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PHYSICS

TIME STABILITY OF LASER EXPOSED PHENOTHIAZINES AQUEOUS SOLUTIONS IN VIEW OF ANTIMICROBIAL RESEARCH

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Abstract. Time-stability studies of two non-antibiotics, chlorpromazine and thioridazine, which possess antimicrobial activity after their exposure to UV pulsed laser beams, are reported. The role of stability testing is to provide information about how the quality of such a drug varies over time, in view of further applications. The dynamics of identified peaks in absorption spectra shows an absorption dependence of the new formed photoproducts with respect to irradiation time. Both chlorpromazine and thioridazine irradiated solutions stabilize after 24 h and 48 h, respectively. The solutions are stable for different periods function of exposure time. Thus, the conclusions show that the samples are stable and can be used in antimicrobial susceptibility assays along one to four weeks after irradiation, depending of irradiation time intervals.

Key words: Chlorpromazine, Thioridazine, Time stability, Laser exposure, Absorption spectroscopy.

1. INTRODUCTION

Time stability is an important property regarding the quality of pharmaceutical products and therefore testing it has an important role in the development of new drugs. The role of stability testing is to provide information about how the quality of a drug varies in time under the influence of environmental factors such as temperature, humidity, or light exposure. Another goal is to set optimal test periods related to storage conditions for drugs [1, 2]. UV-Vis-NIR spectroscopy is frequently used to assess the identity, purity, and concentration of pharmaceuticals, but it can also be applied in stability assessments [3–7].

Large efforts in drug development are directed to efficient ways to combat multidrug resistance in bacterial pathogens. Related to this, phenothiazine derivatives have demonstrated enhanced antimicrobial activity and less toxicity when exposed to UV radiation [8–10]. As for the cytotoxic effects of irradiated solutions, it was demonstrated that their toxicity is lower than in the case of parent compounds when tested on Swiss albino murine fibroblasts cell lines [11, 12]. Recent reports have shown that phenothiazine, hydantoin derivatives, and antibiotics when exposed to pulsed UV laser radiation generate products with different molecular structures and spectral properties, when compared to the respective parent compounds [8, 10, 13–19]. Also, the use of Nd:YAG laser in combination with existing drugs proved to be efficient in treating induced pseudo-tumours in rabbit eye [20, 21], in the treatment of micro-veins [22–25], or even in photodynamic therapy [26]. In short, UV-Vis-NIR absorption spectroscopy has been demonstrated to be an appropriate method to investigate time-stability of the phenothiazine derivative solutions [12, 13, 27].

2. MATERIALS AND METHOD

The investigated solutions were chlorpromazine (CPZ) and thioridazine (TZ), both purchased from Sigma Aldrich. The optimized geometry of CPZ structure is depicted in Ref. [10] and the chemical structure of TZ is presented in Refs. [13, 28]. A volume of 2 mL containing 2 mg/mL CPZ or TZ was irradiated with the fourth harmonic of a pulsed Nd:YAG laser (266 nm). The solvent was ultrapure water and the laser beam average energy was of 6.5 mJ per pulse. The samples were exposed to pulsed UV laser radiation from 1 min to 240 min. The irradiation protocol and the set-up are shown in detail in Ref. [10].

The absorption spectra were recorded between 280 nm and 1,200 nm with a Perkin Elmer spectrophotometer, model Lambda 950, having a standard error of ±0.004%. The standard error, calculated with respect to user manipulation of samples was ±2.174% [18]. The spectrophotometric cell was 1 mm thick. Time-stability studies were performed by recording the UV-Vis-NIR absorption spectra immediately (0 h), at 24 h, 48 h, one week, two weeks, three weeks, and four weeks after the irradiation process was completed. CPZ and TZ samples were stored in dark at 4 °C.

3. RESULTS

Prior to using irradiated phenothiazines on cell cultures, time-stability studies were performed in order to identify the timeframe in which, after irradiation, solutions remain stable and can be used in susceptibility assays. The antimicrobial, antibiofilm, and antifungal activities of unirradiated and irradiated CPZ or TZ are presented in Ref. [29] and show that irradiated CPZ and TZ could be considered an alternative to some of the current, generally accepted, treatments of bacterial resistance with antibiotics.

3.1. CHLORPROMAZINE

During 240 min irradiation of 2 mg/mL CPZ solution with 266 nm laser beam, a gradual change in color was observed. After the first 5 min irradiation, the color changed from initial clear solution to a light-yellow one and after 240 min of irradiation, the solution presented a dark brown color. The changes in color of the irradiated samples are shown in Fig. 1.

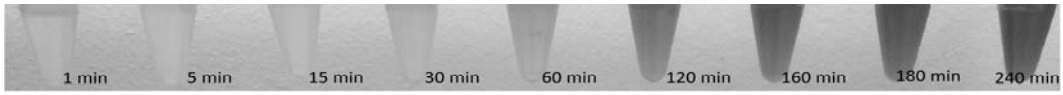

Fig. 1 – Gradual change in color of 2 mg/mL CPZ solution in ultrapure water exposed to 266 nm laser beams from 1 min to 240 min.

The absorption spectra of 2 mg/mL unirradiated and irradiated CPZ solutions recorded immediately and 24 h after irradiation are shown in Fig. 2. The 306 nm peak shows bathochromic and hyperchromic shifts during 240 min irradiation (Fig. 2A_1). In the 400–600 nm spectral range (Fig. 2B_1) two peaks (503 nm and 540 nm) were present corresponding to CPZ and promazine oxidized forms [30].

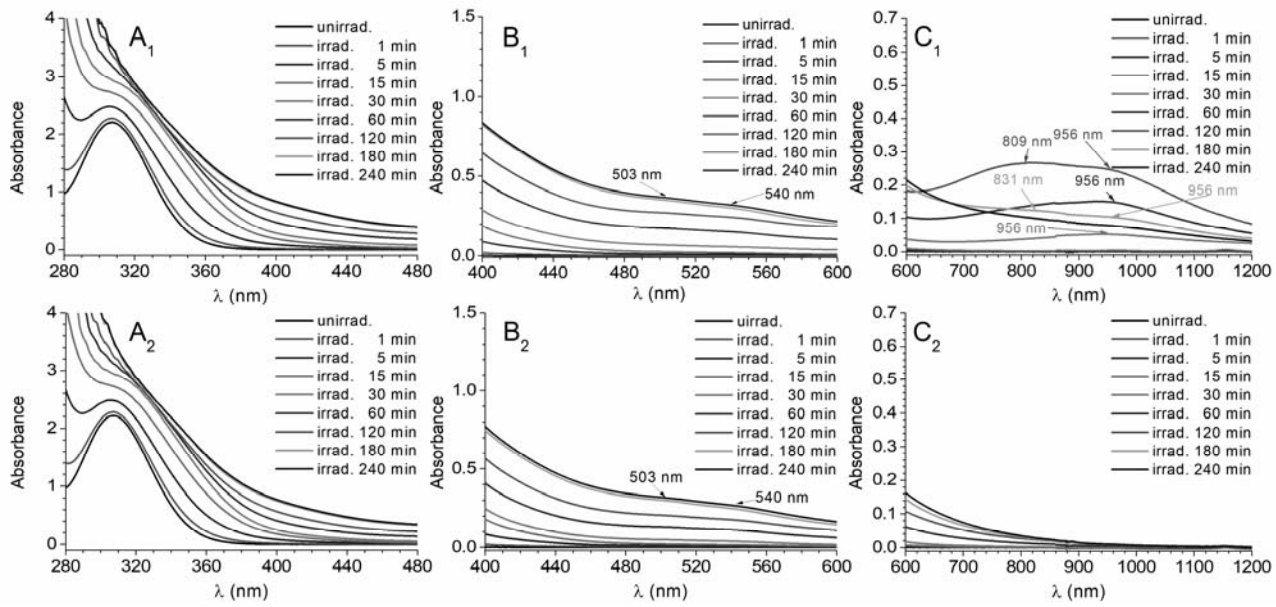

Fig. 2 – Absorption spectra of 2 mg/mL unirradiated CPZ and irradiated CPZ solutions, in ultrapure water, between: 280-480 nm, recorded after irradiation at 0 h (A_1), 24 h (A_2); 400-600 nm, recorded after irradiation at 0 h (B_1), 24 h (B_2); 600-1,200 nm, recorded after irradiation at 0 h (C_1), 24 h (C_2).

For 30 min exposed CPZ, immediately after irradiation a wide band appeared centered on 956 nm (Fig. $2C_1$). After 60 min irradiation, another band at 831 nm showed-up accompanied by a peak at 956 nm. The maximum absorbance intensity for the two peaks was obtained for 120 min irradiated CPZ. After 240 min exposure to 266 nm laser beam, the bands disappeared. Absorption spectra recorded after 24 h showed no changes in 280–600 nm spectral range (Fig. $2A_2$ and Fig. $2B_2$), however between 600 nm and 1,200 nm peaks at 831 nm and 956 nm vanished (Fig. $2C_2$).

These modifications occurred due to generation of a variety of free radicals during irradiation of CPZ, including the corresponding cation radical (*via* photoionization), chlorine atom, neutral promazinyl radical, hydroxyl radical and sulfur centered peroxy radical [10, 31–34].

To establish the time-stability of unirradiated and irradiated CPZ samples, the absorption spectra were analyzed during four weeks.

In Fig. 3 are shown absorption spectra of unirradiated and irradiated CPZ solutions for 1 min, 5 min and 15 min, respectively. For unirradiated CPZ and 1 min irradiated CPZ there were no changes in the spectra at wavelengths higher than 480 nm. Unirradiated CPZ (Fig. 3A) was stable four weeks, while 1 min (Fig. 3B) and 5 min (Fig. 3C) irradiated CPZ were stable two weeks, the absorbance intensity remaining within error limits.

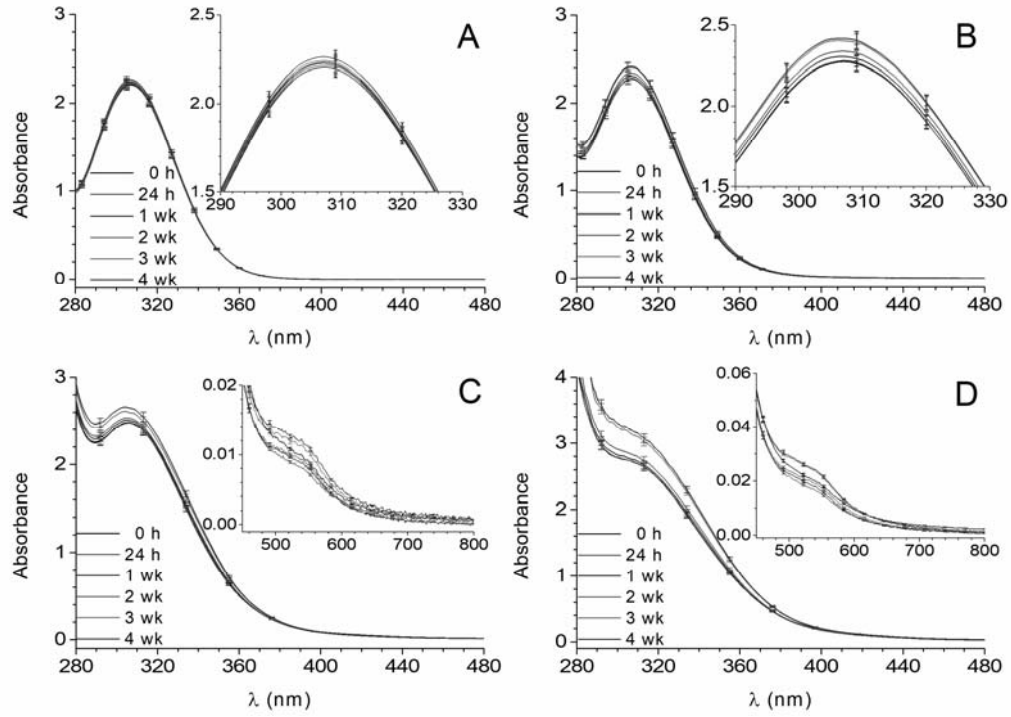

Fig. 3 – Absorption spectra, recorded during four weeks, of 2 mg/mL CPZ solution, in ultrapure water, irradiated: A) 0 min (unirradiated); B) 1 min; C) 5 min; D) 15 min.

For 15 min irradiated CPZ (Fig. 3D), the time stability was determined to be also two weeks, whereas in the 280-480 nm range after two weeks the band intensity increased, exceeding the error limits. The sample analyzed after three weeks showed an increase of absorbance of 15%, and a 17% increase for the solution tested after four weeks was measured.

Figure 4 presents the absorption spectra of 30 min to 240 min irradiated CPZ. The 30 min irradiated CPZ (Fig. 4A) was stable two weeks. For the sample analyzed after three weeks, the absorbance increases by 11.6% and after four weeks by 13.1%. The 60 min irradiated CPZ (Fig. 4B) stabilized after 24 h and remained stable during two weeks.

The absorption spectra recorded for CPZ irradiated 120 min (Fig. 4C) showed that the peaks at 809 nm and 956 nm vanished after 24 h and the solution remained stable two weeks. Both 180 min (Fig. 4D) and 240 min (Fig. 4E) irradiated CPZ were stable two weeks.

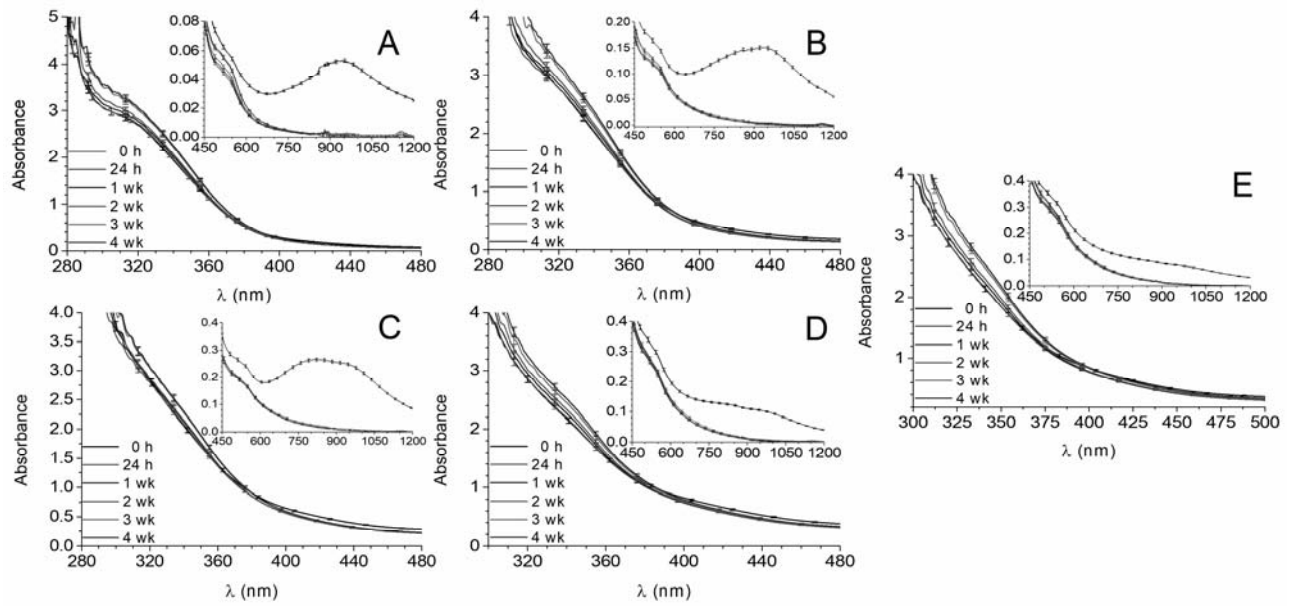

Fig. 4 – Absorption spectra, recorded during four weeks, of 2 mg/mL CPZ solution, in ultrapure water, irradiated: A) 30 min, B) 60 min, C) 120 min, D) 180 min, E) 240 min.

3.2. THIORIDAZINE

The 2 mg/mL TZ in ultrapure water was exposed to 266 nm laser beam and it was observed (Fig. 5) that after the first minute it changed color from a clear solution (unirradiated TZ) to a light turquoise (15 min irradiated TZ). After 60 min irradiation, the color of the sample changed significantly, from turquoise to light yellow. When the exposure time was increased, a gradual change in color was observed, ranging from a light yellow (60 min irradiated TZ) to a light brown (240 min irradiated TZ).

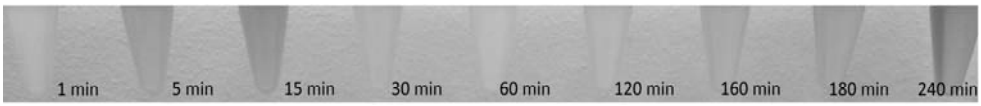

Fig. 5 – Gradual change in color of 2 mg/mL TZ solution in ultrapure water exposed to 266 nm laser beams from 1 min to 240 min.

The absorption spectra of unirradiated and irradiated TZ between 1 min and 240 min recorded immediately, at 24 h, and at 48 h after irradiation, respectively, are shown in Fig. 6.

The band with peak at 315 nm suffered both a hypsochromic and a hypochromic shift during 240 min irradiation (Fig. $6A_1$). After 1 min exposure, two peaks at 454 nm and 486 nm appeared and after 30 min irradiation the two bands vanished (Fig. $6B_1$). Thus, one may conclude that photoproducts formed in the first 15 min of irradiation were responsible for 454 nm and 486 nm absorption bands and the turquoise color of the solutions. Besides the above-mentioned spectroscopic modifications, other two bands appeared: a narrow one, with a peak at 637 nm, and a wide one at 882 nm, both being visible after the first minute of irradiation and having a maximum absorption intensity for 5 min irradiated TZ solution. Disappearance of bands after 180 min of TZ exposure to 266 nm was also observed (Fig. $6B_1$). The same two bands were obtained for TZ irradiated with 355 nm, where highest absorption intensities were observed for 5 min irradiated samples [27].

The spectra measured 24 h after irradiation process ended, showed in the 280–480 nm spectral range (Fig. $6A_2$) shifts to longer wavelengths, as well as between 400 nm and 1,100 nm the disappearance of 882 nm peak (Fig. $6B_2$). Still, the peak at 637 nm can be observed in the absorption spectra only for 1 min and 5 min irradiated TZ. After 48 h (Fig. $6A_3$), no spectral changes in 280–480 nm spectral range were observed when compared to 24 h spectrum. In the 400–1,100 nm range, the above-mentioned bands vanished (Fig. $6B_3$). These phenomena may be due to the free radicals found in TZ solution exposed to UV radiation [35].

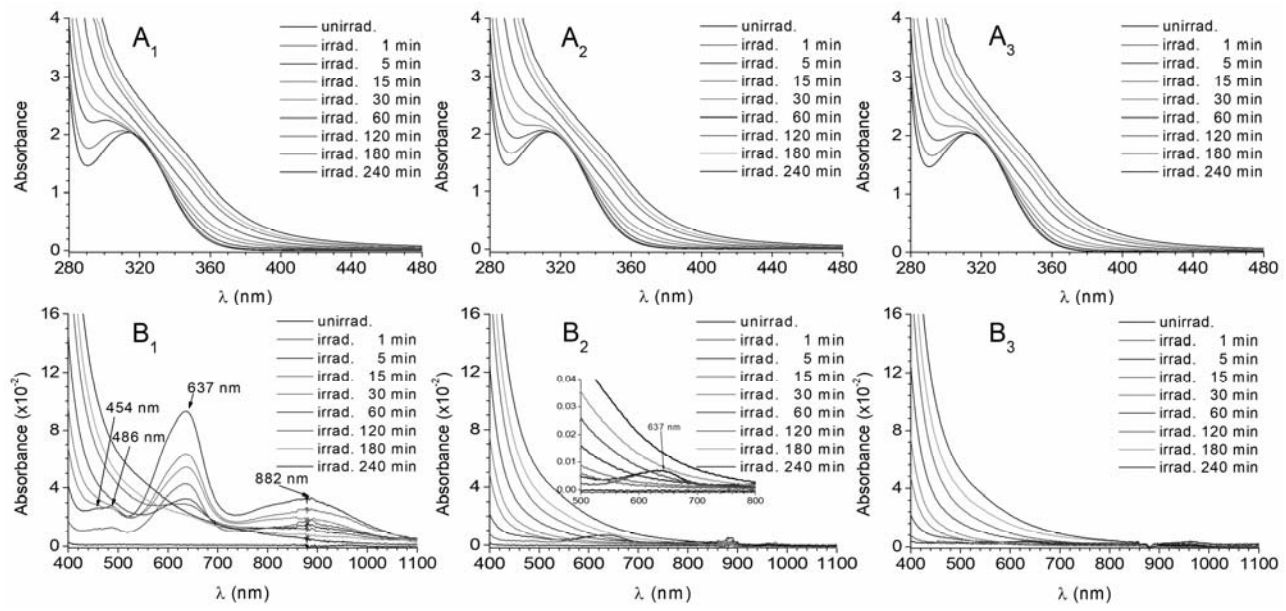

Fig. 6 – Absorption spectra of 2 mg/mL unirradiated TZ and irradiated TZ solutions in ultrapure water between: 280–480 nm, recorded after irradiation at 0 h (A_1), 24 h (A_2), 48 h (A_3); 400–1,100 nm, recorded after irradiation at 0 h (B_1), 24 h (B_2), 48 h (B_3).

To evaluate the time-stability of unirradiated and irradiated TZ samples over four weeks, the absorption spectra were analyzed as in the CPZ case.

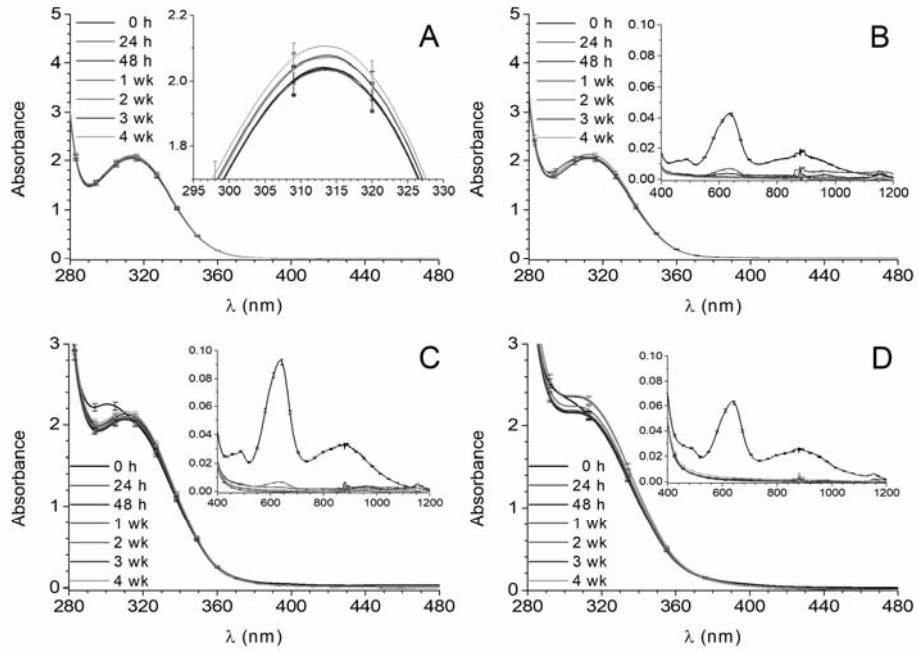

Fig. 7 – Absorption spectra, recorded during four weeks, of 2 mg/mL TZ solution, in ultrapure water, irradiated: A) 0 min (unirradiated); B) 1 min; C) 5 min; D) 15 min.

Thus, Fig. 7 shows the stability study of unirradiated TZ solution and 1 min, 5 min, and 15 min irradiated TZ. For unirradiated TZ (Fig. 7A) the analysis showed that the sample was stable for up to three weeks. The 1 min irradiated TZ was stable for two weeks (Fig. 7B). TZ irradiated 5 min (Fig. 7C) recorded during four weeks was stable for only three weeks. For 15 min irradiated TZ (Fig. 7D), absorption spectra recorded immediately after exposure showed a peak at 300 nm, which was shifted to 313 nm after 24 h, this position remaining unchanged along the four weeks of testing. The irradiated solution stabilized after 24 h, when the bands in 400–1,100 nm spectral range disappeared, and remained stable two weeks. The absorption

spectrum, measured after three weeks, presented an absorbance intensity increasing by 9% and was no longer within the limits of experimental errors.

Figure 8 presents the absorption spectra of 30 min to 240 min irradiated TZ. The absorption spectrum of the irradiated TZ for 30 min stabilized after 24 h and the sample remained stable for two weeks (Fig. 8A).

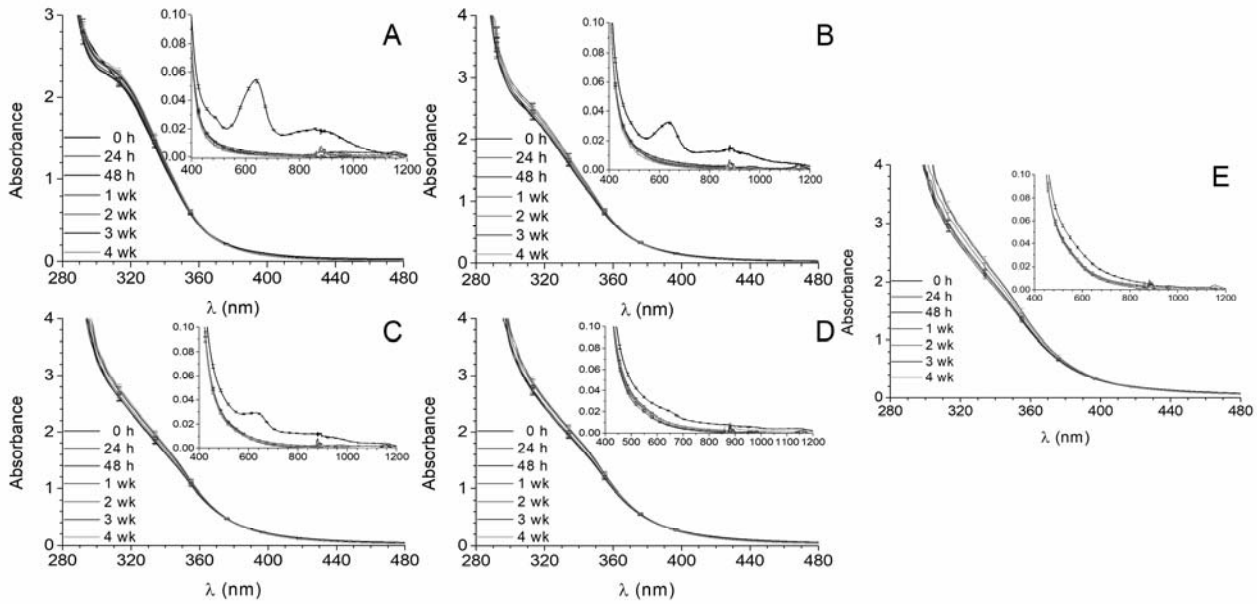

Fig. 8 – Absorption spectra, recorded during four weeks, of 2 mg/mL TZ solution, in ultrapure water, irradiated: A) 30 min; B) 60 min; C) 120 min; D) 180 min; E) 240 min.

In the case of 60 min irradiated TZ (Fig. 8B), the bands with peaks at 637 nm and 882 nm vanished after 24 h. Thus, the sample stabilized and remained stable two weeks. Absorption spectra of TZ irradiated 120 min (Fig. 8C) showed that the 637 nm and 882 nm peaks vanished 24 h after irradiation ended; the solution remained stable during two weeks. Both, TZ irradiated 180 min (Fig. 8D) and 240 min (Fig. 8E) stabilized after 24 h and remained stable during one week.

4. CONCLUSIONS

Based on time-stability studies, unirradiated CPZ solution can be stored in dark and at 4 °C during four weeks without changing its properties. CPZ samples irradiated between 1 min and 240 min stabilized after 24 h and remained stable only two weeks after the irradiation process ended. In addition, during the irradiation, a change of color was observed after the first 5 min showing that the samples display light-yellow color. By the end of the 240 min irradiation of CPZ a gradual change to dark brown occurred.

The time-stability study for the unirradiated TZ solution suggests that the sample was stable three weeks. TZ solution irradiated 1 min and 5 min stabilized after 48 h and remains stable two weeks; solutions irradiated between 15 min and 120 min stabilize after 24 h and remain stable two weeks. The samples exposed to 180 min and 240 min are stable between 24 h and one week.

The dynamics of the peaks identified in the absorption spectra shows an absorption dependence on irradiation time and all changes in spectra compared with those of unirradiated samples suggest the photo-degradation of the parent compound, which leads to the formation of new photoproducts.

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REFERENCES

1. K. HUYNH-BA, *Handbook of stability testing in pharmaceutical development: regulations, methodologies and best practices*, Springer, New York, 2009.
2. J.P. HAMMOND, *The use of spectrophotometry in the pharmaceutical industry*, in: *Experimental Methods in the Physical Sciences*, Elsevier, pp. 409–456, 2014.
3. A. RIEDEL, C.S. LEOPOLD, *Quantification of omeprazole degradation by enteric coating polymers: an UV-VIS spectroscopy study*, Pharmazie, **60**, 2, pp. 126–130, 2005.
4. D. CASTRO, M.A. MORENO, S. TORRADO, J.L. LASTRES, *Comparison of derivative spectrophotometric and liquid chromatographic methods for the determination of omeprazole in aqueous solutions during stability studies*, J. Pharm. Biomed. Anal., **21**, 2, pp. 291–298, 1999.
5. N.M. EL-KOUSY, L.I. BEBAWY, *Stability-indicating methods for determining omeprazole and octylonium bromide in the presence of their degradation products*, J. AOAC Int., **82**, 3, pp. 599–606, 1999.
6. A. MILITARU, A. SMARANDACHE, A. MAHAMOUD, S. ALIBERT, J.-M. PAGES, M.-L. PASCU, *Time stability studies of quinazoline derivative designed to fight drug resistance acquired by bacteria*, Lett. Drug Des. Discov., **8**, 2, pp. 124–129, 2011.
7. A. SMARANDACHE, A. SIMON, T. TOZAR, V. NASTASA, M.L. PASCU, *Stability studies on Promethazine unexposed and exposed to UV laser radiation*, Proceedings SPIE, 954916, 2015.
8. M.L. PASCU, B. DANKO, A. MARTINS, N. JEDLINSZKI, T. ALEXANDRU, V. NASTASA, M. BONI, A. MILITARU, I.R. ANDREI, A. STAICU, A. HUNYADI, S. FANNING, L. AMARAL, *Exposure of chlorpromazine to 266 nm laser beam generates new species with antibacterial properties: contributions to development of a new process for drug discovery*, PLoS One., **8**, *2*, e55767, 2013.
9. T. ALEXANDRU, A. ARMADA, B. DANKO, A. HUNYADI, A. MILITARU, M. BONI, V. NASTASA, A. MARTINS, M. VIVEIROS, M.L. PASCU, J. MOLNAR, L. AMARAL, *Biological evaluation of products formed from the irradiation of chlorpromazine with a 266 nm laser beam*, Biochem. Pharmacol. Open Access, **02**, 01, 2013.
10. T. ALEXANDRU, A. STAICU, A. PASCU, E. RADU, A. STOICU, V. NASTASA, A. DINACHE, M. BONI, L. AMARAL, M.L. PASCU, *Characterization of mixtures of compounds produced in chlorpromazine aqueous solutions by ultraviolet laser irradiation: their applications in antimicrobial assays*, J. Biomed. Opt., **20**, *5*, 051002, 2014.
11. A.M. ARMADA, T. ALEXANDRU, D. MACHADO, B. DANKO, A. HUNYADI, A. DINACHE, V. NASTASA, M. BONI, J. RAMOS, M. VIVEIROS, J. MOLNAR, M.L. PASCU, L. AMARAL, *The in vitro activity of products formed from exposure of chlorpromazine to a 266nm laser beam against species of mycobacteria of human interest*, In Vivo, **27**, *5*, pp. 605–610, 2013.
12. M.C. MORÁN, T. TOZAR, A. SIMON, A. DINACHE, A. SMARANDACHE, I.R. ANDREI, M. BONI, M.L. PASCU, F. CIRISANO, M. FERRARI, *Toxicity study in blood and tumor cells of laser produced medicines for application in fabrics*, Colloid Surf. B-Biointerfaces, **137**, pp. 91–103, 2016.
13. M.L. PASCU, V. NASTASA, A. SMARANDACHE, A. MILITARU, A. MARTINS, M. VIVEIROS, M. BONI, I.R. ANDREI, A. PASCU, A. STAICU, J. MOLNAR, S. FANNING, L. AMARAL, *Direct modification of bioactive phenothiazines by exposure to laser radiation*, Recent Patents Anti-Infect. Drug Disc., **6**, *2*, pp. 147–157, 2011.
14. A. SMARANDACHE, V. NASTASA, M. BONI, A. STAICU, J. HANDZLIK, K. KIEC-KONONOWICZ, L. AMARAL, M.L. PASCU, *Laser beam resonant interaction of new hydantoin derivatives droplets for possible biomedical applications*, Colloid Surf. A-Physicochem. Eng., **505**, pp. 37–46, 2016.
15. A. SMARANDACHE, A. PASCU, I.R. ANDREI, J. HANDZLIK, K. KIEC-KONONOWICZ, A. STAICU, M.L. PASCU, *Study of the optical properties of 2-thiohydantoin derivatives*, Rom. Rep. Phys., **68**, *2*, pp. 673–683, 2016.
16. A. HUNYADI, B. DANKO, M. BONI, A. MILITARU, T. ALEXANDRU, V. NASTASA, I.R. ANDREI, M.L. PASCU, L. AMARAL, *Rapid, laser-induced conversion of 20-hydroxyecdysone and its diacetonide – experimental set-up of a system for photochemical transformation of bioactive substances*, Anticancer Res., **32**, *4*, pp. 1291–1297, 2012.
17. I.R. ANDREI, T. TOZAR, A. DINACHE, M. BONI, V. NASTASA, M.L. PASCU, *Chlorpromazine transformation by exposure to ultraviolet laser beams in droplet and bulk*, Eur. J. Pharm. Sci., **81**, pp. 27–35, 2016.
18. A. SIMON, T. ALEXANDRU, M. BONI, V. DAMIAN, A. STOICU, V. DUTSCHK, M.L. PASCU, *Interaction of solutions containing phenothiazines exposed to laser radiation with materials surfaces, in view of biomedical applications*, Int. J. Pharm., **475**, *1–2*, pp. 270–281, 2014.
19. A. DINACHE, M. BONI, T. ALEXANDRU, E. RADU, A. STOICU, I.R. ANDREI, A. STAICU, L. LIGGIERI, V. NASTASA, M.L. PASCU, M. FERRARI, *Surface properties of Vancomycin after interaction with laser beams*, Colloid Surf. A-Physicochem. Eng., **480**, pp. 328–335, 2015.
20. M.L. PASCU, M. BREZEANU, L. VOICU, A. STAICU, B. CARSTOCEA, R.A. PASCU, *5-fluorouracil as a phosensitiser*, In Vivo, **19**, *1*, pp. 215–220, 2005.
21. M.L. PASCU, B.D. CARSTOCEA, A. STAICU, M.A. IONITA, S. TRUICA, R. PASCU, *Spectroscopic studies of drugs used in the treatment of malignant tumors in ophthalmology*, Proceedings SPIE, pp. 52–60, 2001.
22. A. SMARANDACHE, *Laser beams interaction with polidocanol foam: molecular background*, Photomed. Laser Surg., **30**, *5*, pp. 262–267, 2012.
23. J. MORENO-MORAGA, E. HERNANDEZ, J. ROYO, J. ALCOLEA, M.J. ISARRIA, M.L. PASCU, A. SMARANDACHE, M. TRELLES, *Optimal and safe treatment of spider leg veins measuring less than 1.5 mm on skin type IV patients, using repeated low-fluence Nd:YAG laser pulses after polidocanol injection*, Lasers Med. Sci., **28**, *3*, pp. 925–933, 2013.
24. V. NASTASA, K. SAMARAS, C. AMPATZIDIS, T.D. KARAPANTSIOS, M.A. TRELLES, J. MORENO-MORAGA, A. SMARANDACHE, M.L. PASCU, *Properties of polidocanol foam in view of its use in sclerotherapy*, Int. J. Pharm., **478**, *2*, pp. 588–596, 2015.
25. M. BONI, V. NASTASA, A. MILITARU, A. SMARANDACHE, I.R. ANDREI, A. STAICU, M.L. PASCU, *Laser beams interaction with liquids in optofluidic experiments*, Rom. Rep. Phys., **64**, pp. 1179–1194, 2012.

26. A. STAICU, A. PASCU, M. BONI, M.L. PASCU, M. ENESCU, *Photophysical study of Zn phthalocyanine in binary solvent mixtures*, J. Mol. Struct., **1044**, pp. 188–193, 2013.
27. M.L. PASCU, A. SMARANDACHE, M. BONI, J. KRISTIANSEN, V. NASTASA, I.R. ANDREI, *Spectral properties of some molecular solutions*, Rom. Rep. Phys., **63**, pp. 1267–1284, 2011.
28. A. SMARANDACHE, J. KRISTIANSEN, J.B. CHRISTENSEN, M.L. PASCU, *Optical studies of the spectral properties of phenothiazines*, Lett. Drug Des. Discov., **9**, *4*, pp. 352–360, 2012.
29. T. TOZAR, A. STOICU, V. NASTASA, M. POPA, A. SMARANDACHE, M. COSTACHE, M.C. CHIFIRIUC, M.L. PASCU, *Application of laser modified medicines in fighting multiple drug resistance acquired by microorganisms*, in: *Laser Optofluidics in Fighting Multiple Drug Resistance*, Bentham Science, Sharjah, UAE, 2017, pp. 338–365.
30. J. KARPINSKA, B. STARCZEWSKA, H. PUZANOWSKA-TARASIEWICZ, *Analytical Properties of 2- and 10-Disubstituted Phenothiazine Derivatives*., Anal. Sci., **12**, *2*, pp. 161–170, 1996.
31. C.F. CHIGNELL, A.G. MOTTEN, G.R. BUETTNER, *Photoinduced free radicals from chlorpromazine and related phenothiazines: relationship to phenothiazine-induced photosensitization*, Environ. Health Perspect., **64**, pp. 103–110, 1985.
32. A. FELMEISTER, C.A. DISCHER, *Photodegradation of chlorpromazine hydrochloride*, J. Pharm. Sci., **53**, *7*, pp. 756–762, 1964.
33. G.R. BUETTNER, A.G. MOTTEN, R.D. HALL, C.F. CHIGNELL, *Free radical production by chlorpromazine sulfoxide, an ESR spin-trapping and flash photolysis study*, Photochem. Photobiol., **44**, *1*, pp. 5–10, 1986.
34. C. GARCIA, L. PINERO, R. OYOLA, R. ARCE, *Photodegradation of 2-chloro substituted phenothiazines in alcohols*, Photochem. Photobiol., **85**, *1*, pp. 160–170, 2009.
35. H. TAKIWAKI, K. TSUCHIYA, M. FUJITA, Y. MIYAOKA, *Thioridazine induces immediate and delayed erythema in photopatch test*, Photochem. Photobiol., **82**, *2*, 523, 2006.

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