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Original Article

The Stability Studies of Penicillin and Ampicillin following γ-Irradiation in the Solid State

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Abstract

The effect of 10 and 25 kGy γ-irradiation on penicillin G plus procaine and sodium ampicillin has been assessed by different chemical and microbiological analytical methods. According to the chemical results obtained, the antibiotics showed a slight reduction in doses applied but the microbiological assays revealed that the activity of irradiated antibiotics did not reduce. Microbiological studies were carried out in order to determine the minimum absorbed dose required to achieve Sterility Assurance Level (SAL) of 10^{-6}. Briefly, lower irradiation doses seem to be useful for decontamination purposes.

Keywords: γ-irradiation; Sterilization; Penicillin; Ampicillin; Stability; SAL.

Introduction

Radiation sterilization of medical products is one of the ideal applications of high energy radiation. It has been accepted as an industrial process of sterilization worldwide. Sterilization of several polymeric and metallic medical devices is carried out using high energy γ-irradiation from Cobalt-60, Cesium-137 radioisotopes and electron beam from electron accelerator. During recent years, the results from numerous experiments of radiation sterilization of pharmaceuticals have been published (1). A major application of this technique is for sterilizing solid pharmaceutical compounds which are unstable in traditional methods of sterilization employing heat and ethylene oxide (EtO). However, EtO sterilization is seriously criticized because of its toxicity. Microfiltration is another technique routinely employed for sterilization of EtO and heat susceptible solids and solutions. During manufacturing process, steps such as filtration, filling and lyophilization are performed under aseptic condition which is difficult to maintain (2). While, radiation sterilization could be carried out on finally packaged product, it is clean and well-controlled, and higher SAL of 10^{-6} is normally achieved at 25 kGy, which is a dose generally applicable to products manufactured under good manufacturing practice (3). Pharmaceuticals, especially injectables, need to be sterilized. The main concern of using ionizing radiation for sterilization of pharmaceuticals is that radiolysis products may be formed and even small changes in the structure of drug molecules may affect its physicochemical, microbiological and toxicological properties. The difficulty in predicting the radiolysis of various antibiotics necessitates analyzing each compound individually. There are few reports on the effects of γ-irradiation on penicillins (4-6).

γ-irradiation is sometimes used to sterilize bulk powders prior to formulation. The present investigation aimed at studying the effect of

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different doses of γ-irradiation on natural and semi-synthetic members of penicillins, i.e. penicillin G and sodium ampicillin.

They have been irradiated in the dry-state and the probable changes in physicochemical and microbiological properties were studied. Our findings have been related to the feasibility of their radiation sterilization.

Experimental

Materials

Antibiotics used were penicillin G plus procaine (800,000 IU/vial) and sodium ampicillin (100 mg/vial) with batch numbers of 3L247 and 3C026, respectively. Jaber-ebne-Hayan Pharmaceutical company kindly provided the antibiotics.

Methods

Irradiation

Antibiotic powders were irradiated in original glass vials with 10 and 25 kGy at room temperature in a ^{60}Co source chamber (Gamma Cell-220) at a dose rate of 0.447 GyS^{-1} determined by Perspex dosimeters.

HPLC

The Reverse Phase HPLC system which was used consisted of a Knauer Compact chromatograph with 20 µl injectable loop, spectral photometer set at 254 nm and 180×3 mm analytical column packed with 10 µm packing L1. The mobile phase was a mixture of water, acetonitrite, phosphate buffer solution and glacial acetic acid (3600:360:40:4). The flow rate was set at 2 mL min^{-1} and the column temperature was maintained at 40°C (7).

UV spectrophotometry, pH and specific rotation

UV spectrophotometric determinations were carried out on aqueous solutions of the irradiated drug at appropriate concentrations in 430 nm using Perkin Elmer 2201.

The pH was measured using Hanna pH meter at the concentration recommended in the labeling. Specific rotation was measured using Atago polarimeter. Solubility, identification, appearance, syringibility and clean of suspension tests were performed on both antibiotics (7).

Plate cultivation technique

Mueller-Hinton Agar (Himedia) plates were inoculated with 2 to 8-h cultures of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 with a density adjusted to 1.5×10^8 cell mL^{-1} (0.5 McFarland). Sterile paper disks (6 mm in diameter) containing 10, 16, 20, 25 and 30 IU penicillin (dissolved in distilled water) and 5, 8, 10, 15, 20 and 25 µg ampicillin (dissolved in phosphate buffer, pH 6) were placed on the agar surface. After incubation at 35°C for 18 h, the zones of inhibition (ZOI) were measured as the complete inhibition of bacterial growth around the disks as judged by the unaided eye (8).

Macrodilution technique

One milliliter of 5×10^7 cell mL^{-1} of *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 was spiked into penicillin G and ampicillin samples which had already been diluted (1:2) using Mueller-Hinton Broth (Himedia) (9). The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of antibiotics capable of inhibiting the growth of organisms completely after incubation at 35°C for 16-20 h, as detected by the unaided eye. The results presented are the mean of 7 replications for antibiotics irradiated at 10 and 25 kGy.

D-value

Bacillus pumilus E601 spores in triple distilled water at a concentration of 10^7 cell mL^{-1} were irradiated with sublethal doses and then cultured in Plate Count Agar medium (Merck). The survivors were counted after incubation at 35°C for 48 h. The D-value was the slope of the inactivation curve obtained versus different doses, which is the required dose to kill 90% of viable bacteria. To determine the radiation resistance of spores in antibiotic suspension, the inactivation curve of *B. pumilus* spores in non-lethal concentrations of antibiotics were evaluated.

Results

HPLC analysis

The chemical analyses results of irradiated

Table 1 . Chemical analysis of penicillin G plus procaine as determined by the USP method.

Test	Unirradiated	10 kGy	25 kGy
Color	White	Yellow	Yellow
Syringe ability	Able	Able	Able
Dispersibility	Uniform suspension	Uniform suspension	Uniform suspension
Clean of suspension	Free from visible impurities	Free from visible impurities	Free from visible impurities
Content of penicillin*	0	-0.051	-0.054
Content of procaine*	0	0.0029	-0.023

* As percentage with respect to estimated total peak area of unirradiated samples.

samples are presented in Table 1 and 2. Quantization of the main sodium ampicillin peaks gave purity values of 95.8 and 97.1% for the samples irradiated at 10 and 25 kGy, respectively, compared to unirradiated standard sample. For penicillin, these values were 95.7% and 95.4% at 10 and 25 kGy, respectively.

The purity of antibiotics peaks was determined by absorbance rationing. The differences in values between irradiated and unirradiated samples suggested the presence of interfering substances, which could contribute to increased radiolysis. Nevertheless, no unusual peaks in HPLC chromatograms of irradiated drugs were seen in conditions used here.

UV and discoloration

Irradiation discolored the antibiotic powders from off-white to yellow (Tables 1 and 2). The intensity of the color rose as radiation dose increased, shown itself by an increased absorption in 430 nm for ampicillin which was more than the threshold of 0.15 mentioned in the US Pharmacopeia 27. In contrast, the syringe and dispersibility of penicillin G suspension as well as solubility and the identification test results of sodium ampicillin revealed no significant changes after irradiation.

pH

Changes in pH of irradiated ampicillin were in normal range of 8-10 (according to USP-28) and not affected by the doses applied.

Specific rotation

The treated ampicillin at 10 and 25 kGy showed specific rotation of 279° and 265°, respectively (i.e., 0.35% and 5.4% reduction compared to unirradiated antibiotic), but it was still in normal range of 258-287 (according to USP-27) (Table 2).

Biological activity of irradiated antibiotics

The biological potency of untreated antibiotics based on its ability to inhibit bacterial growth was assayed first using standard bacterial strains mentioned before. The effect of antibiotic concentrations on growth of tested bacteria, as determined in the test for procedure validity, is shown in Figure 1. The inhibition of bacterial growth was enhanced in disks containing higher concentrations of antibiotics.

Moderate ZOI were observed around disks containing 25 IU penicillin and 20 µg ampicillin. These concentrations of antibiotics were prepared from irradiated antibiotics. The results of biological assays are presented in Table 3.

Table 2. Chemical analysis of sodium ampicillin as determined by the USP method.

Sodium ampicillin	ΔpH	Absorbance at 430 nm	Specific rotation	HPLC*	Color
Unirradiated	0	0.032	280	-	White
10 kGy	-0.07	0.187	279	-0.056	Yellow
25 kGy	-0.06	0.371	265	-0.043	Yellow

*As percentage with respect to estimated total peak area of unirradiated samples.

Table 3. Diameters of growth-inhibition zones (ZOI) determined by the disk diffusion technique and minimum of inhibitory concentration (MIC) determined by the macrodilution test for the irradiated antibiotics, according to NCCLS.

	Diameter of inhibition zone in mm (mean ±2SD)		MIC in µg/mL (mode)	
Antibiotics	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
Sodium ampicillin				
Unirradiated	22.4±0.76	14.14±1.38	-	3.5
10 kGy	22.43±1.07	13.86±1.8	-	3.5
25 kGy	22.14±1.8	13.43±1.95	-	3.5
Penicillin G plus procaine				
Unirradiated	19±2.31	-	2	-
10 kGy	19.33±1.8	-	2	-
25 kGy	19.43±1.95	-	2	-

- Not Determined

The values are the mean of 7 replications. In the dose range studied, the mean of ZOI for irradiated penicillin G and ampicillin were particularly unchanged in comparison with the control antibiotics (t-student, $P<0.05$).

The data obtained from MIC tests were discontinuous, so mode value was used for comparison instead of mean value. The mode value of unirradiated and irradiated ampicillin at 10 and 25 kGy for *E. coli* was 3.5 µg mL^{-1} (all of 7 repetitions for samples unirradiated as well as those irradiated at 10 kGy, and one out of 7 analyses for samples irradiated at 25 kGy showed MICs of 7 µg mL^{-1}).

When tested against *S. aureus*, the mode value of unirradiated as well as penicillin samples irradiated at 10 and 25 kGy was 2 IU mL^{-1} (in 2 repetitions out of 7 for samples unirradiated and those irradiated at 10 kGy, and in 3 out of 7 replicated tests related to the sample irradiated at 25 kGy, the MICs were 1 IU mL^{-1}).

D-value

Non-lethal concentrations of penicillin G and sodium ampicillin for *B. pumilus* E601 spores evaluated by the macrodilution technique were 0.0161 U mL^{-1} and 0.0275 µg mL^{-1}, respectively. The inactivation curves of *B. pumilus* E601 spores in these concentrations of antibiotics are presented in Figure 4. The slope of these inactivation curves (D- value) showed no significant differences compared to the inactivation curve of spores in distilled water, indicating that antibiotic suspensions did not affect the resistance (D- value) of *B. pumilus* E601 spores to radiation.

Discussion

A decrease of 5.1% and 5.4% in penicillin content and 5.7% and 4.4% in ampicillin content occurred following γ-irradiation at 10 and 25 kGy. This finding was in accordance with previous reports (4, 5, 10, 11).

HPLC chromatograms did not show any peaks attributable to degradation under conditions used; however, this could be explained as either the by-products, if any, did not have any absorbance at 254 nm, the wavelength used for detection, or may degradation products have escaped detection. Nevertheless, a decrease in penicillin and ampicillin contents was observed in HPLC chromatograms.

Specific rotation of ampicillin showed a decrease from 280° (before irradiation) to 279° and 265° after treatment at 10 and 25 kGy, respectively. This reduction may be due to the cleavage of hydrogen bounds in antibiotic molecule, which demonstrated itself as a raise in UV absorption in correlation with radiation doses.

A decrease in pH and specific optical rotation of ampicillin treated at 10 and 25 kGy was seen. There was also a reduction in penicillin and ampicillin contents that support the degradation of drugs.

Biological activity of the antibiotics, as evaluated by the plate and macrodilution

susceptibility tests, was not affected significantly ($P > 0.05$). These results were in accordance with Jacobs's report on not finding systematic changes in potency of irradiated semi-synthetic penicillin at 10, 25 and 50 kGy (4).

Other organoleptic properties of compounds i.e., smell and odor remained unchanged; however, a darkening from off-white to yellow color was observed in irradiated antibiotics. There are similar reports on the color changing of chloramphenicol (2), cefotaxime (3) and sodium ampicillin (5) after treatment by γ-irradiation. The darkening of the irradiated glass vials from off-white to yellow may affect the discoloration of contained powders. This might be due to leaching an unknown material, originated from γ-irradiated glass containers, into powders. It could also be related to oxidation reaction. However, the justification of former phenomenon as well as the development of new techniques capable of detecting possible degraded forms of the antibiotic molecules entails further research.

Briefly, this research considered the feasibility of radiation sterilization for filter sterilized antibiotic powders in bulk form. Irradiation in the final package was not taken into account, since if it was the case, vials made of polyethylene which do not show the discoloration in doses applied would be used.

The effect of two antibiotic suspensions on D-value of a radio-resistant test strain spores i.e., *B. pumilus* E 601 was also evaluated. The antibiotic suspensions showed to have no significant effect on the resistance of spores. This finding was in line with a previous report by Botelho et al., who studied the effect of oxytetracycline hydrochloride ophthalmic ointment on the same bacterial spores irradiated at 5-20 kGy (1).

Although both antibiotics were showed minimum instability at 10 and 25 kGy, lower radiation doses may be practicable for decontamination purposes. The D-value of *B. pumilus* was not changed at applied doses. Therefore, this value can be used when applying lower doses to ascertain the dose required for sterilization.

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