European Journal of Pharmaceutical Sciences 81 (2016) 27–35

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Chlorpromazine transformation by exposure to ultraviolet laser beams in droplet and bulk

Ionut Relu Andrei [a,1], Tatiana Tozar [a,b,1], Andra Dinache [a,1], Mihai Boni [a,b,1], Viorel Nastasa [a,b,1], Mihail Lucian Pascu [a,b,*,1]

[a] *National Institute for Lasers, Plasma and Radiation Physics, PO Box MG 36, str. Atomistilor 409, 077125 Magurele, Ilfov, Romania*
[b] *Faculty of Physics, University of Bucharest, PO Box MG 11, str. Atomistilor 405, 077125 Magurele, Ilfov, Romania*

ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form 21 September 2015
Accepted 28 September 2015
Available online 8 October 2015

Chemical compounds studied in the article:
Chlorpromazine
Thioridazine
Promazine
Promazine sulfoxide
2-Hydroxy Promazine
2-Hydroxy Promazine sulfoxide
Chlorpromazine sulfoxide
Water
Chlorpromazine Hydrochloride

Keywords:
CPZ
Microdroplet
Laser irradiation
Photoproducts
CPZ-SO
PZ

ABSTRACT

Multiple drug resistance requires a flexible approach to find medicines able to overcome it. One method could be the exposure of existing medicines to ultraviolet laser beams to generate photoproducts that are efficient against bacteria and/or malignant tumors. This can be done in droplets or bulk volumes. In the present work are reported results about the interaction of 266 nm and 355 nm pulsed laser radiation with microdroplets and bulk containing solutions of 10 mg/ml Chlorpromazine Hydrochloride (CPZ) in ultrapure water. The irradiation effects on CPZ solution at larger time intervals (more than 30 min) are similar in terms of generated photoproducts if the two ultraviolet wavelengths are utilized. The understanding of the CPZ parent compound transformation may be better evidenced, as shown in this paper, if studies at shorter than 30 minute exposure times are made coupled with properly chosen volumes to irradiate. We show that at exposure to a 355 nm laser beam faster molecular modifications of CPZ in ultrapure water solution are produced than at irradiation with 266 nm, for both microdroplet and bulk volume samples. These effects are evidenced by thin layer chromatography technique and laser induced fluorescence measurements.

1. Introduction

The multiple drug resistance (MDR) developed by some bacteria may be overpassed using solutions of medicines modified by exposure to laser radiation (Armada et al., 2013; Pascu et al., 2011). This is particularly applicable for substances which are not antibiotics but may generate photoproducts that might have antibiotic properties resulting from exposure to laser beams. The generation of photoproducts may be obtained by resonant interaction of a laser radiation with medicine molecules i.e., *via* the absorption of the beam by the parent molecules (Alexandru et al., 2013; Dinache et al., 2013).

A particular class of medicines for these studies is constituted by phenothiazines which are used mainly as neuroleptics (Chlorpromazine — CPZ and Thioridazine — TZ, are some of them) but which may work as agents to treat malaria or tuberculosis (mainly TZ) as well (Pascu et al., 2013; Amaral et al., 2010). Recent studies performed on larger volumes (bulk — 1.5 ml sample volume) of CPZ solutions in ultrapure water at several concentrations have shown that hundreds of photoreaction products are obtained by exposure to ultraviolet (UV) laser radiation, namely 266 nm, 337.1 nm or 355 nm and that the application of solutions containing mixtures of the obtained photoproducts on Gram-positive bacteria evidences a higher antibacterial effect of the mixture than that of the CPZ parent compound (Pascu et al., 2013, 2011; Alexandru et al., 2013). Measurements of absorption and laser induced fluorescence spectra of un-irradiated and irradiated water

---

* Corresponding author at: National Institute for Lasers, Plasma and Radiation Physics, PO Box MG 36, str. Atomistilor 409, 077125 Magurele, Ilfov, Romania.

E-mail addresses: ionut.andrei@inflpr.ro (I.R. Andrei), tatiana.alexandru@inflpr.ro (T. Tozar), andra.dinache@inflpr.ro (A. Dinache), mihai.boni@inflpr.ro (M. Boni), viorel.nastasa@inflpr.ro (V. Nastasa), mihai.pascu@inflpr.ro (M.L. Pascu).

[1] Present/permanent address for all authors: National Institute for Laser, Plasma and Radiation Physics, PO Box MG 36, str. Atomistilor 409, 077125 Magurele, Ilfov, Romania.

http://dx.doi.org/10.1016/j.ejps.2015.09.017
0928-0987/© 2015 Elsevier B.V. All rights reserved.

solutions of compounds such as 4,6-bis(2-N,N-dimethylaminoethylthio)-10-methylpyrido[3,2-g]quinoline) [called BG1120] designed and prepared by the Faculty of Medicine from Marseille, CPZ and TZ were reported by Nastasa et al., 2011, showing that there are differences between the absorption and fluorescence spectra of the same medicine solution before and after exposure to laser radiation and concluding that modifications of CPZ and TZ molecular structures and symmetries are produced by their exposure to UV laser radiation. This makes irradiated solutions that contain modified parent CPZ, efficient against the *Staphylococcus aureus* ATCC25923 reference strain on which unirradiated CPZ is not active.

Out of the hundreds of photoreaction products, 5 were identified, namely: Promazine (PZ), Promazine sulfoxide (PZ-SO), 2-Hydroxy Promazine (PZ-OH), 2-Hydroxy Promazine sulfoxide (PZ-OH-SO) and Chlorpromazine sulfoxide (CPZ-SO) (Alexandru et al., 2014, 2013; Pascu et al., 2013, 2011).

In this paper results concerning the interaction of 266 nm and 355 nm pulsed laser radiation with solutions of 10 mg/ml Chlorpromazine Hydrochloride (CPZ) in ultrapure water exposed in microdroplets and bulk form are shown. In both, droplet and bulk, laser induced fluorescence measurements and thin layer chromatography analysis have shown that the parent compound transformation starts in a time interval that is shorter for exposure at 355 nm with respect to 266 nm at the same laser beam fluences. The modifications are similar in bulk and droplet at the same exposure dose, with higher rates at 355 nm. Irradiation, regardless the wavelength, generates compounds that coexist in solution and have higher polarity than the parent compound. Photoproducts less polar than unirradiated CPZ have been observed only at exposure to 355 nm. Results obtained on CPZ samples (droplet and bulk) at 10 mg/ml were compared with those obtained for two commercially available pure substances which were identified as photoproducts generated in exposed CPZ: Promazine (PZ) and Chlorpromazine sulfoxide (CPZ-SO).

The results reported in this paper are complementary with respect to previously presented and interpreted data since the article architecture is based on the detailed graphic interpretation of the TLC data obtained on CPZ water solutions, un-exposed and exposed to laser radiation. At the same time, systematic LIF data on droplet samples are shown only for CPZ water solutions. A particular emphasis is placed on the way in which TLC plates may be interpreted to better identify the photoproducts obtained by exposure of the CPZ solutions to UV laser radiation (at 266 nm, only).

2. Materials and methods

CPZ (Sigma-Aldrich, DE, purity 98%) was dissolved in ultrapure water (MilliQ, Milli-pore) at 10 mg/ml, the solution being kept protected from environmental light.

The methods used to analyze photoproduct formation in droplet and bulk samples were laser-induced fluorescence (LIF) and thin layer chromatography (TLC). LIF allows monitoring the decomposition of a CPZ solution irradiated in pendant droplet or bulk (bulk means in this case liquid volumes of standard spectrophotometric cells, i.e., 1–2 ml) in real time by showing the modifications of the emitted LIF spectra. These were recorded using an Ocean Optics HR4000 spectrometer (optical resolution 0.64 nm) and a Princeton Instruments (Acton SpectraPro 2750) spectrograph with an optical resolution of 0.08 nm.

The Ocean Optics HR4000 spectrometer was used to measure LIF emitted by CPZ solutions and the Acton SpectraPro 2750 spectrograph which was synchronized with an Nd:YAG laser's pulse generator was utilized to measure the LIF emitted by the PZ and CPZ-SO.

In general a recorded LIF spectrum is an average of the same spectrum measured for a number of pumping laser pulses (for 10 s). In the experiments the LIF spectrum acquisition conditions were chosen function of the effects aimed to be outlined.

The TLC is a well known technique described in papers such as Geiss (1987), Lewis and Moody (1989), Sherma and Fried (1991) and Spangenberg et al. (2011) and presented in detail for applications of the same kind as those reported in this paper in Pascu et al. (2013). It allowed to directly compare photoreaction compounds produced by laser irradiation at each exposure wavelength and time of irradiation. The mobile phase solvent system consisted of acetone–methanol–ammonia (150:50:5, v/v/v). For TLC normal phase was used a silica plate (DC-Alufolien Kieselgel 60F254; Merck, Darmstadt, DE). One microliter aliquots were taken from the irradiated samples (droplet or bulk) and applied to normal phase. Using CP ATLAS 2.0 freeware TLC analysis software, each TLC image was transformed into a graph, representing the intensity of the local dots (each dot represents one photoproduct) as a function of position (measured in pixels) along a column on the TLC plate. The direction is usually chosen to evidence the decomposition of one drop of solution containing the constituent photoproducts with different polarities. In this way, one may evaluate, even approximately, how many photoproducts are generated by exposure of the droplet/bulk solution to laser radiation. If CPZ monitoring is aimed, the dots analysis is made along the CPZ line and not along a liquid phase column.

The interaction of a laser beam with the target may be unresonant when the laser beam is not absorbed by the target (which may be droplet or bulky liquid) materials (Pascu et al., 2012) and/or resonant when it is absorbed and produces modifications of the medicine molecules (Pascu et al., 2010). In this paper the resonant interactions of UV laser beams with CPZ solutions in ultrapure water are studied keeping the laser beams energies at levels which do not produce significant unresonant interaction effects (Pascu et al., 2012) or bleaching.

In droplet experiments, TLC measurements were performed using single, independent droplets for each irradiation time interval. For each LIF measurement a single droplet was used/irradiated all along the exposure time.

In Fig. 1 is shown the experimental set-up. The laser beams were provided by a pulsed Nd:YAG laser (Continuum, Surelite II) which emit the third (355 nm) and fourth (266 nm) harmonics of the fundamental beam (1.064 μm). The laser emitted in pulsed regime 6 ns full time width at half-maximum pulses at 10 pps repetition rate. Laser beam energy used for irradiation was 3 mJ in the droplet measurement case, and 7 mJ for the bulk. Laser beams were processed so that on samples they were not focused but quasi-parallel. This was made with the lens L having the focal length 150 mm, whereas the cross-section of the laser beam incident on it was 5.6 mm.

In droplet experiments (set-up variant (a) in Fig. 1), the irradiation energy was 3 mJ in order to obtain fast resonant effects and at the same time to avoid mechanical effects specific to unresonant interaction (Pascu et al., 2012). The droplet was positioned at 220 mm from the lens L so that the beam fully covered the droplet cross-section. The beam waist had around 2.5 mm diameter in the interaction plane with the droplet, i.e., close to the droplet's diameter (considering it spherical) that is around 2.67 mm at 10 μl volume.

Bulk experiments (set-up variant (b) in Fig. 1, replacing the droplet from position (a)) were performed using the same experimental set-up. The 10 mg/ml CPZ solution was poured in a standard spectrophotometer cell (45 × 12.5 × 12.5 mm) that was positioned at 425 mm from L. The laser beam waist was at the input on the spectrophotometer cell around 11 mm. During the bulk measurements samples of 1 ml CPZ solution were used which were continuously stirred using a magnetic bar at 700 rpm to permanently homogenize the solution. The irradiation energy was 7 mJ in agreement with previous experiments (Alexandru et al., 2014, 2013; Armada et al., 2013; Pascu et al., 2013) performed on 10 and 20 mg/ml CPZ in ultrapure water.

The irradiation time intervals were chosen so that irradiation doses were the same for droplet and bulk measurements at the irradiation geometries described above. The irradiation dose was calculated considering the average laser power incident on exposed area (average power density) during the irradiation time. Irradiation area was 0.056 cm^2 in droplet case (circular area described by the droplet radius) and about 1 cm^2 in bulk case. So, the average power density calculated for the