Sterile Topical Dosage Forms I: Laboratory Phase

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Abstract □ A laboratory investigation was conducted to evaluate the use of conventional sterilizing techniques to manufacture sterile topical dosage forms. Four types of topical dosage forms were investigated: (*a*) an ophthalmic ointment, (*b*) a topical cream, (*c*) a topical ointment, and (*d*) a topical lotion. Dry-heat sterilization was found to be an acceptable method to sterilize the ophthalmic ointment vehicle. Moist-heat sterilization was found to be an acceptable method to sterilize the topical cream vehicle, part of the topical ointment vehicle, and also part of the topical lotion vehicle. Membrane filtration sterilization was found to be an acceptable method to sterilize the active ingredients in the topical cream, part of the topical ointment vehicle, and the active ingredients and part of the topical lotion vehicle. Ethylene oxide gas sterilization was found to be an acceptable method to sterilize an insoluble, inactive ingredient and the ointment tubes for all four types of topical dosage forms. Residual ethylene oxide determinations showed that longer "venting" times were required for phenolic and urea–formaldehyde capped tubes than polyethylene capped tubes. A microorganism suspension, consisting of *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger*, and *Candida albicans*, was used in the contamination studies. In all cases, the sterilized contaminated samples passed the sterility test. A variety of analytical techniques was employed to verify that the sterilizing procedures did not degrade the sterilized material. Included in these techniques were GC, color, and rheology. Aseptic processing of the sterilized components, under conventional sterile room conditions and under laminar air flow, did not present any major problems. All samples passed the finished-product sterility test. All sterile products passed the nonsterile finished-product specifications and showed equivalent chemical and physical stability.

Keyphrases □ Topical dosage forms—evaluation of commercial sterilizing techniques, effect of sterilization techniques on various dosage forms □ Sterilization techniques—evaluation of methods used for manufacturing various topical dosage forms, effect on formulation □ Microbiological contamination of topical dosage forms—evaluation of commercial sterilizing techniques

A 1965 report of the Swedish National Board of Health showed various topical dosage forms, such as ophthalmic ointments, topical ointments, and topical creams, to have varying degrees of bacterial contamination (1). The facts that the pathogen, *Pseudomonas aeruginosa*, was found in an ophthalmic ointment and that 11 of the 28 ophthalmic ointments tested contained some sort of bacterial contamination were surprising to many people in the pharmaceutical industry.

Except for a few articles (2–8), the literature contains little information on the preparation of sterile topical dosage forms. In view of this observation and because several pharmacopeias require ophthalmic ointments to be sterile and because at least one country requires all topical steroid dosage forms to be sterile, this study was conducted. The purpose of the study was to determine, at the laboratory scale, if sterile topical dosage forms could be prepared using existing, conventional, sterilizing procedures. Gamma radiation was not included in this study since it was being investigated by another group within these laboratories. Four types of topical dosage forms were investigated: (*a*) an ophthalmic ointment (a petrolatum vehicle), (*b*) a topical cream (an oil-in-water emulsion), (*c*) a topical ointment (an aqueous polyethylene glycol–propylene glycol vehicle), and (*d*) a topical lotion (an aqueous hydroxyethyl cellulose vehicle).

EXPERIMENTAL

The laboratory work was divided in two phases. The objectives of Phase I were: (*a*) to investigate the conventional procedures which could be used to sterilize the complete vehicle, a portion of the vehicle, or a single component of the product; (*b*) to verify that the procedure(s) did indeed sterilize by deliberately contaminating with known amounts of microorganisms before the sterilizing step; and (*c*) to verify that the procedure(s) did not degrade the component(s) sterilized by doing appropriate physical and chemical testing. The objectives of Phase II were: (*a*) to demonstrate that sterile products could be made by aseptically compounding the sterile components in a conventional sterile room (a positive pressure room fed with "Hepa" filtered air and equipped with direct UV lights) or a vertical laminar air flow hood, and (*b*) to determine if the sterile products passed the release specifications of the nonsterile products and had adequate stability.

Ophthalmic Ointments—The ophthalmic ointments consisted of active ingredients and a petrolatum vehicle. The active ingredients were available as sterile solids.

Since the petrolatum vehicle was anhydrous, wet-heat sterilization (121°, 15 lb. pressure, 20 min.) was not considered. However, sterilization by both dry heat and filtration was investigated. The filtration technique was limited to membrane disks, since depth filtration could present flowthrough problems during subsequent pilot plant and production operations. An MF Millipore-type GS filter was used.

The petrolatum vehicle was seeded with a mixture of microorganisms at a level of 100,000 organisms/gram. The mixture consisted of equal parts of *Staphylococcus aureus* ATCC 6588 (representing an airborne organism), *Bacillus subtilis* ATCC 6633 (representing a spore former), *Pseudomonas aeruginosa* ATCC 9027 (representing a Gram-negative organism from which ointments should be free according to USP XVIII), *Escherichia coli* ATCC 8739 (representing fecal contamination), *Aspergillus niger* ATCC 16404 (representing mold), and *Candida albicans* ATCC 10231 (representing yeast). Half of the inoculated samples served as controls; they were not subjected to sterilizing conditions. The USP XVII sterility test was employed using thioglycollate and Sabouraud media. For this particular vehicle, the sample was prepared by heating it to 50° and aseptically mixing it with 5 ml. of a sterile 0.5% aqueous solution of polysorbate 80 which had been heated to 50°.

Topical Creams—The topical creams consisted of water-soluble active ingredients in an oil-in-water emulsion vehicle. Membrane filtration of an aqueous solution of the active ingredients and wet-heat sterilization of the emulsion were investigated. Aqueous solutions of the active ingredients and the emulsion vehicle were seeded with microorganisms to challenge the sterilization procedures. Again, the USP XVII sterility test was employed.

Topical Ointments—The topical ointments were more complicated. They were made up of water-insoluble active ingredients, water-insoluble inactive ingredients, and a water-miscible vehicle. The active ingredients were available as sterile solids. A benzalkonium chloride solution was sterilized by membrane filtration.

Because of its low melting point, zinc stearate could not be sterilized by dry- or wet-heat treatment and had to be sterilized with ethylene oxide gas. The gas sterilization experiments were conducted in a portable tabletop unit[1], using a 10% ethylene oxide–90%

[1] Cryotherm, model 1036, American Sterilizer Co., Erie, Pa.

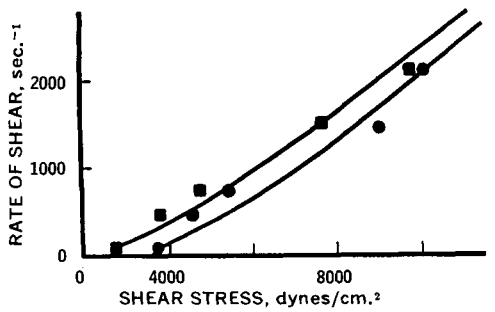

Figure 1—*Typical flow curve before* (■) *and after* (●) *dry-heat treatment of the petrolatum vehicle.*

carbon dioxide mixture[2] at a 50% relative humidity. The relative humidity was controlled by adding a fixed volume of purified water USP to the chamber and was monitored by placing a portable humidigraph[3] in the chamber.

Autoclaving and membrane filtering were explored for the mixture of water-miscible inactive ingredients, which consisted of polyethylene glycol, propylene glycol, and water. Again, the various parts of the vehicles were seeded with microorganisms and the USP XVII sterility test was employed to determine if the procedures did indeed sterilize.

Topical Lotions—This dosage form was essentially an aqueous solution of drugs, buffers, and preservatives with a macromolecule acting as a viscosity-inducing agent. The aqueous solution portion of the formulation was sterilized by membrane filtration. Both membrane filtration and wet wall techniques were explored as possible methods for sterilizing the hydroxyethyl cellulose solution.

As noted previously, both portions of the formulation were seeded with microorganisms and the USP XVII sterility test was performed on control and sterilized samples.

Ointment Tubes—Three sterilization methods were investigated, namely dry heat, wet heat, and ethylene oxide gas. Gas sterilization was carried out as described under *Topical Ointments*.

The sterilization procedure was challenged by deliberately contaminating with 100,000 organisms/tube. The USP XVII sterility test was employed by rinsing the tubes with the thioglycollate and Sabouraud media.

RESULTS AND DISCUSSION

Ophthalmic Ointments—Because of low filtration rates, sterilization of the petrolatum vehicle with membrane disks was abandoned. Dry-heat sterilization, 150° for 2 hr., was successful.

Prolonged heating of the petrolatum vehicle yields low molecular weight aldehydes and peroxides which can be detected by changes in color, rheology, and GLC. None of these changes was noted. Color was measured with a color and color difference meter[4]. The rheology of the petrolatum vehicle was studied with a viscometer[5]. Figure 1 shows a typical flow curve before and after dry-heat treatment. The presence of aldehydes and ketones was checked with GLC[6].

Dry-heat sterilization of the petrolatum vehicle passed the microbial contamination tests, as illustrated in Table I.

A total of 10 1-kg. lots of sterile ophthalmic ointments was prepared successfully using aseptic processing techniques in both a conventional sterile room and in a vertical laminar air flow hood[7]. The samples were prepared for the USP XVII sterility test by heating a tube of ointment to 50°, aseptically removing 1 g., and aseptically mixing it with 5 ml. of a sterile 0.5% aqueous polysorbate 80 solution which had been heated to 50°.

Conventional sterilizing techniques such as wet and dry heat were employed to sterilize all the manufacturing equipment except the roller mill. The roller mill was sterilized by wiping it with a 5% aqueous phenol solution and storing it under UV lights or in the laminar air flow hood for 24 hr. before use. Microbial contamination studies (Table I) supported this procedure.

[2] Carboxide, Linte Co., New York, N. Y.
[3] Model 4044H, The Bristol Co., Waterbury, Conn.
[4] Hunterlab, model D25, Hunter Associates Laboratory, McLean, Va.
[5] Rotovisco, Gebriider Haake K. G., Berlin-Steglitz, Siemen-Strasse 27.
[6] Tracor MT-220.
[7] Dexon, model VF66E, Minneapolis, Minn.

Table I—Efficacy of Sterilization Procedure Employed

Test Sample[a]	Number of Samples—Not Sterilized—Tested	Not Sterilized—Failed USP Sterility Test	Sterilized—Tested	Sterilized—Failed USP Sterility Test
Petrolatum vehicle	2	2	2	0
Stainless steel from roller mill	3	3	3	0
Plastic from roller mill	5	5	5	0
Emulsion vehicle	2	2	2	0
Dexamethasone sodium phosphate solution	3	3	3	0
Neomycin sulfate solution	3	3	3	0
Benzalkonium chloride solution	3	0	3	0
Zinc stearate	2	2	2	0
Water-miscible vehicle	2	2	2	0
Aqueous solution of drugs, *etc.*	3	3	3	0
Aqueous solution of viscosity-inducing agent	3	3	3	0
Tin tube with polyethylene cap	4	4	4	0
Araldite-lined aluminum tube with urea–formaldehyde cap	2	2	2	0
Tin tube with phenolic cap	2	2	2	0

[a] Each test sample was inoculated with a mixture consisting of equal parts *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *A. niger*, and *C. albicans* at a level of 100,000 organisms/g.

Topical Creams—Membrane filtration sterilization of the active ingredients in aqueous solution and wet-heat sterilization of the emulsion vehicle proved to be successful. No changes in color, rheology, or GLC were noted for the emulsion vehicle. Figure 2 shows a typical flow curve before and after wet-heat sterilization of the emulsion vehicle.

Membrane filtration of the active ingredients in aqueous solution and wet-heat sterilization of the emulsion vehicle passed the microbiological contamination tests (Table I). The unsterilized neomycin sulfate solution failed the sterility test.

Four lots, 5 kg. in size, of sterile topical creams were successfully prepared using aseptic processing techniques in both a conventional sterile room and a vertical laminar air flow hood. The samples were tested by the USP XVII sterility test method.

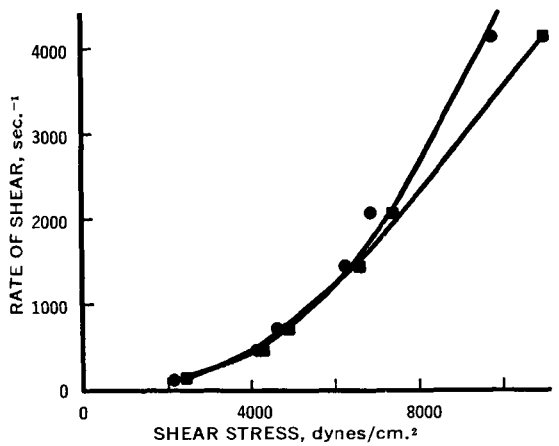

Figure 2—*Typical flow curve before* (■) *and after* (●) *wet-heat treatment of the emulsion vehicle.*

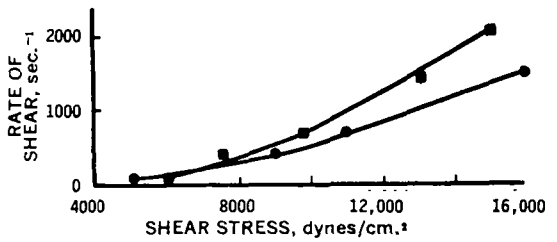

Figure 3—*Typical flow curve before* (■) *and after* (●) *wet-heat treatment of the topical ointment vehicle.*

Stability samples passed the release specifications (appearance, viscosity, pH, preservative assays, and active ingredient assays) and proved to have shelflives equivalent to the nonsterile products.

Topical Ointments—Sterilization of the polyethylene glycol–propylene glycol–water solution by membrane filtration was abandoned because of low filtration rates.

Membrane filtration of the benzalkonium chloride solution, ethylene oxide gas sterilization of the zinc stearate, and wet-heat sterilization of the water-miscible vehicle proved to be successful.

No differences were observed between untreated and treated zinc stearate in color or GLC. Ethylene oxide could react with the zinc stearate, altering the alkyl side-chain distribution of free fatty acids. The sterilized zinc stearate had to be vented for at least 48 hr. at 23° to obtain less than 25 p.p.m. residual ethylene oxide. The residual levels were determined by the potassium acid phthalate–nitrobenzylpyridine colorimetric method.

No changes were observed in color, rheology, or GLC for the water-miscible vehicle. Prolonged heating of polyethylene glycol yields low molecular weight aldehydes, ketones, ethylene glycol, and water. The aldehydes and ketones were colored compounds. Also, negative chemical tests for aldehydes and ketones were observed with Schiff's reagent and 2,4-dinitrophenylhydrazine. Figure 3 shows a typical flow curve before and after wet-heat treatment.

Membrane filtration of the benzalkonium chloride solution, ethylene oxide gas sterilization of the zinc stearate, and wet wall sterilization of the polyethylene glycol–propylene glycol–water solution passed the microbial contamination tests (Table I). The unsterilized benzalkonium chloride solution passed the sterility test.

Four 1-kg. lots of sterile topical ointments were successfully prepared using aseptic processing techniques in both a conventional sterile room and a vertical laminar air flow hood. The samples were tested by the USP XVII sterility test method.

Stability samples passed the release specifications (appearance, viscosity, pH, preservative assays, and active ingredient assays) and proved to have shelflives equivalent to the nonsterile products.

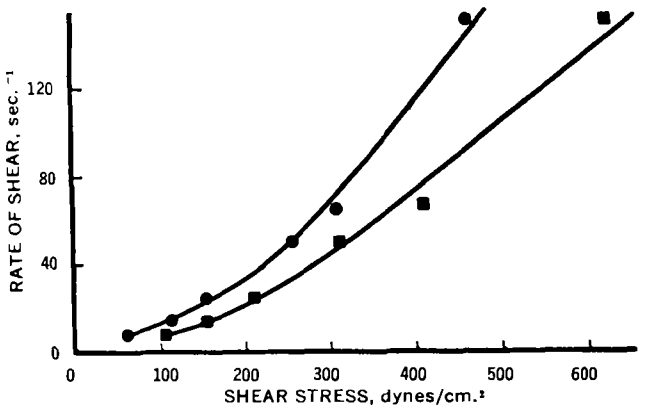

Figure 4—*Typical flow curve before* (■) *and after* (●) *wet-heat treatment of the hydroxyethyl cellulose solution.*

Topical Lotions—Sterilization of the hydroxyethyl cellulose solution by membrane filtration was abandoned because of slow filtration rates. Membrane filtration of the aqueous solution of drugs, buffers, and preservatives and wet-heat sterilization of the hydroxyethyl cellulose solution proved to be successful. No change in color but a decrease in viscosity of the macromolecular solution was observed. Figure 4 shows a typical flow curve before and after wet-heat treatment.

Membrane filtration of the aqueous solution containing the drugs, buffers, and preservatives and wet wall sterilization of the aqueous hydroxyethyl cellulose solution passed the microbiological seeding experiments (Table I).

Two 1-kg. lots of sterile topical lotions were successfully prepared using aseptic processing techniques in both a conventional sterile room and a vertical laminar air flow hood.

Samples placed on a stability testing program showed that the sterile topical lotions passed all release specifications (appearance, pH, preservative assays, specific gravity, and active ingredient assays) for the nonsterile topical lotion except the viscosity specification. Use tests indicated that the viscosity specification could be changed and was adjusted accordingly. Stability samples showed the sterile topical lotions to have a shelflife equivalent to the nonsterile topical lotions.

Ointment Tubes—Dry- and wet-heat sterilization was not possible because: (*a*) the plastic caps (phenolic, polyethylene, and urea–formaldehyde) deformed above 100°; (*b*) the "Westite" seal in the ophthalmic ointment tubes melted at 121°; and (*c*) the "peel-off" labels darkened at temperatures above 100°.

Ethylene oxide gas sterilization of the tubes proved to be successful and passed the microbial contamination test (Table I).

Residual levels of ethylene oxide were determined by the colorimetric thiosulfate–bromthymol blue method. Different venting conditions had to be developed to obtain residual levels below 25 p.p.m. The tin tube with the phenolic cap and the "Araldite"-lined aluminum tube with the urea–formaldehyde cap were vented for 24 hr. at 45°, whereas the tin tube with the polyethylene cap was vented for 48 hr. at 23°.

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