

Dedicated to Professor Valentin I. Vlad's 70<sup>th</sup> Anniversary

## PHENOTHIAZINE DERIVATIVES INTERACTION WITH LASER RADIATION

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*Abstract.* Phenothiazine derivatives are sensitive to light, especially to UV radiation. Chlorpromazine (CPZ) and Promazine (PZ) solutions in ultrapure water were exposed to 266 nm laser radiation in order to modify their structure and to obtain new species active against multidrug resistant bacteria. Modifications of the UV-Vis absorption spectra of the solutions indicate changes of the molecules.

During irradiation, CPZ molecules are modified gradually. The energy absorbed during irradiation was measured. The maximum amount of CPZ molecules that could absorb the energy in order to break each type of bond in the CPZ molecule (C–Cl, C–H, C–C, C–N and C–S) was calculated. A general qualitative picture of the effects of laser radiation on CPZ and PZ is made.

*Key words:* phenothiazine, chlorpromazine, CPZ, promazine, PZ, laser irradiation, UV-Vis absorption spectroscopy, bond dissociation energy.

### 1. INTRODUCTION

Exposure of medicines at ultraviolet (UV) and/or visible (Vis) radiation may modify the structure of the molecules and yield new compounds. Most molecules that are of biological interest absorb in the UV region of the spectra [1]. Compared to incoherent sources, when it comes to expose medicine molecules to light beams, the lasers offer the advantage of specific wavelength, very low beam bandwidth and much higher beam energy.

Studies have shown that several medicines from different classes were modified when exposed to light sources, especially to UV radiation.

The cytostatic activity on an *in vivo* experimental model of 5-Fluorouracil (5-FU) was enhanced under the influence of UV-Vis radiation. 5-FU solutions were exposed to a Hg lamp (emission spectral range: 300–600 nm) and N<sub>2</sub> laser (wavelength: 337.1 nm) and then applied on rabbit pseudotumour eye tissues, with measurable results. Similar results were obtained in the case of Methotrexate (MTX). Both medicines acted as photosensitizers [2, 3].

Another study reports an improvement of the effect of Polidocanol, (that is a pharmaceutical active ingredient of commercially available Aethoxysklerol 2%, used in sclerotherapy), if the medicine is injected as foam in a varicose tissue and if the so impregnated tissue is exposed to 1.06  $\mu\text{m}$  laser beam [4,5].

An enhancement of the bioactivity of medicines may be possible in the case of some molecules if they are exposed to laser radiation outside the biological targets, i.e. not necessarily in cells, bacteria or tissues impregnated from the beginning with the photosensitive medicine. In other words, we may expose to laser radiation the solutions of medicines, modify the medicines molecules and apply them on the biological targets studying the results of their action. This may be more effective on eye pseudotumour tissues than the exposure to laser radiation of the tissue impregnated with the unirradiated medicine [4,5].

In another case, two ecdysteroids, equivalent with the insect molting hormone, were considered promising candidates for photo-transformation by exposure to a 266nm laser radiation [6].

As for the phenothiazine derivatives, laser irradiation of medicines solutions was proposed as a method of generating new species active against multidrug resistant bacteria [7,8]. In this case, the antibacterial activity of the Chlorpromazine (CPZ) solutions exposed at 266nm laser beam was evaluated against several Gram-positive and Gram-negative bacterial strains. The activity of the products of irradiation against *Staphylococcus aureus* and *Escherichia coli* strains was enhanced, but the activity against *Salmonella enterica* serovar Enteritidis was slight [9].

Studies were performed in order to identify the modifications that arise in phenothiazines molecules during exposure to laser radiation.

In [7], the structural changes of CPZ induced by 266nm laser radiation were investigated and several irradiation products were identified as Promazine (PZ), hydroxypromazine or PZ sulfoxide, hydroxypromazine sulfoxide, CPZ and CPZ sulfoxide. Correlated with the results reported in [7], this paper constitutes a first step in elucidating the interaction mechanisms of the phenothiazine derivatives molecules with the laser radiation.

## 2. MATERIALS AND METHODS

The phenothiazine derivatives utilized in this study are CPZ and PZ, both medicines being normally used as neuroleptics. According to previous literature reports, they are photosensitive and may produce phototoxic and/or photoallergic reactions to patients [10, 11, 12].

CPZ and PZ are both in hydrochloride form and, for our studies, were purchased from Sigma-Aldrich as biological grade powders (purity over 98%). The chemical formulas of CPZ and PZ are  $\text{C}_{17}\text{H}_{19}\text{ClN}_2\text{S}$  and  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{S}$ , respectively.

The chemical structures of CPZ (Fig. 1a) and PZ (Fig. 1b) are similar, the only difference being in CPZ a chlorine atom (represented with green color) attached to the tricyclic phenothiazine part of the molecule. In Fig. 1, the carbon atoms are represented with grey color, nitrogen atoms with blue, sulfur atoms with yellow and hydrogen atoms with white color.

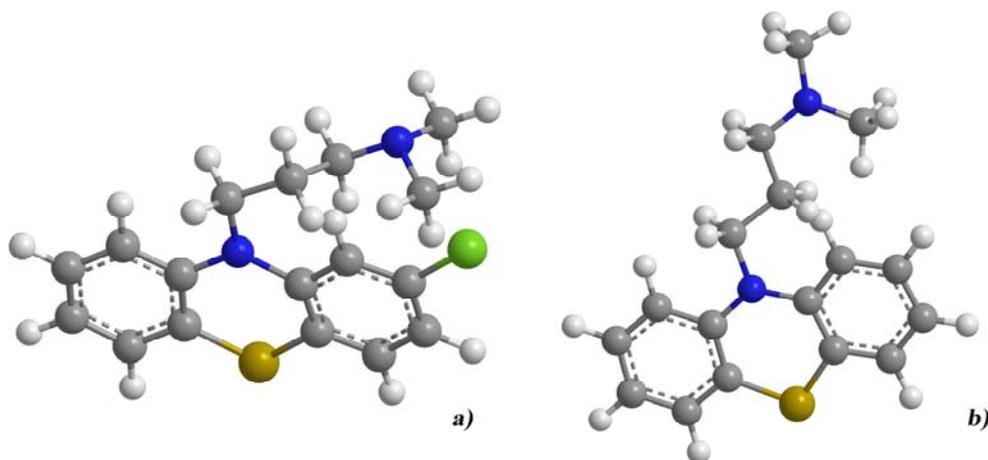


Fig. 1 – Chemical structures of: a) CPZ; b) PZ.

The solutions of CPZ and PZ were prepared at a concentration of  $10^{-4}$ M in ultrapure deionized water immediately before the irradiation. This concentration was chosen after recording the absorption spectra at different concentrations of CPZ and PZ. At  $10^{-4}$ M the absorption signal is not saturated and the shape of the absorption spectra could be clearly analyzed. On the other hand, the concentration of  $10^{-4}$ M is below the critical micelle concentration (*cmc*) or the concentration at which the polymerization takes place; in this way, in each solution only phenothiazine monomers were present [7, 13, 14]. To avoid measuring errors due to photosensitive properties of the CPZ or PZ, the vials containing the solutions were protected from light.

The ultrapure deionized water was obtained *via* a sterile filter (TKA Pacific UP/UPW6) and TKA Genpure ultra-pure water system accessory. Its bacterial content is <1CFU/ml, the particle content <1 particle/ml at a resistivity of  $18.2\text{M}\Omega\times\text{cm}$  and  $0.055\mu\text{S}/\text{cm}$  conductivity, at  $25^\circ\text{C}$ .

The experimental set-up used for exposing the samples to laser radiation is described in Fig. 2.

Volumes of 2ml of CPZ or PZ solutions were exposed to laser radiation for 2 hours, in a cuvette having 10mm width. The wavelength of the radiation is 266nm, which is the fourth harmonic of a Nd:YAG laser radiation emitted at  $1.064\mu\text{m}$ . Laser pulses repetition rate is 10pps and the full time width of the pulse

is 10ns. The average energy of the laser radiation was 6.5mJ, so that at the concentration of  $10^{-4}$ M the beam was not entirely absorbed by the sample, as observed experimentally. A second powermeter was placed after the cuvette in order to measure the value of the energy that is transmitted through the sample.

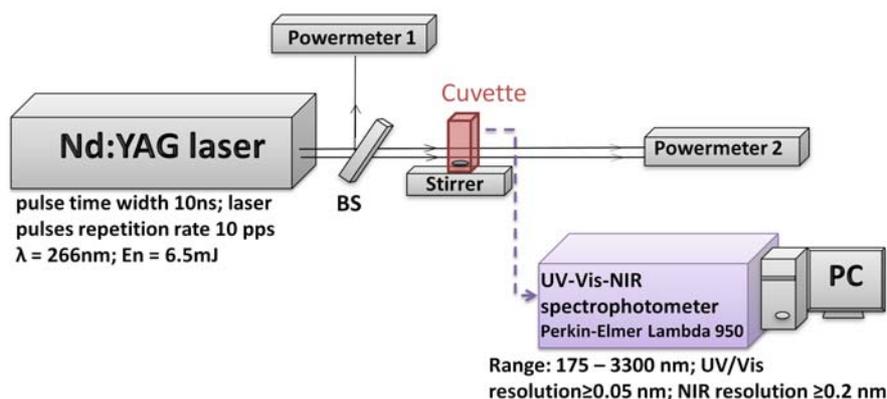


Fig. 2 – Experimental set-up used for irradiation of the phenothiazines.

A magnetic bar was inserted in the cuvette and the laser beam was focused above it; the cuvette was placed on a magnetic stirrer to ensure a continuous stirring during the irradiation time interval (two hours) of 600 revolutions per minute.

Before and after the irradiation the absorption spectra of the phenothiazines solutions were measured using a Perkin Elmer Lambda 950 UV/Vis/NIR spectrophotometer, with an error bar of  $\pm 0.004\%$ . The thickness of the optical cells used for absorption spectra measurements was 1 mm.

### 3. RESULTS AND DISCUSSIONS

Since the phenothiazine derivatives CPZ and PZ are photosensitive drugs their absorption spectra were recorded to identify the appropriate wavelength to be used for exposure to laser beams.

In Fig. 3 are displayed the absorption spectra of two CPZ  $10^{-4}$ M solutions in ultrapure deionized water: first unirradiated, recorded immediately after preparation, and the second, the spectrum of the solution exposed for 2 hours at 266nm laser radiation, with an average energy of 6.5mJ.

The absorption spectrum of unirradiated CPZ  $10^{-4}$ M in ultrapure deionized water solution recorded immediately after preparation presents three absorption bands: a partially resolved band below 205nm, that may originate from the atmospheric oxygen, and bands at 254nm and 307nm, respectively.

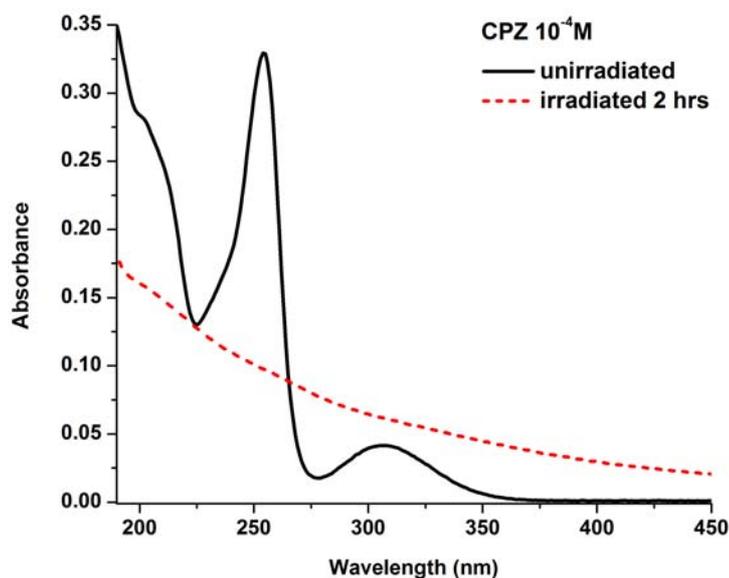


Fig. 3 – Absorption spectra of CPZ  $10^{-4}$ M solutions in ultrapure water before and after exposure to laser radiation.

The absorption peak at 254nm is probably due to a  $\pi - \pi^*$  transition, because its high molar absorptivity. Also, the relative broad absorption band at 307nm is most likely a result of a  $n - \pi^*$  transition, having a low molar absorptivity.

The modifications induced by exposure to laser radiation are obvious from the absorption spectrum recorded after 2 hours of irradiation.

The shape of the spectra is totally modified and the absorption bands are no longer visible. The value of the absorbance at 254nm decreased after irradiation from 0.329 to 0.097. In the case of the broader peak at 307nm, the absorbance value increased from 0.041 to 0.061, but the band cannot be differentiated, probably because the CPZ molecules are separated in smaller fragments that have large absorption bands with low absorbance in the same spectral range.

The absorption spectra of PZ  $10^{-4}$ M solutions in ultrapure deionized water recorded before and after exposure to laser beam are shown in Fig. 4.

The irradiation conditions were identical with those in the previous experiment; the sample was irradiated for 2 hours at 266nm. The energy of the laser beam was 6.5mJ.

Because the structures of CPZ and PZ are very similar, the UV-Vis absorption spectra of the two molecules are almost identical.

The absorption spectrum of the unirradiated PZ  $10^{-4}$ M solution in ultrapure water has the general shape similar to the CPZ absorption spectrum; the partially resolved band originating from the oxygen is present below 205nm, but the absorbance maxima of the two bands are at 252nm and 301nm.

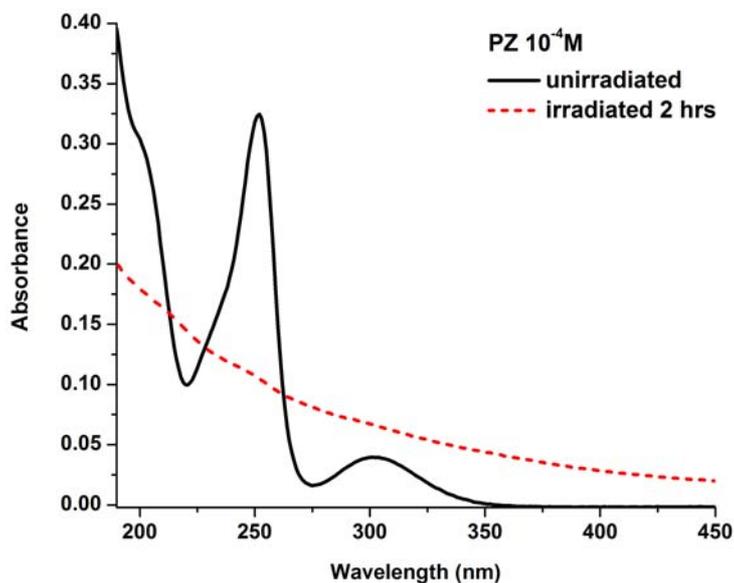


Fig. 4 – Absorption spectra of PZ  $10^{-4}$ M solutions in ultrapure water before and after exposure to laser radiation.

As in the case of CPZ, the narrow peak with high molar absorptivity at 252nm is due to a  $\pi - \pi^*$  transition and the broad peak, but with a low molar absorptivity, at 301nm, is due to a  $n - \pi^*$  transition.

During irradiation, the structure of the PZ molecules is modified so that, after 2 hours of laser exposure, the absorption bands are no longer visible, most likely because the fragments formed have large absorption bands with low absorbance. The absorbance at 252nm decreased after exposure to laser radiation from 0.324 to 0.105 and for the peak at 301nm, the absorbance value increased from 0.039 to 0.066.

The changes that appear in the absorption spectra of the irradiated CPZ and PZ are in agreement with the data reported in [7] and the production of promazyl radical and of sulfoxides is likely. The exposure to laser radiation may produce changes in the phenothiazines molecular structure and may affect the  $S_0 \rightarrow S_1$  and  $S_0 \rightarrow S_n$  transitions, as suggested for Thioridazine (TZ) in [15].

In [7], attempts were made to identify the products yielded during irradiation. PZ, hydroxypropromazine or PZ sulfoxide, hydroxypropromazine sulfoxide, CPZ and CPZ sulfoxide were identified, but the presence of other 200 unidentified compounds was noticed. Some mechanisms of modification were proposed, but the processes of interaction of laser radiation with the molecules in solution have not been fully clarified, yet.

The conditions of irradiation were chosen so that, at the concentration of  $10^{-4}$  M, the average energy of the laser beam (6.5mJ) is not totally absorbed by the sample. A volume of 2ml of solution is placed in a cuvette of 1cm width.

For the CPZ  $10^{-4}$ M solution, the energy absorbed during irradiation decays exponentially, as shown in Fig. 5.

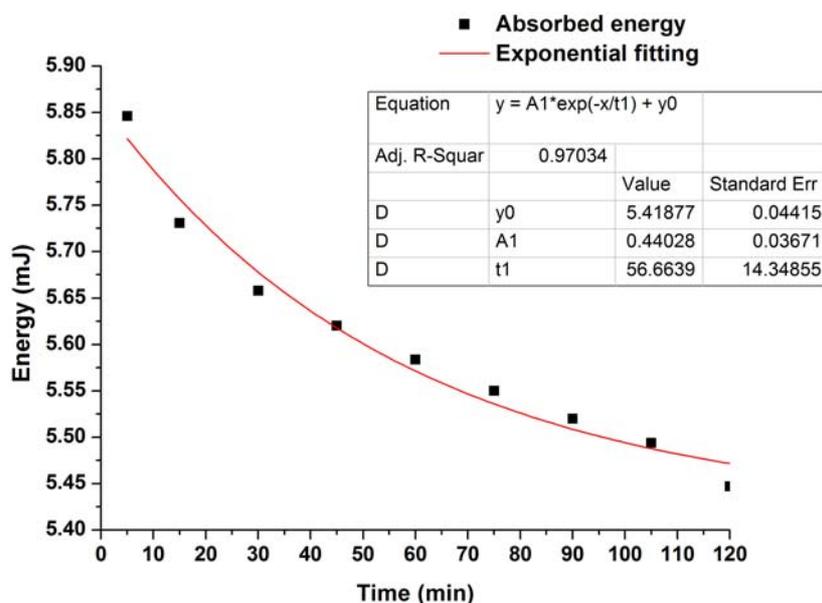


Fig. 5 – Energy absorption by a CPZ  $10^{-4}$ M solution during 2 hours exposure.

The average energy absorbed by the CPZ sample measured at 5 minutes after starting the irradiation was 5.846 mJ and it decreased to 5.447 mJ at the end of 2 hours of irradiation.

The volume of solution in the cuvette that is crossed by the laser beam is, considering the diameter of the laser beam 5mm and the optical length 10mm,  $196.25\mu\text{l}$ . At the given concentration, in this volume, we have  $196.25 \times 10^{-10}$  moles of CPZ and consequently, the number of CPZ molecules in this volume is  $11.81845 \times 10^{15}$ .

The solution in the cuvette was stirred during irradiation at 10 rotations per second and the laser pulse repetition rate was 10 pulses per second (100ms time interval between two successive pulses). According to our observations, due to the solution viscosity, the liquid agitation produced by stirring was not transmitted to its free surface in the spectrophotometer cell where the interaction with the laser beam was produced. Though, the solution was rotated so that within its volume practically all the molecules crossed the laser beam path and were placed in the conditions to interact with the laser beam photons. On the other hand, the Brownian motion and the tendency of molecules to migrate to regions with lower concentration at room temperature is a known process which leads to molecules

mixture in the liquid. It is the CPZ molecules case as well, since the molecules dissociate after interaction with the laser beam and their concentration decreases with the increasing of the exposure time to the laser beam. So, it is to be expected that CPZ molecules have the tendency of migrating in the liquid volume (i.e. the cylinder) directly exposed to the laser radiation. At the speed of the CPZ molecules produced by the Brownian movement, it would take more than  $10^9$  seconds for a molecule to cross the diagonal of the cylinder described by the intersection of the laser beam with the cuvette. This is very long time with respect to the laser pulse lifetime (10ns) as well as with respect to the time interval of 100ms between two successive laser pulses. For this reason we assume that the speed of the molecules in the laser beam volume is equal to the speed of the stirring magnet and that during 10ns there is not a significant diffusion of new CPZ molecules in the volume of the laser beam. Even more, we consider that for the entire duration of one rotation (one full rotation of the magnet takes 100ms at 600 rotations per minute) we have only one laser pulse that interacts with  $196.25 \times 10^{-10}$  moles of CPZ (the pulse repetition rate is 10 pps).

The average measured energy absorbed after 5 minutes was 5.846 mJ. Consequently, the corresponding average energy absorbed by the total moles of CPZ in the volume of the laser beam was  $297.885 \text{ kJ mol}^{-1}$ . At the end of 2 hours of irradiation, the average energy absorbed by the total moles of CPZ was  $277.554 \text{ kJ mol}^{-1}$ .

For the structural changes of molecules to be induced by 266nm laser radiation as described in [7] the energy absorbed must be at least equal to the bond-dissociation energy for each bond involved. For example, a first product identified in a large quantity in the irradiated CPZ solutions is PZ. For the CPZ molecule to be transformed in a PZ molecule it is necessary for the C–Cl bond to break. For this to happen, the energy absorbed by the CPZ molecule must be at least equal to the bond-dissociation energy (BDE) for the C–Cl bond.

The bond-dissociation energy is equal to the enthalpy change that appears at a bond cleavage and it depends on the structure of the molecule. BDE is usually calculated at standard pressure and at the temperature of 298 K [16, 17].

In general, the BDE values for C–Cl vary function of the type of molecule and in the case of substituted aromatic compounds the value also varies with positioning of the chlorine atom with respect to the radical. The BDE value for Cl–CH<sub>3</sub> (chloromethane) is  $350 \text{ kJ mol}^{-1}$ ; for the substituted chlorobenzene, C–Cl BDE varies with the position of the radical from  $376 \text{ kJ mol}^{-1}$  to  $399 \text{ kJ mol}^{-1}$ . Due to the similarity with the CPZ molecules we have chosen in our case an average BDE for C–Cl of  $385 \text{ kJ mol}^{-1}$ .

For other dissociations to happen we need to consider the BDE for the other type of bonds in the CPZ molecules, like C–H, C–C, C–N and C–S. The considerations regarding BDE variations apply for these bonds as for the C–Cl bond.

The BDE for C–H in methane is 439 kJ mol<sup>-1</sup>, in pyridine ring 468 kJ mol<sup>-1</sup> and in a substituted pyridine ring it is 362, 365 or 378 kJ mol<sup>-1</sup>, function of the position of the radical. Considering these values we have chosen for the calculations in this paper the BDE average value of 400 kJ mol<sup>-1</sup>.

For the C–C bond, the BDE in ethane is 377 kJ mol<sup>-1</sup>, and in methylbenzene BDE equals 426 kJ mol<sup>-1</sup>. In biphenyl the C–C bond enthalpy is 478 kJ mol<sup>-1</sup> and the energy required to break a radical from a pyridine ring is between 362 kJ mol<sup>-1</sup> and 433 kJ mol<sup>-1</sup>, given the positioning of the radical on the phenyl ring. Taking into account the similarities with the CPZ molecule, we selected a value of 430 kJ mol<sup>-1</sup>.

For a substituted nitrobenzene, the BDE for C–N bond ranges between 276–302 kJ mol<sup>-1</sup>. For a phenyl methylamine C–N BDE is 420 kJ mol<sup>-1</sup> and for a phenyl dimethylamine is 390 kJ mol<sup>-1</sup>. Taking into account the positioning of the C–N bonds in the CPZ molecules we considered for further calculations a lower value for BDE, namely 300 kJ mol<sup>-1</sup>.

The S–C bond has different enthalpy values for different molecules for the same reasons as mentioned above. In diphenyl sulfide, BDE is 327 kJ mol<sup>-1</sup> and in benzenethiol it is 362 kJ mol<sup>-1</sup>, but for methanethiol the enthalpy is 312 kJ mol<sup>-1</sup>. A mean value of BDE was chosen for the S–C bond, in our case 330 kJ mol<sup>-1</sup> [17].

The average energy absorbed by the CPZ molecules divided to the number of moles present in the area of the cuvette crossed by the laser radiation is at any time lower than any bond dissociation energy in the molecule. Considering this and the fact that modifications occur (bond break and new compounds are formed during irradiation, as shown by the absorption spectra) it results that the energy is not absorbed by all the molecules present in the interaction volume during one laser pulse, which is in agreement with the quantum mechanics approach of these processes.

The maximum number of moles that absorb during one pulse in order to break a bond is:

$$N = \frac{E}{\text{BDE}},$$

where  $N$  is the number of moles in the considered volume,  $E$  is the average absorbed energy and BDE is the bond dissociation energy.

Further, one may find out the maximum number of molecules in the considered volume that absorb the energy so that a bond is dissociated, according to the equation:

$$n = N \times N_A,$$

where  $n$  is the number of molecules and  $N_A$  is Avogadro's number.

The values obtained for the maximum number of CPZ molecules in the area of the cuvette crossed by the laser beam that absorb the amount of energy needed to break each type of bond during different time intervals are given in Table 1.

Table 1

Maximum number of CPZ molecules that could absorb the energy needed to break different types of bonds

Time (min)	$E \times 10^{-6}$ (kJ)	$n$ for C-Cl to break $\times 10^{15}$ (molecules)	$n$ for C-H to break $\times 10^{15}$ (molecules)	$n$ for C-C to break $\times 10^{15}$ (molecules)	$n$ for C- N to break $\times 10^{15}$ (molecules)	$n$ for C-S to break $\times 10^{15}$ (molecules)
5	5.846	9.14427	8.80136	8.18731	11.7351	10.6683
15	5.731	8.96439	8.62822	8.02625	11.5043	10.4585
30	5.658	8.8502	8.51832	7.92402	11.3578	10.3252
45	5.62	8.79076	8.46111	7.8708	11.2815	10.2559
60	5.584	8.73445	8.40691	7.82038	11.2092	10.1902
75	5.55	8.68127	8.35572	7.77276	11.141	10.1281
90	5.52	8.63434	8.31056	7.73075	11.0807	10.0734
105	5.494	8.59367	8.27141	7.69434	11.0285	10.026
120	5.447	8.52016	8.20065	7.62851	10.9342	9.94018

The second column in Table 1 represents the amount of energy absorbed by the CPZ molecules recorded at different times of irradiation. The total number of molecules present in the volume of solution crossed by the laser radiation is  $11.8185 \times 10^{15}$ . In columns 3 – 7 are listed the values for the maximum possible number of molecules that could absorb in order to break each type of bond present in a CPZ molecule: C–Cl, C–H, C–C, C–N and, C–S, respectively. These values were calculated considering the bond dissociation energies:  $385 \text{ kJ mol}^{-1}$  for C–Cl bond,  $400 \text{ kJ mol}^{-1}$  for C–H bond,  $430 \text{ kJ mol}^{-1}$  for C–C bond,  $300 \text{ kJ mol}^{-1}$  for C–N bond, and  $330 \text{ kJ mol}^{-1}$  for C–S bond. The maximum number of absorbing molecules was calculated considering that for the duration of one pulse occurs the dissociation of only one type of bond.

The behavior of the maximum number of molecules that could absorb the energy of the laser pulse in order to dissociate the C–Cl bond is given in Fig. 6 for the entire duration of irradiation, computed from the measurements of the energy absorbed by the solution at different irradiation times. An exponential decrease of the number of molecules is observed from  $9.14427 \times 10^{15}$  to  $8.52016 \times 10^{15}$  molecules. These values depend on the amount of energy absorbed during irradiation.

The same exponential decrease is observed also for all other types of bonds in the CPZ molecule based on the measured absorbed energy, only.

This continuous decrease of absorbing molecules number is an indicator of the fact that the number of the initial CPZ molecules present in the irradiated volume is decreasing with the increasing of the exposure time. This was evidenced in [7] through chromatography methods, when the amount of CPZ disappeared after 30 minutes of irradiation and new species were formed, some of them being identified.

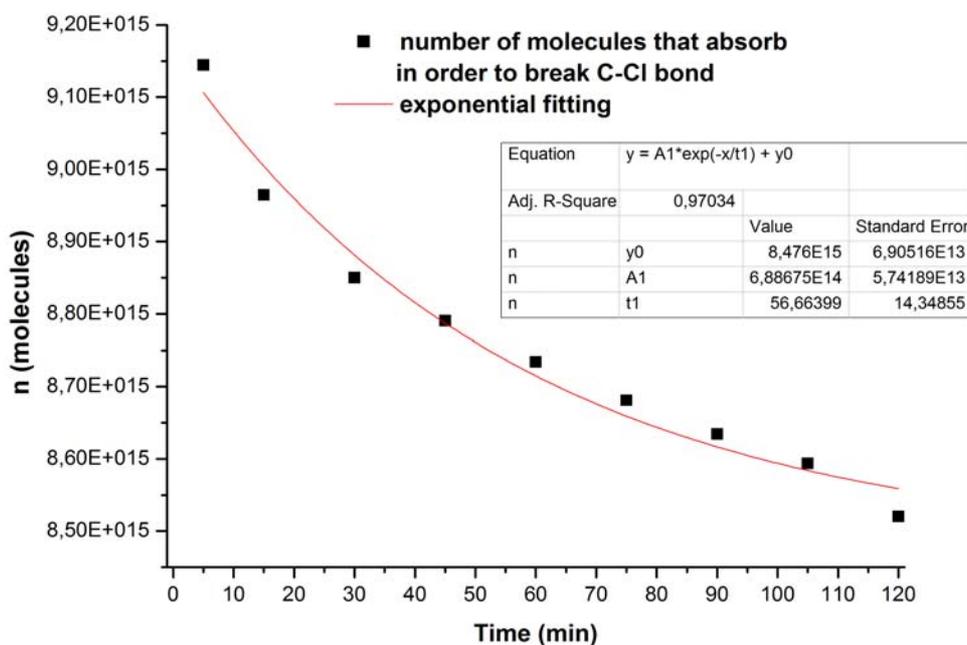


Fig. 6 – Maximum number of molecules that could absorb the energy of the laser pulse in order to dissociate the C–Cl bond during 2 hours of irradiation.

The energy of the photons was calculated according to:

$$E_{\text{photon}} = \frac{h \times c}{\lambda},$$

where  $E_{\text{photon}}$  represents the energy of one photon,  $h$  is Planck's constant ( $6.62606957 \times 10^{-34}$  J s),  $c$  is the speed of light in air ( $2.99792458 \times 10^8$  m/s) and  $\lambda$  is the wavelength of the laser radiation (266 nm).

The number of photons ( $n_{ph}$ ) absorbed by the CPZ  $10^{-4}$ M solution at different times during irradiation is given in Table 2.

The order of magnitude of the number of photons absorbed by CPZ  $10^{-4}$ M samples is in the same range as the maximum number of molecules that absorb the energy of the laser radiation in order to dissociate a bond.

Table 2

Number of photons absorbed in a CPZ  $10^{-4}$ M solution at different times of irradiation

Time (min)	$E$ (mJ)	$E_{\text{photon}}$ (mJ)	$n_{ph}$ (photons)
5	5.846	$0.7472 \times 10^{-15}$	$7.8238 \times 10^{15}$
15	5.731	$0.7472 \times 10^{-15}$	$7.6699 \times 10^{15}$
30	5.658	$0.7472 \times 10^{-15}$	$7.5722 \times 10^{15}$

Table 2 (continued)

45	5.62	$0.7472 \times 10^{-15}$	$7.5214 \times 10^{15}$
60	5.584	$0.7472 \times 10^{-15}$	$7.4732 \times 10^{15}$
75	5.55	$0.7472 \times 10^{-15}$	$7.4277 \times 10^{15}$
90	5.52	$0.7472 \times 10^{-15}$	$7.3875 \times 10^{15}$
105	5.494	$0.7472 \times 10^{-15}$	$7.3527 \times 10^{15}$
120	5.447	$0.7472 \times 10^{-15}$	$7.2898 \times 10^{15}$

This would be a good explanation for the gradual decrease of the CPZ molecule number and the gradual production of the new species in irradiated solutions, if one considers that the probability that a CPZ molecule to absorb laser photons and further to dissociate is very far from unit.

Modifications during exposure to laser radiation occur in the PZ  $10^{-4}$ M solutions and the same behavior was observed for the CPZ molecules, as well. The energy absorbed by the molecules present in the solution during 2 hours of irradiation decreased in exponential manner, as well. Regarding the fact that the only difference between the CPZ and PZ molecule is a chlorine atom, implicit the lack of a C–Cl bond, the same calculations could be applied for PZ molecules considering the same values for the bond dissociation energies and the values recorded for the energy absorbed in the PZ solution during irradiation.

#### 4. CONCLUSIONS

Phenothiazine derivatives CPZ and PZ solutions in ultrapure water are modified during exposure to 266nm laser radiation and the UV-Vis absorption spectra of the solutions are an indicator of these changes.

In this paper some semi-quantitative theoretical considerations were developed in order to calculate the maximum amount of molecules that absorb the laser pulse energy for these modifications to occur. The maximum amount of CPZ molecules that could absorb the energy for each type of bond in the CPZ molecule (C–Cl, C–H, C–C, C–N and, C–S) to break was calculated. The number of photons absorbed in CPZ  $10^{-4}$ M solutions in ultrapure water was calculated at different times of irradiation.

The results show that at the utilized energies of the laser beam and at the CPZ and PZ working concentrations, one may describe quite exactly the evolution (decreasing) of the number of phenothiazine molecules which are modified by exposure to laser radiation. This is in agreement with literature reports showing these numbers evolution by TLC, HPLC, etc methods.

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