivatives (PTH) show comparatively weak values of quantum yields of fluorescence, but their quantum yields of phosphorescence are always larger than 0.3 and sometimes very near to one. Therefore, it can be deduced that the quantum yields of the intersystem crossing (IC) for phenothiazine derivatives are close to 1.8 [10].

Besides the phosphorescence, two mechanisms can be responsible for the triplet-state deactivation: energy transfer to molecular oxygen, which leads to the generation of $^1O_2$, and PNT specific processes. The PTH photoionization could generate the cation radical and solvated electron (eq. 1) or the neutral PNT and hydrogen radicals (eqs. 2 and 3) [11].

$$PTH \xrightarrow{h\nu} PTH^* \rightarrow PTH\bullet^+ + e_s^- \tag{1}$$

$$PTH \xrightarrow{h\nu} PTH^* \rightarrow PT\bullet + H\bullet \tag{2}$$

(monophotonic homolytic cleavage)

$$PTH \xrightarrow{h\nu} PTH^* \rightarrow PTH\bullet^+ + e_s^- \rightarrow PT\bullet + H\bullet \tag{3}$$

(thermal or photolytic deprotonation of PTH•$^+$)

The PNT photoionization via the singlet manifold process, yielding the cation radical, in competition with intersystem crossing and bond cleavage, has been described by several authors [9, 12, 13]. The cation radical can react with molecular oxygen, generating a phenothiazine sulfoxide derivate [14].

This study reports time evolution absorption measurements of Thioridazine HCl solutions in ultra-pure water in its three forms: rac / (–) / (+), in a concentration range between 10^{-5}M – 10^{-3}M, at three temperature/light conditions: 4°C in dark, 22°C in dark and 22°C in daylight. Also, solutions of 10^{-5}M / 5×10^{-2}M of Thr are exposed to pulsed Nd:YAG laser emitted radiation at different wavelengths, time intervals and beam energies. Stability studies based on the absorption spectra measurements are conducted following exposure to laser beam. The photodegradation process of these medicines is discussed.

1.2. Other studies on the laser beam interaction with solutions containing fluorescent molecules have shown that the changes that occur at the molecular level can be observed faster in small systems, such as micro-droplets, than in large systems, such as bulk [15].

One of the explanations of this behavior could be that in a droplet a larger part of the whole volume of solution is irradiated at the same time, so that the most part of the molecules are excited and they cannot exchange energy between them according to the most usual interaction processes. A complete irradiation of a substance in bulk is very difficult because in this case the laser beam interacts with a small cross section of the sample *i.e.* a small volume containing the molecules of interest found in the solution.

From another point of view, the bead can be associated with a closed resonant spherical cavity which can amplify, in certain conditions, the radiation emitted by the bead's material. This phenomenon will be described in more detail in this paper.

2. EXPERIMENTAL

2.1. PHENOTHIAZINES

2.1.1. Chemicals and solutions

Thioridazine HCl, having the molar mass 407.04g/L, in racemic mixture and its Thr(-) and Thr(+) enantiomers were kindly made available by the Department of Clinical Microbiology, Sønderborg Sygehus, Denmark, where the mixture was separated.

The solvent was ultra-pure de-ionized water delivered via a sterile filter (TKA Pacific UP/UPW6) and TKA Genpure ultra-pure water system accessory; the flow rate (at 15^{o}C) was 6 L/h and the retention of bacteria and particles 99%. Its bacterial content is <1CFU/ml, the particle content <1 at a resistivity of 18.2MΩxcm and 0.055μS/cm conductivity, at 25°C.

Solutions of Thr rac / Thr(–) / Thr(+) in ultra-pure water (pH ≈ 6) in a concentration's range of (10^{-5}–10^{-3})M were prepared. A concentration of 5×10^{-2}M of Thr rac / Thr(–) / Thr(+) in ultra-pure water was used as well in laser beam irradiation studies.

The solutions of Thr rac / Thr(–) / Thr(+) (10^{-5}–10^{-3})M in ultra-pure water were kept in three temperature/light conditions: at 4°C in dark, 22°C in dark and 22°C in daylight.

For the time stability studies after laser beam exposure, the solutions were preserved/stocked in dark, at 4°C.

2.1.2. Methods and instrumentation in PNT studies

In order to evaluate the PNT time behaviour regarding the light absorption properties, the absorption spectra were recorded between 200 nm and 1 300 nm, using a Perkin-Elmer Lambda 950 UV-VIS-NIR spectrophotometer (UV/VIS resolution ≤ 0.05 nm, NIR resolution ≤ 0.20 nm, error limit of ± 0.004%). Optical cells of 1cm thickness were used. For the optical measurement of the ambient light, it was used an Ocean Optics type HR4000 spectrometer working in the spectral range (200 – 1100) nm with a spectral resolution of 0.75nm. The spectrum acquisition was made using a single-mode optical fiber of 1mm core diameter.

Solutions of Thr rac / Thr(–) / Thr(+) at 10^{-5}M were exposed to radiation emitted at 266nm (the fourth harmonic of the Nd:YAG pulsed laser) for 1h, 2h and 3h respectively. The laser beam had the pulse repetition rate 10pps, FTW 5ns and the pulse average energy on the sample 0.263mJ. Following the irradiation, the absorption spectra were recorded using 1cm optical length cells with the aim of estimating the results of the interaction of the drug with the laser beam. The utilised experimental set-up is shown in Fig. 2.

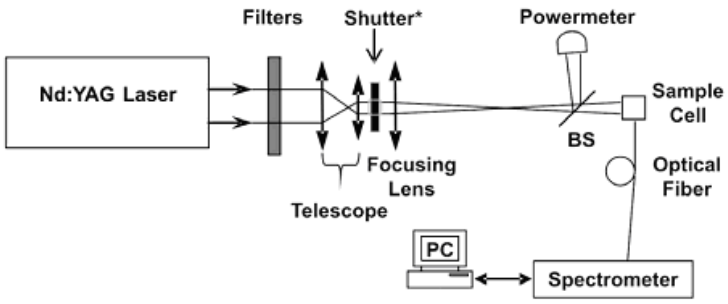


Fig. 2 – Laser irradiation experimental set-up: bulk exposure of compounds in sample cells (2.5 mL). *Shutter speed: < 1ms; Beam splitter (BS) with reflection lower than 1%.

The same experimental arrangement was used in another irradiation session that involved 5×10^{-2}M solutions exposed at 355 nm (third harmonic of the Nd:YAG laser) pulsed laser radiation for time intervals between 1 min. and 30 min. with a pulse average energy on the sample 1.45 mJ.

The absorption spectra measurements after irradiation sessions were performed in bulk with an optical path of 0.1cm.

2.2. MICRODROPLETS LASING EXPERIMENTS

The experiments consisted in the measurement of laser induced fluorescence (LIF) of an organic dye in pendant/hanging droplets.

The experimental set-up is presented in Fig. 3. A pulsed Nd-YAG laser (Panther, Continuum) was used, with laser pulse duration of ~5 ns, laser pulses repetition rate 10 pps and the average energy per pulse 0.3 mJ.

For this experiment pendant/hanging droplets were used, generated with a computer controlled system Hamilton Microlab 500. In order to ensure a quasistatic measurement, the droplets were obtained by means of a programmable dispenser at a slow speed of volume variation [15]. The typical bead volume used in the experiments was 12.5 μL.

The emitted LIF was collected with an optical fiber, and the signal was analyzed utilising an Ocean Optics monochromator.

For these experiments Rhodamine 6G (R6G) was used, which is a common laser dye. It was diluted in ultra-pure water. Although the recommended solvent for R6G is the ethanol, ultra-pure water was chosen because the droplet evaporation occurs at a slower rate than in the ethanol case.

The high purity grade water was produced by a MilliQ (Millipore) ion-exchange purifier provided with a micro filtration stage, and was used as solvent to prepare the solutions and as reference sample for the measurements in order to ensure a quasistatic measurement [16].

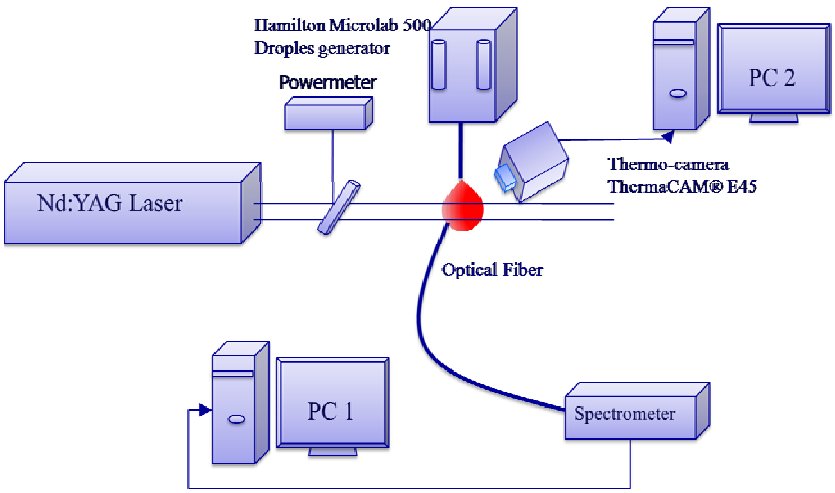


Fig. 3 – Experimental set-up.

A stock solution of R6G at 10^{-2} M was prepared and by dilution with ultra-pure water one obtained 5×10^{-3} M, 10^{-3} M, 5×10^{-4}M, 10^{-4}M, 5×10^{-5}M and 10^{-5}M concentrations.

The temperature variations of the droplet during the interaction with the laser radiation and after the absorption of it was measured using a Thermo-camera ThermaCAM ®E45 which has an accuracy of ± 2°C. The power of the pumping laser radiation was monitored in real time using a dedicated powermeter.

3. RESULTS AND DISCUSSIONS

3.1. PHENOTHIAZINES

The absorption spectra at 10^{-5}M exhibit broad peaks in UV at 262 nm and 314 nm and broader peaks in NIR at 966 nm, 1 153 nm and 1 240 nm, respectively (Fig. 4).

There are differences of the absorption intensities between the Thr rac / Thr(–) / Thr(+) solutions which remain within the measuring errors of the spectrophotometer in the spectral range of the main peak.

The time stability study were conducted for solutions kept in dark at 4°C and 22°C and reveals a slight modification in absorption intensity values for 10^{-5}M solutions.

Some contributions to these modifications could be due to the effect of radiation sources of the spectrophotometer (a deuterium lamp and a halogen lamp, which cover the working spectral range of the equipment) on the investigated solutions.

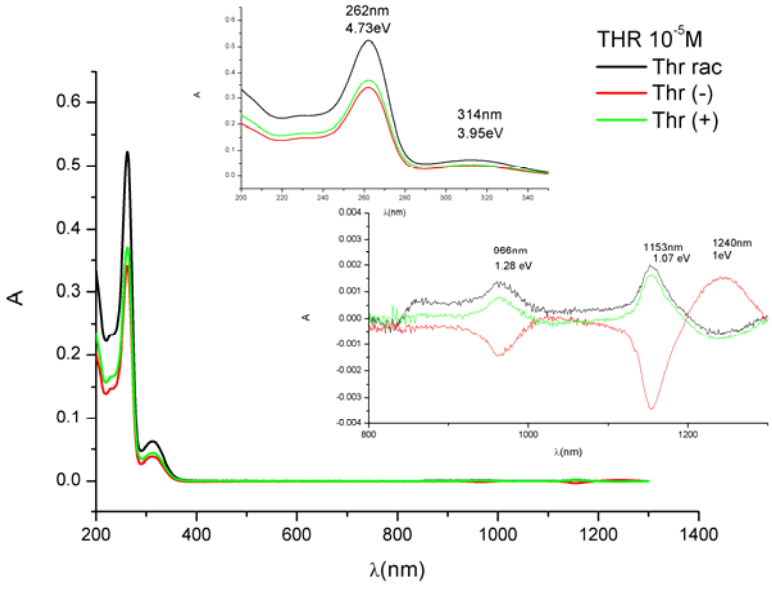


Fig. 4 – The absorption spectra of Thr and its enantiomers.

These very small doses of irradiation could affect the low concentrations of Thr samples.

In daylight conditions the 10^{-3}M solutions change their color as follows: after about 45h in light-blue, after around 70h in blue-green, after 96h in light-yellow and after 150h in light-brown. These color changes are associated with the shift of 314 nm absorption peak to 341 nm and the appearance in the absorption spectra of two peaks at 635 nm and 886 nm (Fig. 5). At the same time the absorption spectra appear to be saturated below 300 nm.

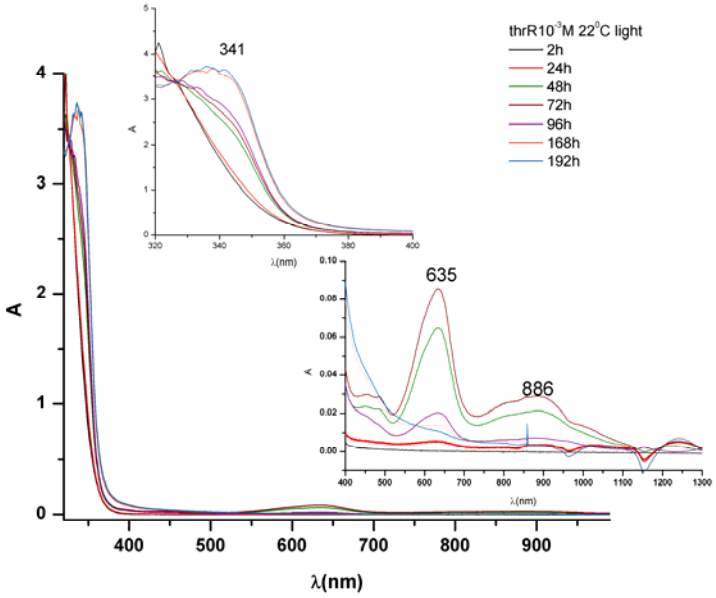


Fig. 5 – The absorption spectra of Thr rac 10^{-3}M at 22^0C in daylight.

The ambient light exposure of solutions led to spectral changes of the investigated samples that suggest the formation of the corresponding sulfoxide derivates [14]. Literature data reveal that shorter side chains in the structure of phenothiazines can favour radical dimerization, leading to hydroxylation regardless the presence of external nucleophiles [17]. Some studies have shown that phenothiazine-oxidized derivates are formed from the reaction of the cation radical with molecular oxygen and in such a way conditions that favoured the formation of the neutral radical could impair the formation of these derivates. Data reported in the literature have excluded the participation of $^1O_2$ in the photo-oxidation process, since the presence of $^1O_2$ quenchers does not modify the rate of sulfoxide formation [18].

The new absorption peaks were assigned to a cation radical stabilized in aggregated forms of the drugs. The appearance of visible bands after the

photoionization of phenothiazines occurs because the removal of one electron from the HOMO (highest occupied molecular orbital) produces a SOMO (solely occupied molecular orbital) and changes the penultimate highest occupied molecular orbital to a new HOMO, which allows a low-energy SOMO → HOMO transition [19].

The formation of the Thr cation radical was accompanied by changes in the UV spectrum of the drug (the shift of 314 nm absorption peak to 341 nm). This result suggests that the aggregation and the photoionization induce alterations in the drug molecular structure and consequently affect the transitions $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_n$.

Following the exposure at 266 nm up to 3h, it can be observed the flattening of Thr absorption spectra (Fig. 6).

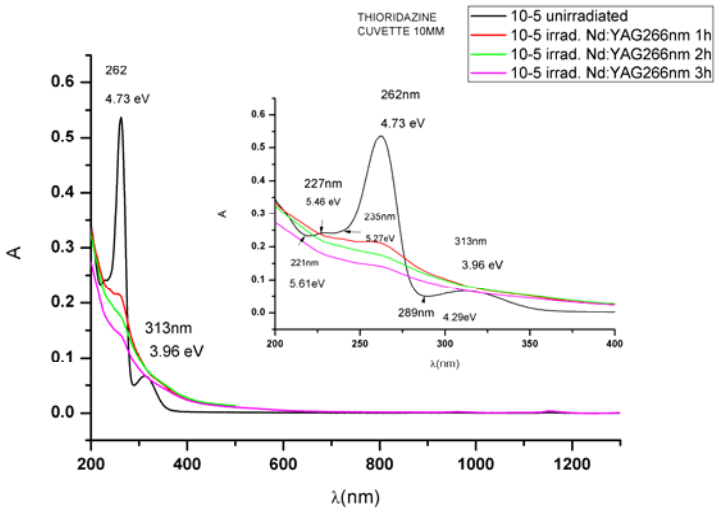


Fig. 6 – The absorption spectra of Thr 10^{-5}M solution unirradiated and exposed to 266 nm Nd:YAG pulsed laser radiation.

The time stability (Fig. 7) of the 3h irradiated drug based on the light absorption measurements reveals that the degradation of Thr continues even at 113h after exposure. A possible explanation of this behaviour could be an irreversible total degradation of Thr samples after 3h of exposure to laser radiation.

The same behaviour was recorded both for the racemic mixture and the enantiomers.

Another set of irradiation experiments at different exposure times was performed on $5x10^{-2}$M ultra-pure water solutions of Thr rac / Thr(–) / Thr(+) using 355 nm wavelength Nd:YAG pulsed laser beam.

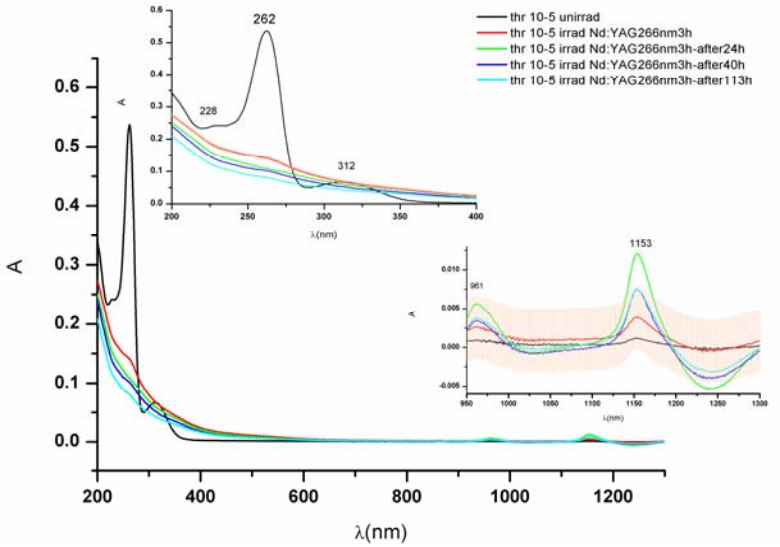


Fig. 7 – Time behaviour of the light absorption properties of Thr.

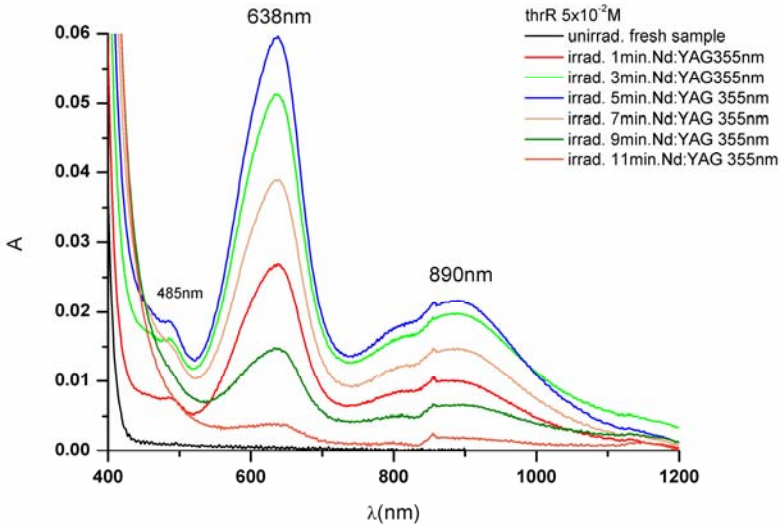


Fig. 8 – The absorption spectra of 5×10^{-2}M solution of Thr exposed to 355 nm Nd:YAG pulsed laser radiation.

The exposure of more concentrated solutions of Thr to 355 nm Nd:YAG pulsed laser radiation leads to the appearance of new absorption peaks at 485 nm, 638 nm and 890 nm (Fig. 8). One may observe the increasing of the absorption intensity for exposure times up to 5 min. followed by the decreasing of it after 11 min. of irradiation.

A previous study demonstrated that no polymerization of Thr took place for concentrations as high as 5×10^{-3} M [20]. Probably, at low concentrations, it was difficult to aggregate two Thr monomers, but with concentration increasing, once the dimers were formed, they acted as a nucleus on which other and Thr monomers could be easily associated. This result suggests a cooperative behaviour for the aggregation of Thr molecules. The increase of the Thr concentration led to the increase of the number of small aggregates and the corresponding increase of the absorbance at 638 nm. The saturation occurred probably because the number of Thr molecules that can be stabilized in the cation radical form in a Thr large aggregate is significantly lower than that stabilized in a small aggregate [11].

These absorption characteristics are transitory, the absorption spectra returning to the initial shape after a few hours following irradiation session (Fig. 9).

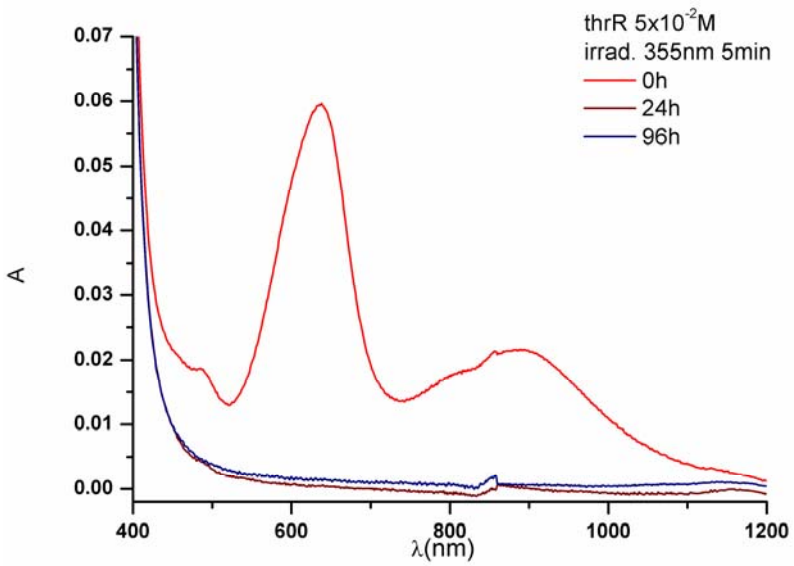
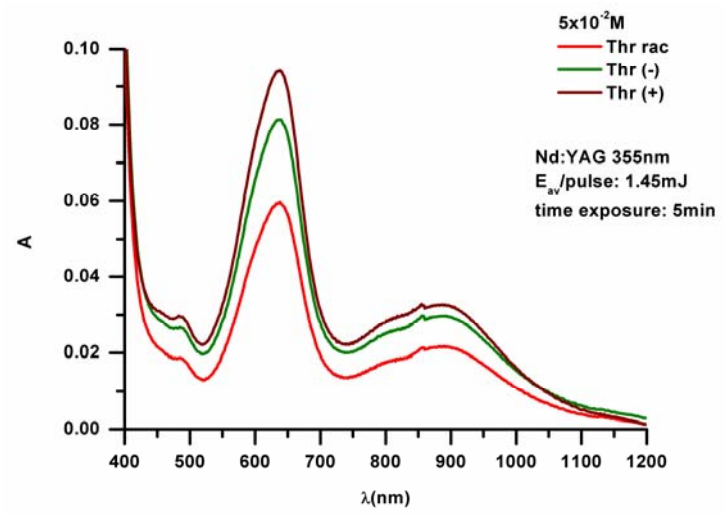


Fig. 9 – Time stability characterization of irradiated Thr solution.

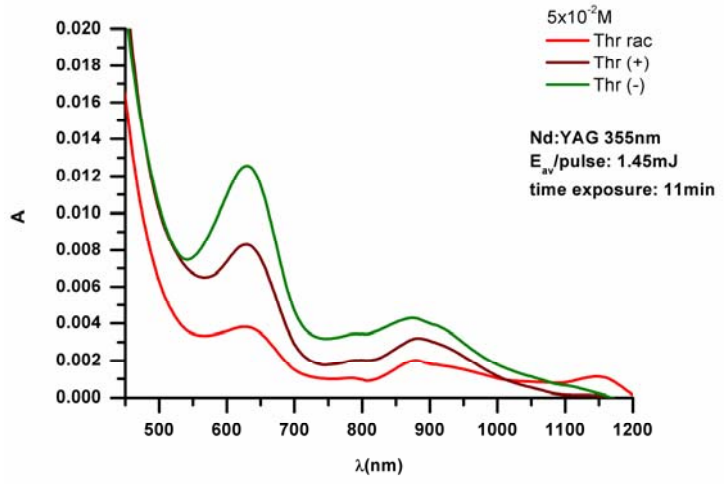
As for the behaviour of the three compounds at 5 min exposure to laser radiation, it can be observed that Thr (+) solution reached the maximum absorbance (Fig. 10.a); during the relaxation process after 11 min of exposure the Thr (–) compound has still the highest absorption intensity peaks in the visible, but shifted with 8 nm (Fig. 10.b). This behaviour reveals one more time the different response of the two enantiomers to light exposure.

3.2. MICRODROPLETS LASING EXPERIMENTS

For the study of the lasing effect of R6G in water solutions different sets of measurements were performed, in which several parameters were modified, such as: dye concentration, droplet size, area and point of signal collection, incidence angle of the laser beams on the droplet.



a



b

Fig. 10 – Comparison of absorption spectra of 5 min (a) and 11 min (b) irradiated Thioridazine solutions (rac / (-) / (+)).

The lasing threshold is defined as the lowest excitation level at which a laser's output is dominated by stimulated emission rather than by spontaneous emission. Below the threshold, the fluorescence output power increases in a slow manner when excitation is increased. Above the threshold, the slope of the curve power vs. excitation is orders of magnitude greater. At the same time the line width of the lasing emitted radiation becomes orders of magnitude narrower above the threshold than under it.

The first parameter that was modified was the concentration of the laser dye. For this type of measurements the concentration of the R6G was varied in the range 10^{-2}M to 10^{-5}M.

At lower concentration (10^{-5}M) one can observe the excitation laser line and a broadband of fluorescence spectrum of R6G (Fig. 11).

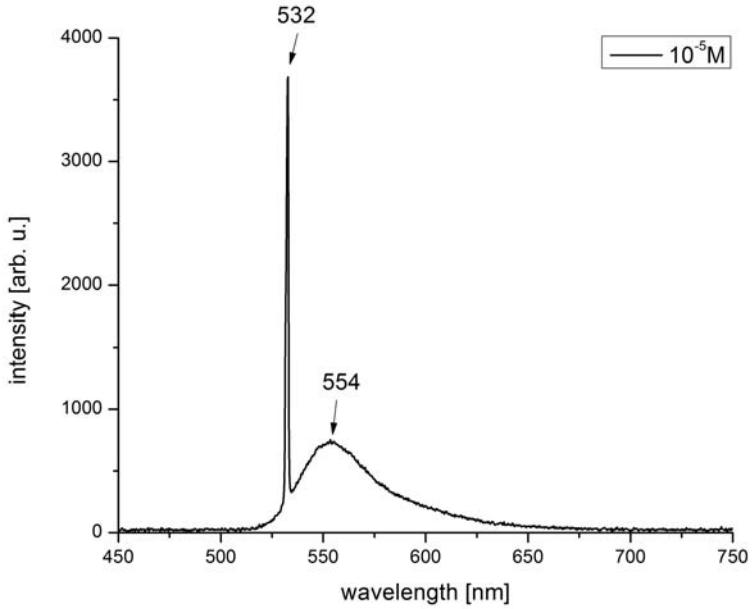


Fig. 11 – The fluorescence spectrum emitted by R6G ($c = 10^{-5}$M) measured on a pendant droplet at 10^{-5}M concentration.

By increasing the R6G concentration, the absorption becomes stronger, the fluorescence spectrum is increasing in intensity, and the peak of the fluorescence spectrum moves to higher wavelengths. The pumping laser line is embedded in the spectrum, as well (Fig. 12).

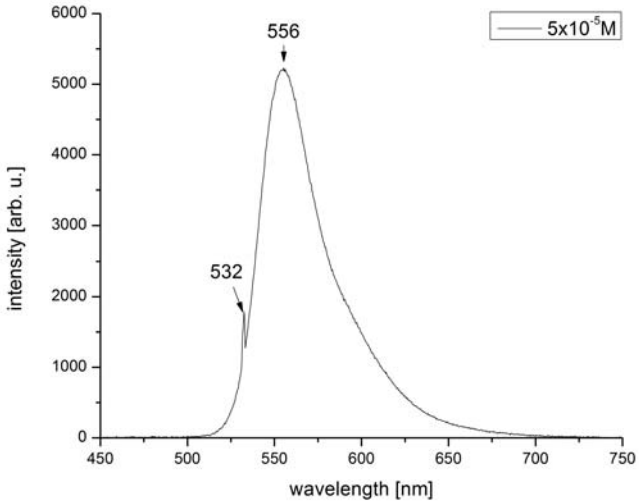


Fig. 12 – The fluorescence spectrum of R6G measured in droplet at 5×10^{-5}M.

By further increasing the concentration, the auto-absorption becomes more important and the intensity of the fluorescence spectrum is affected, so that in order to obtain the lasing one should increase the pumping beam intensity. For 5×10^{-4}M concentration, the lasing emission is assigned to the peak at 620 nm (Fig. 13) observing that with the increase of the dye concentration the lasing peak shifts towards longer wavelengths; this observation is in agreement with reported data which show that the fluorescence spectra peaks move towards longer wavelengths while the fluorophore concentration is increased [21].

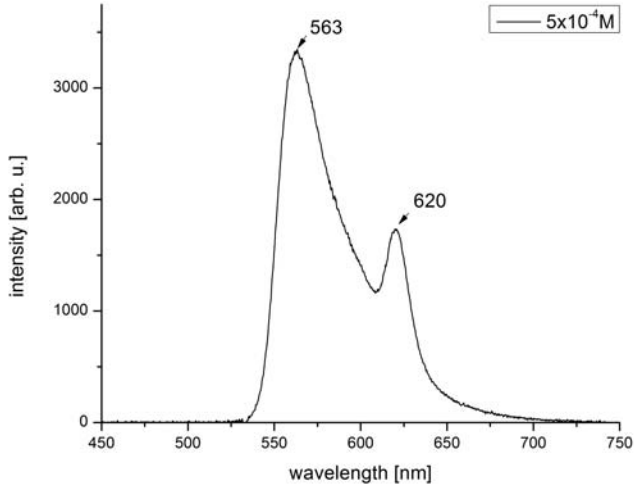


Fig. 13 – The fluorescence spectrum of R6G measured in droplet at the 5×10^{-4}M.

When the concentration was increased further to reach 10^{-3}M, the fluorescence peak decreased simulating a quenching effect. The shift to the red of the lasing peak that begins to be well separated from the fluorescence and becomes narrower are also observed (Fig. 14).

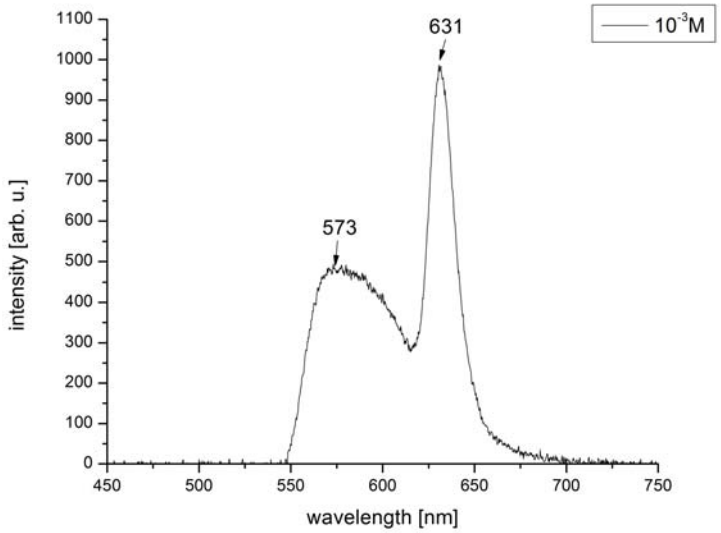


Fig. 14 – The fluorescence spectrum of R6G measured in droplet at 10^{-3}M.

The shift to the red that appears for both fluorescence and lasing line with the increasing of the R6G concentration can be explained by the fact that increased concentration increases the refractive index of the bead material; the optical path of the lasing radiation is changed and this induces changes of the amplification within the spherical cavity constituted by the droplet. In other words, the fluorescence spectrum of the dye is shifted to smaller energies when the dye concentration is increased.

3.3. DROPLET TEMPERATURE EVOLUTION

Although the ethyl alcohol is a good solvent for R6G in these experiments ultra-pure water was chosen as solvent. The reason was that the water evaporates slower than the ethyl alcohol and there is not a modification of the droplet diameter during the interaction with the laser pumping beam.

The droplet volume and shape are sensitive to any (even very small) evaporation process, so that the temperature for the irradiated and non-irradiated droplets was measured with a thermal camera ThermaCAM ®E45. As mentioned above, the laser irradiation was performed with a pulsed Nd:YAG at $\lambda = 532$ nm.

The results showed that the substances that do not absorb at 532 nm and do not emit fluorescence radiation (such as ultra-pure water) do not show major changes in temperature (less than 1°C - temperature changes may be correlated with variations in room temperature and remain within the measuring device errors) during or immediately after the interaction with the pumping beam. To limit air currents that could influence drop characteristics (temperature, shape) the measurements were performed in a chamber with a small access window for the thermo-camera.

The measurements on R6G were made on beads with an equatorial radius of 1.5 mm. The droplets are formed at the end of a capillary through which the substance is injected. Measurements made on R6G showed an increase of temperature with 3°C during the irradiation process.

4. CONCLUSIONS

4.1. The presence of pharmaceutical compounds in river and surface waters has frequently been reported in recent years [22–25]. The presented data give a strong indication of the importance of the investigation of the environmental behaviour of drugs, especially those known to be phototoxic. Photodegradation of PNT produced by light (natural, uncoherent or laser beam) exposure may be of major significance in their elimination process.

The inferences of the study on PNT can be synthesized as follows:

– the ambient light exposure of solutions led to spectral changes of the investigated samples that suggest the formation of the corresponding sulfoxide derivates;

– the photochemically generated cation radicals exhibit absorption visible bands due to a HOMO → SOMO transition;

– the formation of the Thr cation radical was accompanied by changes in the UV spectrum of the drug. This result suggests that the aggregation and the photoionization induce alterations in the drug structure and consequently affect the transitions $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_n$;

– after 3h of 266 nm Nd:YAG pulsed laser beam exposure the irreversible total degradation of the samples was observed. This could be considered a potential removed mean of this drug from water environment;

– the exposure of more concentrated solutions of Thr to 355 nm Nd:YAG pulsed laser radiation leads to the appearance of new VIS absorption peaks. The increase of the Thr concentration led to the increase of the number of small aggregates and the corresponding increase of the absorbance at 638 nm.

4.2. As for the lasing by droplets containing R6G in water, the experiments have shown that there is possible to obtain 4π lasing emission from a liquid active medium in air which constitutes at the same time a closed spherical optical

cavity/resonator. Until now, reports were made on obtaining laser and lasing from active media introduced in open optical resonators.

The changes in the temperature of the bead/laser active medium may be due to the nonradiative processes which are associated to the deexcitation of the R6G molecules excited on singlet states after the absorption of the pumping radiation at 532 nm.

Acknowledgements. The research was funded by the Romanian ANCS projects: PALIRT, 41-018/2007, PN 09 39 01/2009, LAPLAS 3-PN 0933 and the COST Action BM0701 (ATENS).

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