Effects of gamma irradiation on the physico-chemical and biological properties of levofloxacin

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Abstract: The aim of the present study was to examine the effect of gamma radiation on levofloxacin. Powder form of levofloxacin was subjected to different radiation doses (25, 50, 75, 100 and 125kGy) of Cobalt-60 source in a Gammacell-220 at a rate of 8.5 Gray/hr. The effect of radiation has been investigated with the aid of different spectroscopic techniques (UV-Vis, FT-IR), scanning electron microscopy (SEM), X-ray diffraction (XRD), and by antibacterial activities. UV data did not reveal significant changes in the structure of levofloxacin which is supported by scanning electron microscopy. However, X-rays diffraction shows a change in crystallinity of levofloxacin to an amorphous structure and this has been reflected on the morphology of this compound as indicated by SEM images. The antibacterial activities, on the other hand, reveal resistance of irradiated levofloxacin against bacteria, where some bacteria were highly affected by the irradiated drug. Similarly, FT-IR data show some changes in the functional groups principal absorption bands, in the IR spectrum, at frequencies 3286, 2846, 1716 and 1620 cm^{-1} for the O-H stretching band of quinolone, C-H stretching band, and C=O stretching band of carboxylic and pyridine. In addition, new peaks appeared which were not seen in the non-irradiated spectrum. In conclusion, some changes occurred in levofloxacin drug with the passage of radiation but the drug was chemically stable.

Keyword: Levofloxacin, Gamma irradiation, Antibacterial activity, Crystallinity and functional group.

INTRODUCTION

Levofloxacin (fig. 1) belongs to the second generation of fluoroquinolone. It exhibits the same activity as ciprofloxacin against both gram positive and gram negative bacteria and acts as a broad spectrum antibiotic. (Nelson *et al*., 2007) These antibacterial drugs are responsible for the cure of different types of diseases, such as respiratory tract infections, gastrointestinal, abdominal, and urinary tract infections. In addition, these drugs are used to treat infections and diseases caused by different types of Gram negative bacteria, such as, *Haemophilus influenzae*, *Escherichia coli*, *Legionella pneumophila*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Moraxella catarrhalis*, and *Pseudomonas aeruginosa.* (Hirai *et al*., 1986) Similarly, they also treat diseases caused by types of Gram positive bacteria, such as methicillin sensitive but not methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*. (Lafredo *et al*., 1993) Furthermore, levofloxacin is acknowledged for its excellent tissue penetration and broad spectrum of activities. Additionally, this drug exists in two formulations, oral and intravenous (Yamane *et al*., 1994; Wispelwey and Schafer 2010).

Levofloxacin is an antibiotic that is active against both Gram-positive and Gram-negative bacteria. It is commonly used to treat a number of bacterial infections, including urinary tract infections, cellulitis, respiratory tract infections, anthrax, prostatitis, (Paradise, Diliberto *et al*. 2008) meningitis, endocarditis, pelvic inflammatory disease, tuberculosis and plague, and traveler's diarrhea. (Bronstein *et al*., 2011).

Both gram-(+) and gram-(-) bacteria are greatly affected by the broad spectrum antibiotic, levofloxacin. Levofloxacin and other quinolones act by inhibiting the two type II topoisomerase enzymes, DNA gyrase and topoisomerase IV. Topoisomerase IV plays a role in replication of DNA replication during division of bacterial cells. Inhibition of this enzyme stops DNA synthesis as a result bacterium cell cannot divide. The other topoisomerase enzymes, DNA gyrase, is responsible for supercoiling of DNA in the bacterium cell. In conclusion, levofloxacin can cause death of bacterium cells and can be used as a bactericidal agent against the bacteria. (Guptha *et al*., 2011).

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Levofloxacin as a compound is the isomeric form of ofloxacin, another antimicrobial quinolone. This indicates that levofloxacin consists of 50% ofloxacin-containing compound which displays a very high activity against bacteria, whereas the remaining 50% consists of other quinolone parts. In chemical terms, levofloxacin is a chiral fluorinated compound of carboxy-quinolone and also is the (S)-enantiomer of the racemic mixture of ofloxacin (Morrissey *et al*., 1996).

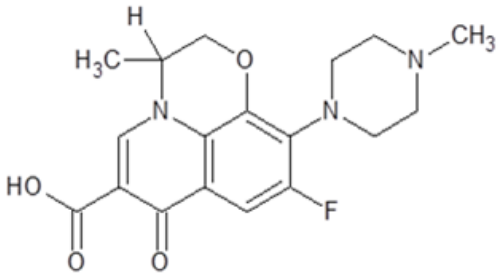

Fig. 1: The basic structure of levofloxacin

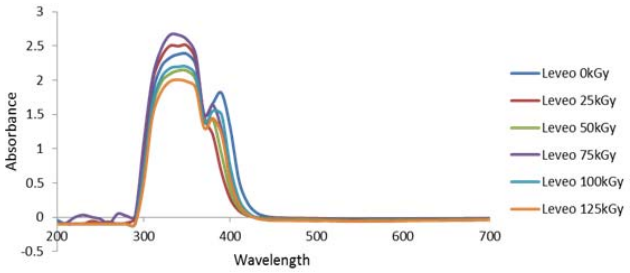

Fig. 2: UV spectra of levofloxacin after gamma irradiation at different doses

The levofloxacin drug contains a hemihydrate material, which has the molecular mass of 370.38 g/mol and the empirical formula $C_{18}H_{20}FN_3O_4 \cdot ½ H_2O$. Levofloxacin is a light-yellowish-white to yellow-white crystal powder in color. (Smith *et al*., 2011)

Sterilization Technique is a process that effectively kills or destroys transmissible agents (such as fungi, bacteria, viruses and prions) from any surface, foods, equipment, medications, or from different biological culture medium. In experimental way sterility is achieved by the exposure of medicine or chemical or physical agent to sterilize for a specified time. Various sources or agents are used for sterilization method are: ionizing radiation, elevated temperature, chemical liquids or different gases etc. The achievement of any process depends upon the choice of that method which is adopted for sterilization. In our work we used gamma irradiation of cobalt 60 different types of radiation sources are used for sterilization methods one of them is electromagnetic radiation (e.g. gamma rays and UV light), and the other is particulate radiation (e.g. accelerated electrons). The main target for all this radiation technique is changes in microbial DNA (Sultana 2007).

Gamma irradiation are one of the most important techniques for the sterilization of pharmaceuticals drugs, due to the high penetrating ability of this rays to sterile packaging of pharmaceutical drugs and cosmetic material, and it is fact that the there is no heat concept during the process did not increase their traceability or doubt to deliver effectively and helpful for the heat-sensitive products in packaging materials or operations. Gamma rays are also used easier to secure, control, reliable and provide a fast process and the product obtained has no harmful effect on the environment (Abuhanoğlu and Özer 2010) The gamma irradiation techniques was proposed by the British Pharmacopoeia (Pharmacopeia 2005) as a proper sterilization techniques for sterilizing certain surgical materials, chemical and equipment with a dose requirement of 25 kGy which was the standard recommendation of International Atomic Energy Agency (Jacobs and Wills 1988) but the choice of sterilizing material with this radiation dose is no longer fixed at 25 kGy but recommendation was instead based on the initial microbial load coupled with the preferred sterility assurance level (Valvo *et al*., 1999). The USP (Ross *et al*., 2000) 24 states that "Although 25 kGy dose of radiation was historically recommended by IAEA; it acceptable in some cases to only employ lower doses for different devices (Saper *et al*., 2004) in other cases, however, higher doses are essential.

MATERIALS AND METHOD

Samples collection

Antibiotics levofloxacin was purchased from a local enterprise Max Pharmaceutical Company Islamabad Pakistan in a powder form and analytical grade.

Sample preparations

Irradiation of samples was performed on 6 wails, obtained from a local medical store. There were 6 antibiotic samples 5 of which were irradiated, whereas the sixth sample was not irradiated for comparison. An amount of 1.0g each of the samples was taken on a wails and sealed with dockhand, and all 6 samples were placed in a gamma source for irradiation (Al-Mohizea *et al*., 2007).

Irradiation procedure

All irradiations were accomplished, under standard conditions (25-30°C, at room temperature) in the dark, using a ^{60}Co gamma cell (2400^{o}Ci, Hungary) which provides a dose rate of 8.5kGy·h^{-1} as an ionizing radioactivity source. Irradiations were performed at the Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan, which is part of the Pakistan Atomic Energy Commission. Investigations, including UV, FT-IR, SEM, X-rays diffraction, HPLC, and biological activities were carried out on levofloxacin irradiated at five different doses stages (25, 50, 75, 100 and 125kGy). Non-irradiated samples were used as

controls to identify physicochemical and biological activity changes resulting from ionizing radiation on studied samples.

Irradiation time

Duration of time is a very important factor for the maintenance of irradiation doses of antibiotics. In the current investigation, the antibiotics were subjected to radiation doses of 25kGy (2h and 56min), 50kGy (5 h and 53 min), 75kGy (8 h and 50min), 100kGy (11 h and 46 min), and 125kGy dose (14h and 42 min) (Tyburski *et al*., 2009).

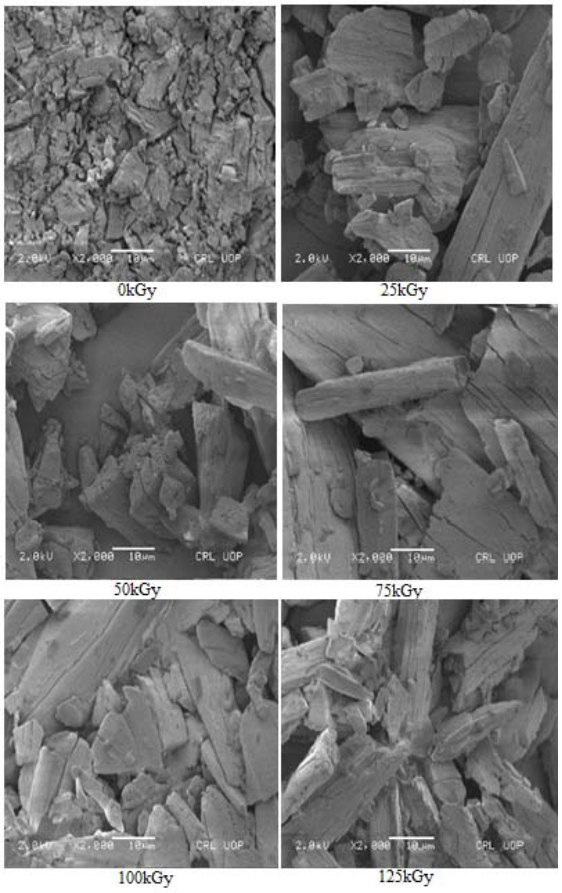

Fig. 3: SEM images of levofloxacin after gamma irradiation at different doses.

Scanning electron microscopy (SEM)

The surface, shape, size, and physical characteristics of the investigated antibiotics were examined by scanning electron microscopy (SEM). A quantity of 0.2g of levofloxacin powder was mounted by means of double-sided adhesive carbon tape and aluminum stub. Then the crystal powder samples were coated with gold palladium (Au/Pd) by means of a vacuum evaporator and studied with a scanning electron microscope JSM-5910 (Jeol Ltd., Tokyo, Japan) at the Central Resources Laboratory (CRL) at the Physics Department, University of Peshawar, Pakistan with a digital camera, at 2.0 kV accelerating voltage (Carja *et al*., 2008).

X-ray diffraction

The X-ray diffraction powder patterns of non-irradiated and gamma-irradiated levofloxacin samples were obtained using an X-ray diffractometer system JDX-3532 (Jeol Ltd Japan)(Ribeiro, Loftsson *et al*. 2003) which is also located in the Central Resources Laboratory (CRL) at the Physics department, University of Peshawar, Pakistan using a Ni filtered CuK (α) radiation at a scanning rate of 20/m under a current of 30 mA and voltage of 40 kV for the generator. Diffractograms were recorded from 5 to 40 degree (2θ) at a time continual of 1.0 s and a step size of 0.040 (Bhattacharjee *et al*., 2009).

UV-Visible Spectroscopy

By means of UV-visible spectroscopy some chemical changes in the non-irradiated and irradiated samples were evaluated with the aid of spectrophotometer model M-350 present at the Institute of Chemical Sciences, Organic Chemistry lab, the University of Peshawar, Pakistan. Spectra were obtained in the wavelength range of 200-700 nm of aqueous solutions of the drug, levofloxacin, made by dissolving 0.5 g of the solid drug in 10 mL of distilled water; changes were observed from absorption spectra (Liang *et al*., 2002).

FT-IR spectroscopy

FT-IR spectra of non-irradiated and irradiated levofloxacin samples, were recorded at the Geology center, University of Peshawar. Pellets for FT-IR were made at the Material Research Laboratory (MRL) at the Physics Department, University of Peshawar. At first, potassium bromide (KBr) was crushed with the help of a mortar and then dried for 15 min in an oven. Pellets were made by mixing 6 mg of sample and 194 mg of KBr powder in a pellet-making disk with a spatula and then pressed with a pellet machine with a pressure of 4000 metric ton; mass of pellet was 200 mg and diameter was 13 mm. Spectra were obtained with a BIO-RAD FT-IR, model FTS-165, IR spectrophotometer (Macias *et al*., 2002).

Microbiological assay

The effect of gamma irradiation on levofloxacin was tested against bacteria using an agar diffusion technique by means of a Mueller Hinton agar (Oxoid Ltd., London, UK).

Culture media and microorganisms used

The media used for antibacterial assessment was nutrient agar medium was prepared according to guidelines set by the manufacturer and transferred onto 20 × 20 cm sterile petri-plates or dishes (approximately 80 mL per plate). The final agar width was 0.4 cm on each petri-plate. The petri-plates were left to solidify by cooling overnight (Yousuf *et al*., 2012).

The following stands of bacteria were used in the present study: *Staphylococcus aureus, Pseudomonas, Escherichia*

coli, *Citromonous*, and *Methicillin-resistant Staphylococcus aureus* (Acar and Goldstein, 1996).

RESULTS

Gamma irradiation is an excellent method for sterilization of pharmaceutical raw materials and products. One of the most important possible disadvantages of radio-sterilization, however, is the possible production of different radiolytic intermediates through the irradiation technique. Sterilization is used in many industries and is a strictly essential process for some products, such as medical devices and parenteral drugs. While there are many types of sterilization techniques according to the physicochemical properties of the substance in question, the use of irradiation has many advantages depending on its significantly reduced toxicity. Above are some results and parameters used to evaluate the effect of irradiation on levofloxacin.

DISCUSSION

UV-Vis. Spectroscopy analysis

The powder form of levofloxacin of irradiated and non-radiated samples was dissolved in distilled water and the UV-Vis spectra were recorded with a UV-Vis spectrometer. Displayed in fig. 2 are spectra of levofloxacin irradiated at different doses. For irradiated samples, spectra exhibit two absorption peaks at 330, 340, 330, 340, 330nm and the second peak at 370, 370, 380, 370, 380nm. On the other hand, the non-irradiated sample shows a peak at 390 nm. Differences in the wavelengths in absorption spectra are due to high availability and small concentration of radiated degradable products. (Avhad and Bonde, 2009) There is a slight bathochromic shift of the maximum wavelength of levofloxacin and its irradiated samples indicating slight different peaks. Although UV-Vis spectra of irradiated levofloxacin show a shift of maxima wavelengths with a different irradiation, results indicate no radiolytical intermediates formed during irradiation and that levofloxacin is chemically stable to radiation.

Scanning electron microscopy analysis (SEM)

Shown in fig. 3 are SEM photographs that show the morphology of levofloxacin in the non-irradiated powders crystals compared irradiated powders at different doses. SEM photographs of irradiated levofloxacin displayed crystalline and heterogeneous porous surfaces. This morphology resulted from surface changes due to radiations. Among different doses of irradiation, results revealed significant differences in the morphology between samples irradiated with 25 kGy and 125 kGy, and between irradiated and non-irradiated samples (Somayaji *et al*., 2010).

X-ray powder diffractometry (XRD)

XRD diffractograms of pure levofloxacin revealed a series of sharp and intense peaks which shows its crystalline characteristic (fig. 4). Levofloxacin is crystalline as observed from diffraction peaks, whereas irradiation with 25kGy to 50kGy led to the disappearance of some peaks which indicates its amorphous character. However, irradiation with 100kGy resulted in the formation of very sharp and broad peaks, suggesting an increase in crystallinity of the compound. Meanwhile, X-ray diffraction profiles for some of the irradiated levofloxacin show a reduction of crystallinity, however, at 100kGy dose some of levofloxacin become significantly more crystalline in comparison to other irradiated and un-irradiated samples (Empey *et al*., 2001).

Antibacterial essay

Irradiated and un-irradiated levofloxacin were tested against five bacterial strains, and results are given in table 1. Results in table 1 reveal variations in activities between irradiated and non-irradiated samples, and effects on different types of bacteria depend on the irradiation dose as in the case of *Escherichia coli* (*E. coli*) (Fu *et al*. 1992), where non-irradiated and irradiated levofloxacin with at 2 kGy dose have basically the same activity. However, increasing the dose above 25k Gy led to a decrease in activity with an increase of irradiation. The same applies to the other strains of bacteria but to various degrees. In *Citromonous* (Citro) bacteria, a small decrease occurs with the increase of irradiation, whereas in Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria show a very large change at 100 kGy and 125 kGy doses in which the activity is decreased as shown in table 1 (Rodrıguez *et al*., 2002).

FT-IR analysis

Levofloxacin showed the characteristic absorptions bands for the non-radiated and radiated samples at 3286 cm^{-1} assigned to O-H and N-H stretching of the piperazinyl moiety, which is carboxylic and amide moities, 2848 cm^{-1} for aliphatic C-H stretch, 1716 cm^{-1} and at 1620 cm^{-1} for (C=O) stretching, and 650 to 740cm^{-1} for C-F stretching. (Sultana *et al*., 2013) With irradiation, no significant changes were observed, however, but some new peaks appeared in the spectra, such as 1304cm^{-1} at 25kgy, 1336 cm^{-1} at 50 kGy and 1394cm^{-1} at 75 kGy dose which were not seen in the non-irradiated spectrum; these new peaks indicated that some excitations take place with irradiation but the drug is chemically stable (Drevenšek *et al*., 2006).

CONCLUSION

In summary, it is concluded that levofloxacin drug is chemically stable towards with the irradiation with cobalt-60, although some minor changes occur as a result of these radiations. In addition, this study revealed some effects of radiation on the activity of the drug against different bacterial strains.

Table 1: Antibacterial activity of levofloxacin irradiated at different doses against bacteria

Serial No	Radiation Strength	*E coli* Mm	*Staph* mm	*Pseudo* Mm	*Citro* mm	*MRSA* Mm
1	0 kGy	19	23	15	28	20
2	25 kGy	19	22	13	28	19
3	50 kGy	18	22	13	27	19
4	75 kGy	17	20	12	27	18
5	100 kGy	17	19	12	26	15
6	125 kGy	16	18	10	25	12

Table 2: FT-IR data of treated levofloxacin

Compound	(O-H)	(C-H)	(C=O) carboxylic	(C=O) pyridone	(C-F)
0 kGy	3286	2846	1716	1620	680
25 kGy	3286	2848	1716	1620	660
50 kGy	3285	2845	1720	1620	650
75 kGy	3248	2835	1716	1620	650
100 kGy	3285	2886	1716	1620	740
125 kGy	3285	2848	1716	1620	651

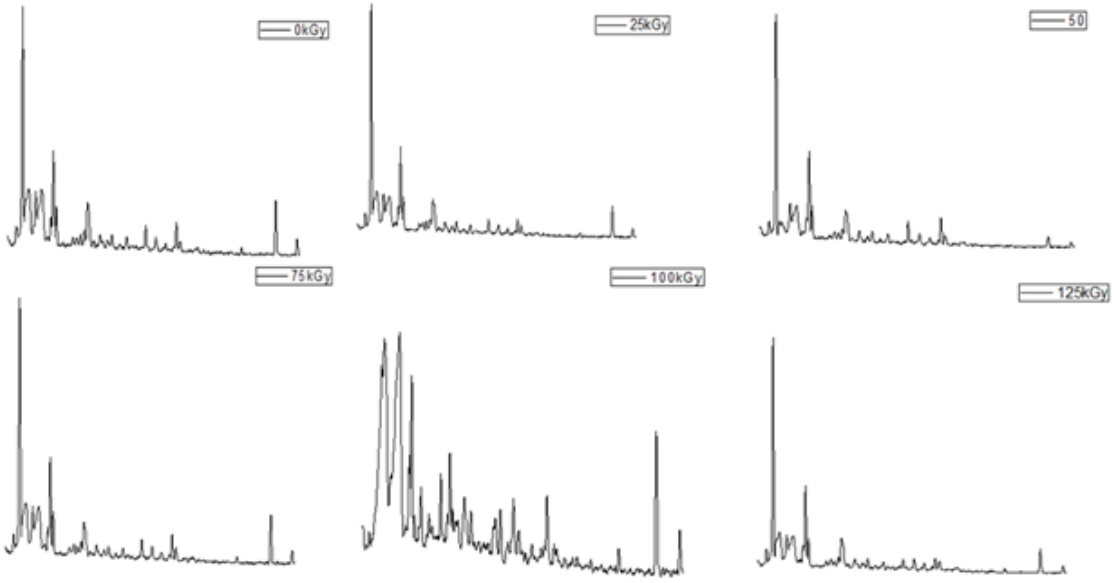

Fig. 4: X-ray graphs of irradiated levofloxacin with different irradiation doses

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