UV Spectrophotometric Method for the Analysis of Tinidazole after Exposure to Superficial X-Rays

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Abstract

The aim of the present work is to assess UV-spectrophotometric method for the effect of superficial X-rays on the tinidazole compound in two forms, solid and aqueous solution. Both acceleration potentials of 120 kV_p and 160 kV_p exert a pronounced effect on aqueous solution of tinidazole at different concentrations represented by changes in optic density of tinidazole. The effect of superficial X-rays on the solid form of tinidazole is less than that observed with the aqueous preparation. It concluded from these that tinidazole is radiosensitive compound and several protection measurements should be used to prevent its hydrolysis.

Key words : Tinidazole, superficial X-rays, acceleration potential, optic density.

Abbreviations: O.D.-optic density, kV_p-kilo volt peak, nm-nanometer,Gy-gray, F.S.D.-focal surface distance.

Introduction

Tinidazole, chemically l[2(ethylsulfonyl)ethyl]-2methyl-5-nitroimidazole, as well as metronidazole and ornidazole, are 5-nitroimidazole drugs which are commonly used against amoebiasis, giardiasis, trichomoniasis, anaerobic bacterial infections and peptic ulcer against H. pylori in humans [1 -5].

Stability studies on nitroimidazoles are important not only for the correct preparation and storage of drug compounds and preparations, but also they provide useful information on degradation products and pathways, as well as valuable material for the detection of their radio-sensitivity [6,7] .

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Stress testing should establish the effect on stability of temperature, humidity, oxidation and photolysis; hydrolytic stability over a wide range of pH values should also be studied for substances in solution or suspension [8,9]. The photolytic disintegration of 5-nitroimidazoles is a versatile reaction [10], it involves several rearrangements of the 5-nitroimidazole structure [11-14] .

Tinidazole is known to decompose photolytically and its degradation kinetics has been found to be the first order both in solution [15] and in the solid state [16]. Some of nitroimidazoles , in particular 2-nitroimidazoles, are used as radiosensitizers for increasing sensitivity of the hypoxic cells against the radiation but their radio-stability havn't been tested frequently in literature. 'Therefore it is interesting to test the stability of tinidazole solution and solid state when it exposed to superficial X-rays at different acceleration potentials.

Materials and Methods

This work is conducted in department of Physics, college of Medicine, diyala university in cooperation with Radiation and Nuclear Medicine Hospital -Baghdad.

Tinid.izole {l-[2-(Ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole C8H13N3O4S=247.3}, pure substance in microionized powder form obtained from Dofar Pharmaceuticals (Baghdad-Iraq) was used in this work . It dissolved in 0.1N HCl and its maximum absorbance was detected at wavelength of 320 nm and 1 cm light path by Pye UNICAM sp 1750 UV-Spectrophotometer .

Experimental Protocol

Group I: included samples of an aqueous solution of non-radiated tinidazole in concentrations of (1.25, 2.5, 3.75, 5, 6.25(µg/mL)). The optic density (O. D.) at wavelength 320 nm, of 24 samples for each concentration, was recorded .

Group II included samples of an aqueous solution of irradiated tinidazole in similar concentrations of group I. The samples were exposed to superficial X-rays at acceleration potentials of 120 and 160 kVp for 30 and 60 seconds. Then the O.D. of tinidazole solution (six samples for each concentration at each acceleration potential and each exposure time) was recorded.

Group III: included samples of irradiated solid (powder form) tinidazole. Samples of solid form of tinidazole were exposed to superficial X-rays as in group II. Then, the irradiated tinidazole powder

was dissolved in 0.1 N HC1 and the same concentrations (as in group I and II) were prepared and their optic densities were recorded by UV-spectrophotometer .

Superficial X-rays

The equipment of X-rays included:

X-rays machine. The specifications of superficial X-rays machine were:

The applied acceleration potential across the tube is 120-300 kVp

The circuit current was 20 mA .

Exposure time was 50 - 87.6 second .

F.S.D. is 30 cm and field size of irradiation was (8x6)cm^2 .

The thimble ionization chamber type 31003. It is water tight, used mainly for relative measurement with a water phantom or air scanner for characterization of the radiation fields of therapy accelerators and teletherapy cobalt sources in addition to use in solid state phantoms . It is connected to computerized instrument with digital display to determine the measurements of temperature and pressure for accurate readings. The initial calibration using Sr-90, the check reading was 35.75 x 10^3 Gy.

Tissue equivalent phantom. Test-tubes containing solid form or an aqueous solution of tinidazole were embedded horizontally in a hollow metal cubic box of 15 cm length with circular opening at the upper face containing bolus grains. Tissue equivalent phantom has mass attenuation coefficient (μ/ρ) and mass energy absorption coefficient (μen/ρ) equal to that of water and tissue that is mainly consist of water . The calculated effective energies for the acceleration potential of 120 kV_p and 160 kV_p (represented the doses of 0.95 and l.95 Gy respectively) were 30.976 and 62.373 keV with mass energy attenuation coefficient for water 10^{-3} (m^2/kg) were 15.2998 and 3.1825 .

Statistical analysis

The data are presented as mean ± SD of number of experiments. The results are analyzed by using student's "t" test and simple correlation test taking $p < 0.05$ as the lowest limit of significance.

Results

Table 1 shows that, the optic density (O.D.) of tinidazole solution exposed to X-rays at 120 kV_P for 39 second is significantly increased, and a further increment is also observed when the exposure time is extended to 60 seconds. Such effect is also observed at 160 kV_p acceleration potential for 30 seconds but not for 60 seconds (table 2). These results indicate that X-rays is possibly induced structural changes in the tinidzole compound leading to form a new byproduct that is detected spectrophotometrically at 320 nm . This new byproduct is degradated when the acceleration potential of 160 kV_p is extended to 60 seconds exposure.

The opposite picture is observed when tinidazole is irradiated in solid state. Superficial X-rays at 120 kV_p for 30 and 60 seconds induced changes in the UV-spectra of tinidazole resulted in decrease of its optic density (Table 3).

When the acceleration potential is increased to 160 kV_p, the UV-spectra of tinidazole showed bizarre changes (table 4). These results give an idea that tinidazole is unstable compound when it is subjected to radiation.

The effect of radiation seems to be related to the nature of tinidazole compound i.e. whether it is in solid or aqueous state as well as to the duration of radiation exposure , the calculated optic densities of tinidazole solutions irradiated for 30 and 60 seconds at 120 kV_p were 0.044 and 0.046 (fig. 1) to the non-radiated optic density of 0.040. These numbers are further increased to 0.048 and 0.047 when the exposure time became 60 seconds (fig. 2). However, in solid state these numbers are decreased to 0.037 and 0.036 at 120 kV_p for 30 and 60 seconds exposure respectively (fig. 3), and 0.0368 and 0.037 at 160 kV_p for 30 and 60 seconds exposure respectively (fi.g.4) .

Table (1): Shows the effect of X-rays , at acceleration potential of 120 kV_p for 30 and 60 seconds, on the aqueous solution of different concentrations of tinidazole powder

Concentration (µg/mL)	Optic density (O.D.) at 320 nm		
	Non-radiated	Irradiated for 30 seconds	Irradiated for 60 seconds
1.25	0.04491±0.0074	0.057833±0.0060†	*0.0611±0.01732

2.5	0.09108±0.01111	0.130167±0.0033†	X-**0.10433±0.01129
3.75	0.13026±0.02703	0.168±0.006082†	*0.14416±0.00189
5	0.18925±0.013466	0.19233±0.00711	0.19433±0.01198
6.25	0.24704±0.02653	0.247±0.01169	0.250±0.017036

The results are expressed as mean ± SD of number of observations (n=6).

† $P<$ O.001 , ** $P<$ 0.02 , $p<$ 0.05

Table (2): Shows the effect of X-rays, at acceleration potential of 160 kV_p for 30 and 60 Seconds, on the aqueous solution of different concentrations of tinidazole powder

Concentration (µg/mL)	Optic density (O.D.) at 320 nm		
	Non-radiated	Irradiated for 30 seconds	Irradiated for 60 seconds
1.25	0.04491±0.0074	*0.0685±0.02463	0.05116±0.00029†
2.5	0.09108±0.01111	0.1470±0.03527†	**0.09766±0.00202
3.75	0.13026±0.02703	**0.181167±0.0448	*0.14334±0.00028
5	0.18925±0.013466	0.24233±0.02532†	0.19023±0.00375
6.25	0.24704±0.02653	0.262±0.02392	0.2413±0.000982

the results are expressed as mean ± SD of number of observations (n=6).

† $p < 0.001$,** $P < 0.02$,* $p < 0.05$

Table(3): shows the effect of X-rays, at acceleration potential of 120 kV_p for 30 and 60 seconds, on the tinidazole powder (solid form).

Concentration(µg/mL)	Optic density (O.D.) at 320 nm		
	Non-radiated	Irradiated for 30 seconds	Irradiated for 60 seconds
1.25	0.04491±0.0074	0.04583±0.00028	0.0478±0.00611
2.5	0.09108±0.01111	0.090±0.0005	0.0938±0.00275
3.75	0.13026±0.02703	0.141±0.0005	0.13683±0.00028
5	0.18925±0.013466	0.187±0.001732	0.1865±0.00576
6.25	0.24704±0.02653	**0.23316±0.0015	***0.2303±0.00340

The results are expressed as mean ± SD of number of observations (n=6).

*** $P < 0.01$, ** $P < 0.02$

Table (4): Shows the effect of X-rays, at acceleration potential of 160 kV_p for 30 and 60 seconds, on the tinidazole powder (solid form).

Concentration (µg/m)	Optic density (O.D.) at 320 nm		
	Non-radiated	Irradiated or 30 seconds	Irradiated for 60 seconds
1.25	0.04491 ± 0.0074	0.056 ± 0.00360†	0.04466 ± 0.00505
2.5	0.09108±0.01111	0.0945 ± 0.0005	0.0915 ± 0.003464
3.75	0.13026± 0.02703	0.148334 ± 0.000208	0.1515 ± 0.00304 13†
5.0	0.18925 ± 0.013466	0.18866 ± 0.00236	0.1865 ± 0.00476
6.25	0.24704 ± 0.02653	0..2388 ± 0.011536	0.23283 ± 0.00592**

The results are expressed as mean ± SD of number of observations (n=-6).

† $P < 0.001$

*** $P < 0.01$

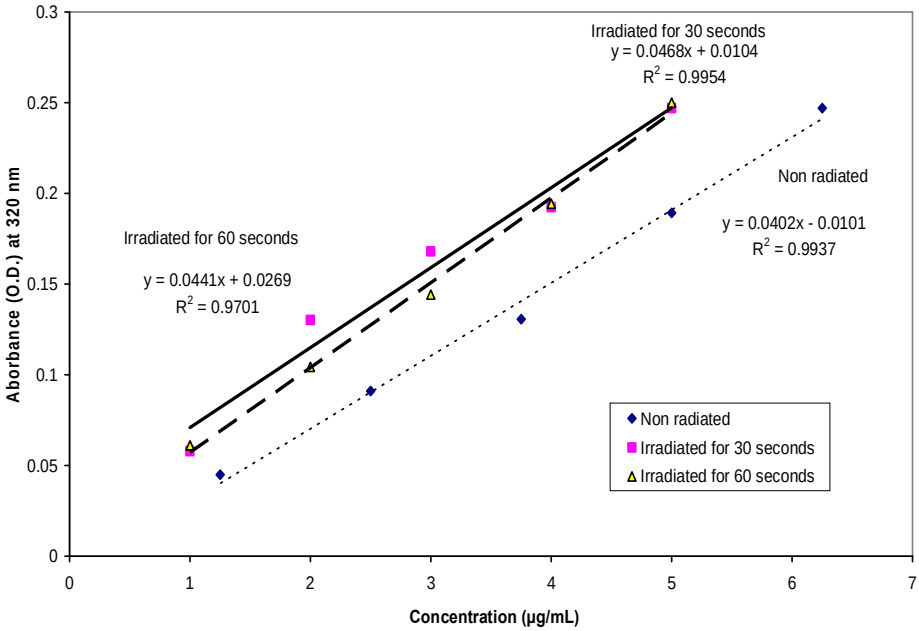

Fig.1. Effect of superficial X-rays at acceleration potential of 120 kVp on aqueous solution of tinidazole

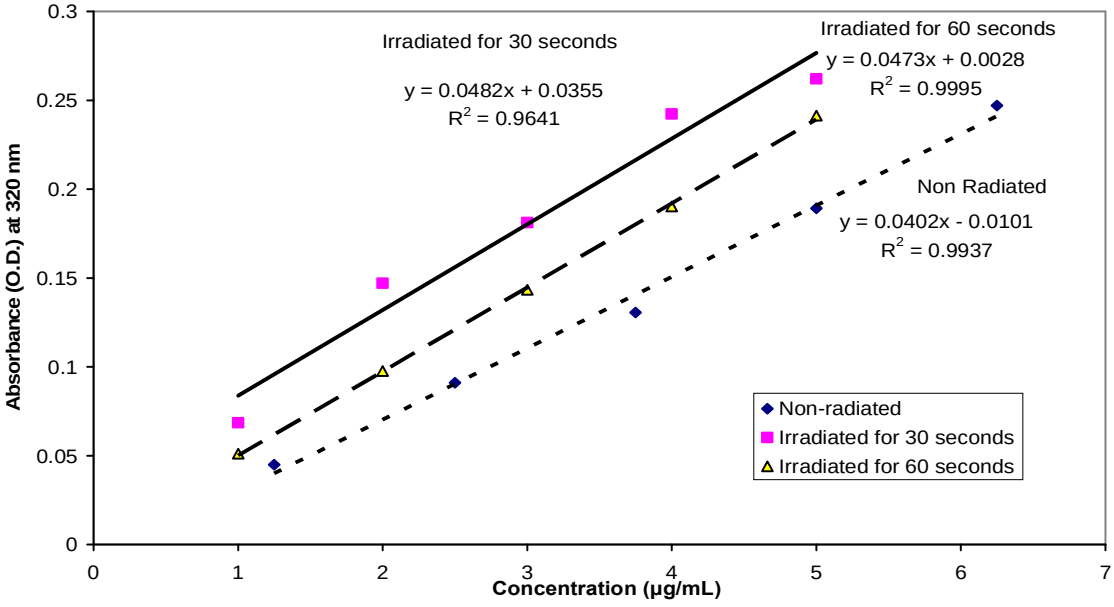

Fig.2. Effect of supereficial X-rays at acceleration potential of 160kVp on aqueous solution of tinidazole

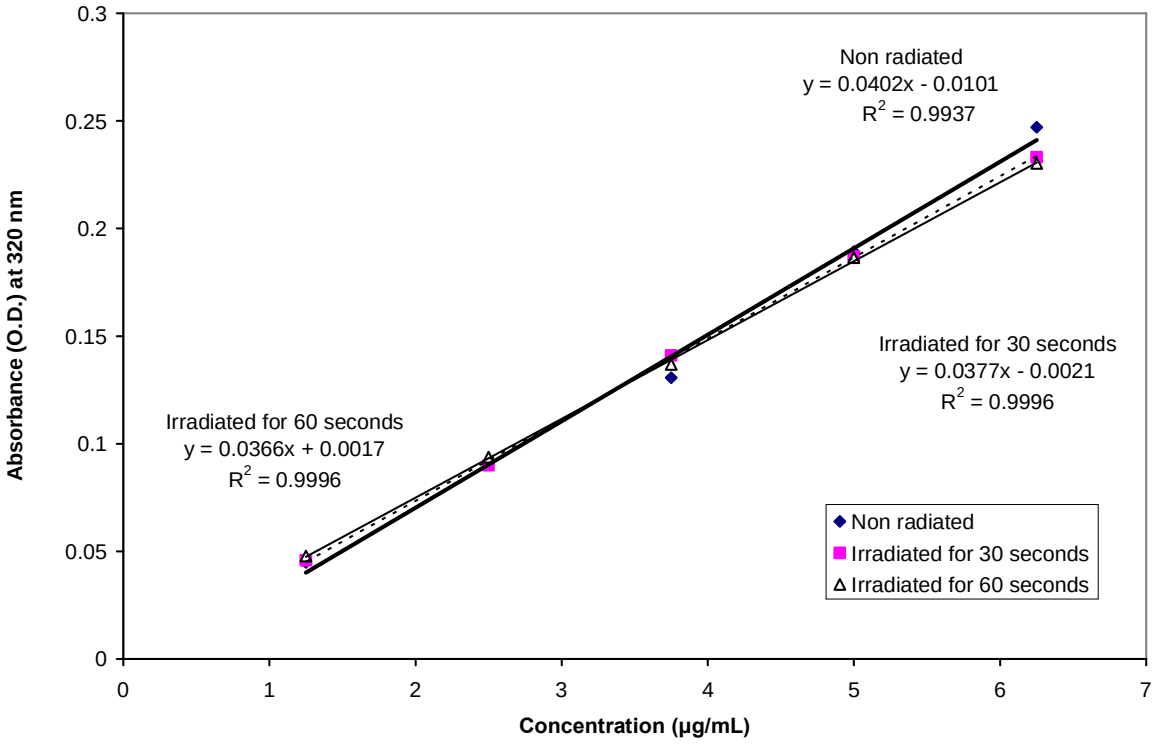

Fig.3. Effect of superficial X-rays at acceleration potential of 120 kVp on solid form tinidazole

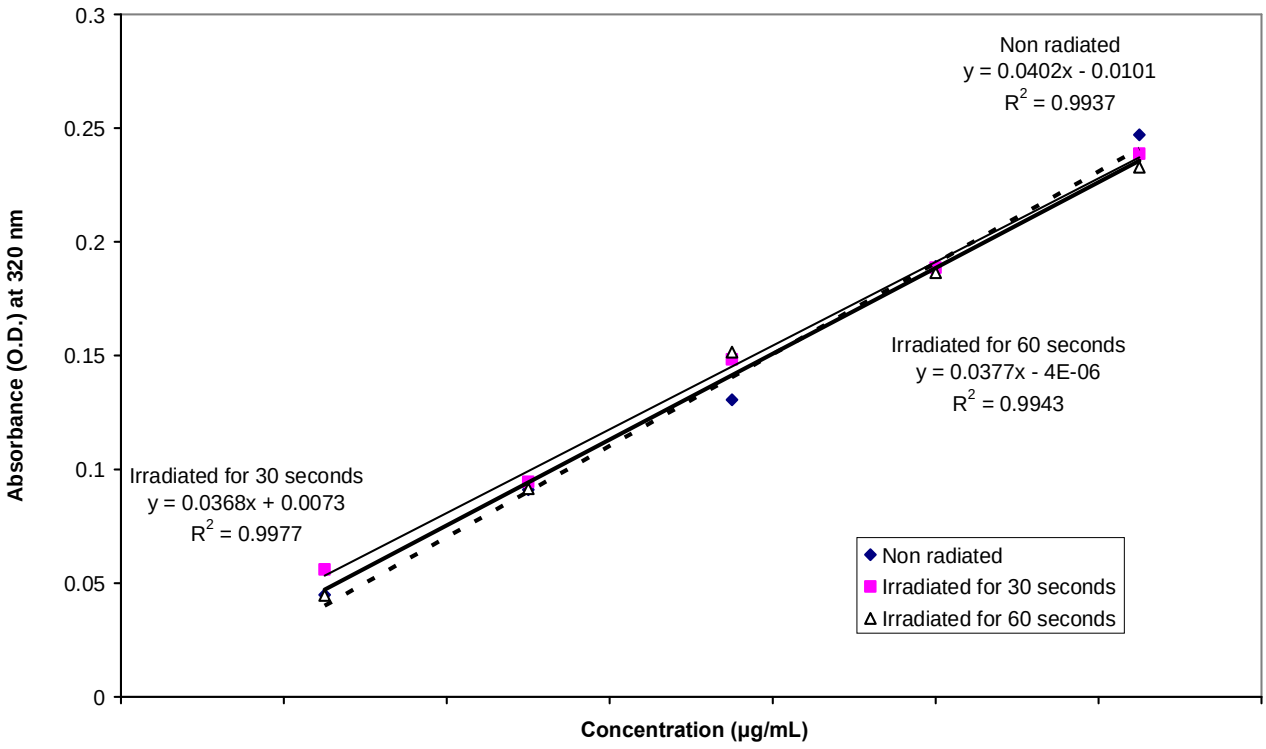

Fig.4. Effect of superficial X-rays at acceleration potential of 160 kVp on solid form tinidazole.

Discussion

The current results show that tinidazole compound is sensitive to radiation whether in its solid form or dissolved in aqueous solution. It is well known that nitroimidazoles including tinidazole are hydrolyzed in

extremely acidic or alkaline media as well as at high temperature [17]. Such hydrolysis is followed apparent first-order kinetics. In this study, the potential acceleration as well as the duration of radiation is not sufficient to alter the pH of aqueous solution of tinidazole but it is sufficient to generate heat which may play a role in hydrolysis of tinidazole compound [18].

The increase in optic density of aqueous tinidazole solution after radiation may give an idea of formation byproducts related to tinidazole. Such byproducts could involve into two important clinical considerations:

First: these byproducts may involve in adverse reaction when the tinidazole in biological fluids and the subject is exposed to ionizing radiation [19].

Second: the therapeutic efficacy of tinidazole is lost when the subject is exposed to radiation [20,21].

From the pharmaceutical point of view, the aqueous pharmaceutical preparations should be protected from radiation in case of marketing, storage and transport [22].

The effect of radiation on solid form of tinidazole compound is less than that observed with aqueous form. Therefore, the precaution measurements could be applied less firmly than that considered with aqueous pharmaceutical preparations [23].

In conclusion, tinidazole is radiosensitive and several protection measurements should be used to prevent its hydrolysis.

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