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Sterilization of topical products by cobalt-60 (coord.progr. in practices for the radiation sterilization of medical supplies in countries of Asia and the Pacific Region)

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Sterilization of Topical Products by Cobalt-60

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Microbial limits and tests of topical products and cosmetics have been tightened in Korea. Controlling or eliminating troublesome microbial contaminants during the manufacturing and packing operations have also received attention. There is no doubt that gamma irradiation is a useful technique for certain pharmaceutical applications where the conventional sterilization processes are impractical. Co-60 radiosterilization is especially useful for the topical products and ophthalmic ointments. The major problem is the unpredictable nature of its effect upon the material being treated. Therefore, extensive trials including stability and toxicity testing have to be undertaken for each substance for which this processes is applied.

The use of ionizing radiation for killing microbes is widely recognized(1). Cobalt-60 has been shown to sterilize tubing, gloves(2) and various medical equipments(3), and inactivated bacterial spores in penicillin(4). Many pharmaceuticals have been reported to be safely sterilized by a 2.5 Mrad dose of Cobalt-60(5). These include multivitamins, ascorbic acid, heparin, pregnenolone, cortisone acetate, potassium penicillin G, chlorotetracycline, oxytetracycline, ergonovine malate, and morphine. Not all pharmaceuticals can be irradiated with loss of potency. In aqueous solutions, ascorbic acid, multivitamins, and heparin show a loss of activity. Insulin and atropine sulfate also suffer severe loss(5). The chemical stability of antibiotics(6) and colorants(7) have been reported.

when choosing gamma radiation as means of sterilization. While it is generally agreed that 2.5 Mrad is sufficient(8), the dose of Cobalt-60 irradiation required for sterilization is still a subject of discussion for the pharmaceutical preparations and their vehicles. In 1967, several papers(9,10,11) were presented, which indicated that radiosterilization of selected ophthalmic and parenteral products was both possible and practical. Wargo(12), O'Neill(13) and Nash(14) suggested the possibility of gamma ray sterilization of topical products. Hartman et al(15) and Jacobs et al(16) studied the physical characteristics of pharmaceuticals which might be affected due to gamma radiation in the decrease of their viscosity. It has been recently investigated to a great extent for some cosmetic raw materials and preparation(17, 18), and for pharmaceuticals and polymers(19).

The objective of this work is to determine the requirements for safe sterilization of topical products and ointment bases with references to specific product criteria and the microbial contaminants to relevance to Korean Pharmacopea.

EXPERIMENTAL

Preparation of ointment

Four ointment vehicles tested were formulated. The composition of these vehicles per gram are as follows.

Vehicle I	Stearic acid	126.7 mg
	Liquid paraffin	134.4 mg
	Propylen glycol	48.0 mg
	Spermaceti	19.2 mg
	Cetyl alcohol	14.4 mg
Vehicle II	Sperm Whale Cachalet	38 mg
	Stearyl alcohol	116 mg
	Polyethylene glycol	38 mg
	Glycerin	192 mg
	Sodium laurylsulfate	9 mg
Vehicle III	White petrolatum	800 mg
	Lanolin	125 mg
	Paraffin	75 mg
Vehicle Vi	Cetanol	30 mg
	Glycerin	124.9 mg
	Paraffin	100.o mg
	Stearyl alcohol	100.0 mg
	Sodium lauryl sulfate	10 mg
	Aqua dista	qs

Ointment tested were prepared with four vehicles. Its active ingredient as model compound is gentamicin 1 mg/gram and bufexamac 50 mg/gram, respectively.

Preparation of testing sample for sterilization

For the selection of the contaminating microbials, two cases were considered.

1. Germs which are likely to come from the surroundings and may contaminate the ointment during manufacturing.
2. Germs which are artificially contaminated in ointment for testing the sensitivity of radiosterilization.

On the basis of these considerations the ointment samples were prepared.

Case 1.

150-200 gram of ointment vehicles were spreaded on the glass plates and were contaminated from the surroundings for one day.

Case 2.

150 grams of each ointment vehicles were artificially contaminated with Bacillus pumilus E601 obtained from ATCC and Bacillus subtilis obtaind from the NIH of Korea.

For the determinations of the sensitivity of the B. pumilus and B. subtilis test strains to irradiation, testing samples of known spore count were prepared by mixing and lavigating aqueous suspensions with ointment vehicles. Samples contained bacteria 10^3- 10^4 germs/g vehicle.

The gentamicin and bufexamac ointments manufactured under the hygienic conditions had its total germ count measured by the K.P. method(20). From the examinations made of several samples, the ointment contained bacteria to a maximum level of 10^2 germs/g ointment.

Irradiation

100 grams of each testing samples were placed in ointment jar or plastic ointment tube. Samples were irradiated with gamma-ray at a dose rate of 2.67 krad/min or 11.56 krad/min by use of 9,000 Ci Co-60 panoramic irradiator or 15,000 Ci Co-60 BNL's shipboard irradiator, respectively, installed Korean Atomic Energy Research Institute. All samples were irradiated with 2.5 Mrad.

Viable cell count

The irradiated spore ointments were tested and bacterial count were carried out by the K.P. method.

Physical and chemical tests

Hardness was measured with Nissei Rheometer Model 2001J. Each measurement was made in triplicate. Change of appearance was observed. Stability of active ingredient was measured spectrophotometically.

RESULTS

The purpose of our test was to determine whether ointment can be sterilized or not by Co-60 irradiation. To evaluate the effect of radiosterilization, biological and physico-chemical tests have been performed. The effects of the Co-60 irradiation have always been compared with non-irradiated standard.

The results showed that the ointment vehicles, which were exposed in the air and contaminated from the surroundings, contained no survivor germs after the irradiation with 2.5 Mrad doses. B. subtilis and B. pumilus which were artificially contaminated in ointment vehicles could not be detected after the irradiation with 2.5 Mrad doses. Others mentioned that B. pumilus have resistance under this dose conditions. We will continue to investigate further in this part.

The quantitative determinations of two ingredients(gentamicin and bufexamac) in ointments did not show changes between before and after irradiation. For the ointment vehicles, no appearance changes have been observed, except slight discoloration. The originally slightly pale yellowish become slightly yellowish. Further work will follow to determine the degree of discoloration quantitatively. A change in hardness was observed after irradiation. Vehicle I, II, III did not show changes, but vehicle IV was soften 5- 10 %. Further detail data will be presented in the final report.

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STATEMENT OF PROJECT EXPENDITURES

Salaries and wages	$ 3,210
Chemicals & culture mediums	$ 1,500
Glassware	$ 462
Office supplies	$ 176
Service charge for Co-60 irradiation	$ 562
Transportation from SNU to KAERI	$ 180
Maintenance of lab. equipment, micellaneous	$ 650
Total	$ 6,740

PROJECT FINANCING

IAEA	$ 2,000
Industry(Yu-Yu Pharm. Co.)	$ 1,700
Total	$ 3,700
Paid by other project	$ 3,040

PROJECT FINANCIAL DEFICIENCY $ 3,040

Young S. Woo

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