The Radiation Sterilization Of Amoxicillin and Cefaclor Antibiotics By Gamma Irradiation

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ABSTRACT

The effect of gamma (γ) irradiation on two antibiotics (amoxicillin and cefaclor) were studied. Antibiotics in solid state were subjected to irradiation sterilization by(γ) at 25 kGy in atmospheric air at room temperature and afterwards they were subjected to some microbiological and analytical determination to check their stability. The results showed that the majority of initially unirradiated compounds had a slight degree of contamination with Bacillus and Micrococcus genera. All irradiated samples were free from any bioburden and their biological activity was mostly preserved, EPR (Electron Paramagnetic Resonance) results showed formation of free radicals. The analytical determination (FT/IR, UV, mass spectroscopy, and Melting point) results proved that the antibiotics analyzed were radio stable and could be sterilized by irradiation with a dose of 25kGy, without any detrimental effect on their properties and antibacterial activity.

**Keywords:** Gamma sterilization; amoxicillin; cefaclor.
1-Introduction

Antibiotics and antibacterial drugs are commonly used in the management of bacterial infections globally. They have been used for more than 50 years to improve both human and animal health since the antibiotic golden age up to date (Flynn, 2012).

One of the most important points in the manufacture of healthcare products is the production of a sterile product.

The field of medical sterilization has become increasingly complex because of the need to prevent patient exposure to infections caused by organisms on any medical devices (MDs) or medical products during their care. Failures in adequate sterilization of medical devices (MDs) result in significant institutional costs related to patient nosocomial infections and mortality/morbidity concerns (Anonymous, 1997 and Rutala and Weber, 1999).

Many conventional sterilization methods were used such as: tempering, cauterization, hot air sterilization, steam sterilization, sterile filtration, radiation sterilization (e.g., by ionizing radiation or UV light), gas sterilization (e.g., by ethylene oxide or formaldehyde), and chemical sterilization (Gibella et al., 2000; Varshney and Dodke...
The more widely used industrial MDs sterilization technologies are steam, ethylene oxide (EO), and $\gamma$ and electron beam irradiation. There are other methods under development, such as low temperature hydrogen peroxide gas plasma, peracetic acid gas plasma; vapor phase hydrogen peroxide, ozone, chlorine dioxide, and high intensity visible light (Fairand et al., 2002).

Historically the pharmaceutical industry has relied on steam, dry heat, ethylene oxide gas, filtration and chemical processes to accomplish microbial reduction requirements.

Nowadays, several pharmaceuticals, raw materials and finished products are being sanitized and/or sterilized successfully with $\gamma$ radiation (Anonymous, 2002).

The selection of a sterilization dose can be done by different approaches, either it is calculated using determination of the number of viable microorganisms or
information obtained by incremental dose or by selection of the sterilization dose of 25 kGy (corroboration of the suitability of this dose is required) (EN ISO 11137, 2006).

Sterilization by \( \gamma \) irradiation has shown a strong applicability for a wide range of pharmaceutical products. Due to the requirement for terminal sterilization Where possible in the pharmaceutical industry, \( \gamma \) sterilization has proven itself to be an effective method as indicated by its acceptance in the European Pharmacopeia (2002) and the United States Pharmacopeia (2009).

Many pharmaceutical products require sterilization to ensure their safe and effective use. Sterility is therefore a critical quality attribute and is essential for direct injection products.

In case of medical substances choice of the sterilization method depends on the type, properties, and production method of the substance in question (Wilczyn´ski et al., 2014).

From industrial point of view radiation sterilization is especially useful in the case of thermolabile products (like antibiotics) because irradiation causes only a small
rise in the temperature of sterilized substances (WHO, 2014; and Wilczyn´ski et al., 2014).

In the case of γ- irradiation, the substance to be sterilized does not directly interact with the reagents and lacks any traces of chemical pollution (Katusˇin-Razˇem et al., 2005).

**Aim of the work**

The main target of the present work to study the availability of γ- rays as a sterilization technique and its impact on certain antibiotics (amoxicillin and cefaclor). Through the following steps:

- Isolation and identification of microbial contaminants of the antibiotics under the investigation (amoxicillin and cefaclor) in the dry state.

- Assessment of the radiation sensitivity of the identified isolated strains obtained from (D10 value).
- Exposure of the antibiotic samples, in dry state, to the recommended dose (25 kGy) of $\gamma$-radiation.

- Isolation of the microbial load after irradiation of the antibiotics under investigation.

- Evaluation of antibacterial activity of the antibiotics before and after irradiation process towards isolated strains and standard strains.

- Assessment of the chemical structure stability of tested antibiotics before and after irradiation by some analytical techniques (melting point, UV spectrophotometer, mass spectroscopy, FT-IR and EPR measurements).

1. Antibiotics:

Antibiotics are metabolic compounds produced by microorganisms, such as moulds and soil bacteria which in low concentration can inhibit the growth of other microorganisms.

Antibiotics may be:
A- **Broad spectrum antibiotics**: which kill or inhibit a wide range of gram positive & gram negative bacteria.

B- **Narrow spectrum antibiotics**: That is effective mainly against gram positive or gram negative bacteria.

C- **Limited spectrum antibiotic**: That is effective against a single organism of a disease.

**Other classification of antibiotics:**

- **Bacteriostatic**: bacterial growth inhibiting.
- **Bactericidal**: bacterial growth destruction.

**Or bactericidal and bacteriostatic**: Affects both mechanisms together (Calderon and Sabundayo, 2007; and Finberg et al., 2004; Cunha, 2009).

**The modes of actions of antibiotics:**

There are many modes of actions of antibiotics on bacteria that can be summarized in Fig (1).
Radiation:

Radiation is a physical phenomenon in which energy resulting from the nuclear disintegration of certain radioactive substances such as the isotopes Co\(^{60}\) and CS\(^{137}\) (Gross, 2007).

1- Types of Radiation:

According to energy emitted is divided into two types.

(Purohit et al., 2009).

2- Non-ionizing radiation: It has enough energy to excite molecules and atoms causing them to vibrate faster which is obvious in a microwave oven, where the radiation causes water molecules to vibrate faster creating heat.
**Ionizing radiation:** It has more energy than non-ionizing radiation; enough to cause chemical changes by breaking chemical bonds. This effect can cause damage to living tissues. The ionizing radiations of primary concern are alpha (α), beta (β) particles, γ, x rays and neutrons.

### 2.2.2. Radiation dose units:

According to the Canadian Nuclear Safety Commission (CNSC, 2012), that the scientific unit of measurement for radiation dose are:

- **Gray (Gy).** The unit of absorbed dose, One gray = 1 J/kg = 100 rad.

- **Rad.** The unit of absorbed dose, One rad = 100 erg/g = 0.01Gy.

- **Sievert (Sv).** The unit of dose equivalent, equal to absorbed dose in gray multiplied by the quality factor. One Sv = 100 rem.

- **Rem.** The conventional unit of dose equivalent, One rem = 0.01Sv.

### 2.2.3. Biological effects of radiations:
Direct and indirect effect of radiation:

- **Direct action** occurs when the radiation interacts directly with cell's essential molecules. The radiation energy may damage cell components such as the cell walls or the deoxyribonucleic acid (DNA) (Shapiro, 2002).

- **Indirect action** occurs when radiation interacts with water molecules which are representing 80% of cells composition.

The energy absorbed by the water molecules can result in the formation of free radicals. Free radicals are molecules that are highly reactive due to the presence of unpaired electrons, which result when water molecules are split. Free radicals may form compounds, such as hydrogen peroxide (Shapiro, 2002) as shown in Fig (5).
**Advantages of gamma sterilization:**

Gamma rays do have some significant advantages (Garcia *et al.*, 2004) that can be summarized as follows:

- Better assurance of product sterility than filtration and aseptic processing.

- Achieves the sterility assurance level SAL $10^{-6}$

- No residue like EtO leaves behind.

- More penetrating than electron beam accelerator.

- Low-temperature process.

- Simple validation process.

- More applicable on large scale under dose control.

**Radiation Effects on Pharmaceuticals:**

The effect of radiation on pharmaceuticals is known in
general, and γ-rays sterilization has an impact on pharmaceuticals to be investigated. The use of γ-rays as a method of pharmaceuticals sterilization was accepted by the IAEA in 1967.

In recent years, for commercial use, an increase in the number of gamma irradiators of medical devices has been seen, and the application of this method has been seen in the pharmaceutical and cosmetic industries.

According to the pharmacopoeia rules currently in force (EP 2002), all drugs produced and introduced for medical therapy must meet standards of microbiological purity. They can contain only a certain number of microorganisms in a unit of mass or volume or on the area unit. Depending on the form of drug and type of its intake, some drugs should be sterile, thus cannot contain any microorganisms or their endospores.

In the present study the impact of gamma radiation sterilization on two antibiotics (amoxicillin and cefaclor) in their dry state were studied. They were subjected to many microbiological and analytical tests to check their antimicrobial activity and their structure stability before and after irradiation.

Isolation and identification of microbial load of random samples of the two antibiotics under study were carried out. The isolated bacteria from amoxicillin were identified as gram positive *Bacillus sphaericus* and *Bacillus pumilus*, but in case of cefaclor the isolated bacteria were *Bacillus subtilis* and
Micrococcus luteus

Estimation of D_{10} value (dose that kills 90% of total microbial population) of isolated bacteria was carried out, it is used to express the degree of radiation resistance of microorganisms, the range of D_{10} of isolated bacteria were from 0.1 kGy to 4.4 kGy.

The antibiotics samples in their solid state were exposed to gamma radiation at dose 25 kGy, then their antibacterial activity and MIC determination were carried out and compared to unirradiated samples. The evaluation was carried out on four standard strains Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Ps. aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633) and on isolated strains. The activity were measured by ELISA reader the results showed that the antibiotics retained their antibacterial activity the MIC values readings were as follows

A) Amoxicillin: Staph. aureus ATCC 25923 (1μg/ml), E. coli ATCC 25922 (1μg/ml), B. Subtilis ATCC 6633 (128μg/ml) before and after irradiation and Ps.aeruginosa ATCC 27853 remained resistant for both unirradiated and irradiated samples. For isolated strains B. pumilus (8μg/ml) and, B.sphaericus (256μg/ml) before and after irradiation.
B) Cefaclor: *Staph. aureus* ATCC 25923 (4μg/ml) before, *E. coli* ATCC 25922 (512μg/ml), *B. Subtilis* ATCC 6633 (512μg/ml) before and after irradiation. *Ps.aeruginosa* ATCC 27853 remained resistant for both unirradiated and irradiated samples. For isolated strains *M. luteus* (128μg/ml) and *B. Subtilis* (16 μg/ml) before and after irradiation. The statistical analysis of this test showed no significant change (P value>0.05).

The isolation of microbial load after irradiation was carried out using filtration method showed lack of any microbial load of irradiated samples.

The stability of the chemical structure of the drugs was evaluated by different techniques and the readings did not change before and after irradiation in case of: UV – IR- Mass spectro- Melting point-and EPR analysis showed that there were no presence of free radicals of unirradiated amoxicillin but in case of irradiated samples free radicals were present, for cefaclor, there were a small singlet of free radicals in unirradiated samples that increased in intensity after irradiation.

The application of gamma irradiation as sterilization technique for these two chemotherapeutic agents; amoxicillin, cefaclor and studying the effect of applied dose on chemical structure of the
irradiated antibiotics compared to non-irradiated samples were studied by ultraviolet (UV), fourier transform infrared spectroscopy measurements (FTIR spectra, EPR, melting point and mass spectroscopy) in addition to antibacterial activity evaluation were done before and after irradiation to probe any change after irradiation.

In general, the results previously available showed that all of the irradiated antibiotics almost kept its antibacterial activity, retaining their structure and activity at dose 25 kGy.

The present work, covered sides of the irradiation effects on antimicrobial activity of antibiotics under study, but the pharmacological activity, decomposition biological studies of the by-products formed in the process need more studies.

Radiation sterilization is still rapid, applicable treatment as new and is not fully recognized. It is safe and successful but requires further detailed study, in future particularly by biological methods that possibility lasts for two years as (toxicological (on animals), pharmacological, mutagenic, teratogenic or carcinogenic tests) and clinical tests for bio-availability in vivo.

We can conclude from this study that these two drugs in the solid phase are resistant to ionizing radiation, and can be safely subjected to gamma irradiation sterilization. But, the problem is reporting detection, identification and determination of the properties of the products of the radiolytic decomposition of drugs, and its effect of therapeutic role of these drugs. That may
acquire further study may extend to 2 years. It can be concluded from this study that these two drugs under study are radio stable and can be sterilized by gamma irradiation in their solid state.