Data analysis and graphical examination of the results were performed on the PROPHET computer system, a specialized resource developed by the Chemical/Biological Information Handling Program of the National Institutes of Health (43).

We wish to thank Ms. Deborah J. Johnson and Mr. Richard Wagner for their able technical assistance during the course of this study and to Imperial Chemical Industries, Ltd. (Macclesfield, England) for their generous supplies of *S*(−)- and *R*(+)-propranolol hydrochloride.

NOTES

Sterility Testing of Fat Emulsions Using Membrane Filtration and Dimethyl Sulfoxide

A. M. PLACENCIA ˣ, G. S. OXBORROW, and J. W. DANIELSON

Received July 28, 1981, from the *Sterility Research Center, Minneapolis Center for Microbiological Investigations, Food and Drug Administration, Minneapolis, MN 55401.* Accepted for publication September 18, 1981.

Abstract □ A method was described for sterility testing of 10% fat emulsions, which consisted of solubilizing the emulsion in dimethyl sulfoxide and filtering the mixture using a polyester membrane. The procedure was rapid and avoided the problems of turbidity and plugging of the membrane filter encountered with other methods.

Keyphrases □ Dimethyl sulfoxide—sterility testing of 10% fat emulsions using membrane filtration □ Membrane filtration—sterility testing of 10% fat emulsions, dimethyl sulfoxide □ Fat emulsions—sterility testing using membrane filtration and dimethyl sulfoxide

Intravenous fat emulsions are used in peripheral and central vein infusions. Since bacterial contamination might be a problem with this kind of human drug product, a reliable method for sterility testing is necessary to ensure that possible contamination is detected.

The two basic methods for sterility testing (direct inoculation and membrane filtration) described in the USP XX (1) are not suitable to test this product. Direct inoculation of the product into growth media renders the media turbid and makes it impossible to observe microbial growth. To detect growth, various transfers must be made from the original inoculated media to fresh media, thus increasing the possibilities of contamination and extending the analysis time. Only small quantities of the product can be tested with this method. Membrane filtration of the product is difficult because the emulsified fat globules will not pass the commonly used membrane filters, and the filter pores become plugged. The present report describes a method for the sterility testing of fat emulsions in which membrane filtration is combined with the use of a solvent, dimethyl sulfoxide.

As part of the study, dimethyl sulfoxide was tested for bacteriostatic and fungistatic properties because of conflicting reports concerning its antimicrobial effects. It was shown (2) that 10% dimethyl sulfoxide protects and aids in the recovery of heat-shocked *Bacillus subtilis* spores. The high recovery of the bacteria has been linked to the capability of the compound to activate dormant spores (3, 4). At concentrations of 10, 20, and 30% in food transport systems, dimethyl sulfoxide acts as a cryoprotective agent and increases the survival of some bacteria (5). A solution of 10% dimethyl sulfoxide yielded a 100% recovery rate of *B. subtilis* phage after storage of the phage for 25 days at −20° (6). During freezing and thawing of *Aerobacter aerogenes* and red blood cells, 10–20% dimethyl sulfoxide increased the organisms' viability (7, 8). Dimethyl sulfoxide (100%) had no diffusible bacteriostatic activity when tested in disk agar diffusion sensitivity studies with various organisms (9). Normal growth of test organisms was obtained after 15-min exposure to 200,000 ppm (20%) dimethyl sulfoxide (10).

Dimethyl sulfoxide has been classified by other researchers as weakly antibacterial and antifungal (11). Bacterial growth was inhibited when 20% dimethyl sulfoxide was used in media (12, 13). Pottz *et al.* (14) found that 5–10% dimethyl sulfoxide was bacteriostatic and 20–80% bacteriocidal. Dimethyl sulfoxide (25%) was reported to inhibit the growth of bacteria isolated from leukemia and cancer patients (15). Dimethyl sulfoxide affects a wide range of bacteria and fungi in concentrations used in antimicrobial testing programs in the pharmaceutical industry (16).

The described method was designed to test the bacteriostatic and fungistatic capabilities of dimethyl sulfoxide to be used in the sterility testing of fat emulsions. Decimal reduction (D values) were determined using various test organisms.

EXPERIMENTAL

Dimethyl Sulfoxide Preparation—Dimethyl sulfoxide[1] was filter-sterilized using a polyester membrane[2] (0.2-μm pore size, 47-mm diameter) and stored in 25- and 50-ml amounts in sterile glass screw-capped tubes.

Sterility Testing of Fat Emulsions—Aliquots (100 ml) of a 10% intravenous fat emulsion[3] were aseptically transferred to sterile 38 × 200-mm glass screw-capped test tubes. To each tube, 25 ml of dimethyl sulfoxide was added. The mixture was vigorously stirred for 30 sec using a vortex mixer and then filtered through a 0.4-μm polyester membrane[2]. The membrane was rinsed with 100 ml of fluid D (1) and two 100-ml portions of fluid A (1), cut into two sections, transferred to fluid thioglycolate and soybean casein digest broth, and incubated as described in USP XX (1).

Bacteriostatic and Fungistatic Testing of Dimethyl Sulfoxide—Bacterial stock cultures containing <100 organisms/ml of the fol-

[1] Mallinckrodt Chemical Co., St. Louis, Mo.
[2] Nucleopore Corp., Pleasanton, Calif.
[3] Cutter Laboratories, Inc., Berkeley, Calif.

0022-3549/82/0600-0704$01.00/0

lowing ATCC test organisms were used to test dimethyl sulfoxide for bacterio- and fungistasis: *Bacillus subtilis* (6633), *Staphylococcus aureus* (6538), *Candida albicans* (10231), *Pseudomonas aeruginosa* (9027), *Clostridium perfringens* (11437), and *Aspergillus niger* (16404).

Six sets of four tubes each, containing 100 ml of fat emulsion[3], were prepared. Each tube was inoculated with 1 ml of a test organism suspension. Dimethyl sulfoxide (25 ml) was added to two tubes per set, and 50 ml to the remaining two tubes. All tubes were vigorously stirred for 30 sec using a vortex mixer. The mixtures were membrane-filtered using a 0.4-μm polyester membrane[2]. The membranes were washed with 200 ml of fluid A (1) and 100 ml of fluid D (1) and then cut into two sections. One section was transferred to fluid thioglycolate and the other to soybean casein digest broth. The fluid thioglycolate was incubated at 33 ± 2° and soybean casein digest broth at 23 ± 2°, both for 7 days (1).

D Value Determination—D values were determined using *B. subtilis* and *C. albicans*. *C. albicans* was used because the results of the bacteriostatic and fungistatic test showed that it was more sensitive to dimethyl sulfoxide at concentrations of 33%.

Spore stock suspensions containing ~10^9 *B. subtilis* organisms/ml of water were used for inoculum.

Cultures of *C. albicans* were grown in 13 × 100-mm trypticase soy agar slants at 37 ± 2° for 72 hr. The slants were washed with 2 ml of phosphate buffer to a final concentration of 10^9 organisms/ml.

Six 25-ml aliquots of a 10% fat emulsion were transferred to separate glass screw-capped test tubes. Three of the tubes were each inoculated with 0.1 ml of a spore stock suspension of *B. subtilis* and the other three tubes with *C. albicans*. The contents of the tubes were vigorously stirred using a vortex mixer, and 6.25 ml of dimethyl sulfoxide was added to each tube. The tubes were stirred as before, and 5-ml aliquots were taken at 0, 15, 30, 45, and 60 min. Each aliquot was filtered through a 0.4-μm polyester membrane. The membranes were washed with 100 ml of fluid A and transferred to 38 × 200-mm test tubes containing 100 ml of fluid A. The tubes were ultrasonicated for 6 min at 55 kHz and dilutions plated using trypticase soy agar. The plates were incubated at 35 ± 2° for 48 hr.

RESULTS

The results show that 20% dimethyl sulfoxide was not bacteriostatic or fungistatic to the test organisms used in this study. The growth rate of the sample was comparable to that of the control samples. A concentration of 33% dimethyl sulfoxide showed some fungistasis only against *C. albicans* after 7 days incubation at 23 ± 2°.

When 20% dimethyl sulfoxide was used, the D values of *B. subtilis* and *C. albicans* were >60 min.

The polyester membrane had good filtration rates, taking ~4 min to filter dimethyl sulfoxide and the emulsion. No problem was noted in the rinsing of the membrane with fluids A and D. Other membranes tested, such as acetate cellulose, pyroxylin, mixed esters of cellulose, and mixed cellulose acetate pyroxylin, were either dissolved by dimethyl sulfoxide or the pores were plugged by the fat emulsion.

In contrast to the direct inoculation of a product, the entire product could be analyzed in this membrane filtration method, with no problem of the media showing turbidity upon inoculation. The use of dimethyl sulfoxide as a solvent and of polyester membrane filters for the filtration of the product and rinsing fluids provided a rapid method of analysis. Since dimethyl sulfoxide showed no bacteriostasis or fungistasis and the analysis time was short, the probability of obtaining a better bacterial recovery is greatly increased by using the described method.

REFERENCES

(1) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Inc., Rockville, Md., 1980, pp. 873–882.

(2) L. B. Quesnel, B. L. Scott, and J. L. Taylor, in "Spore Research," J. Wolf, Ed., Academic, New York, N.Y., 1971, pp. 303–314.

(3) D. A. Cotter, J. W. Morin, and R. W. O'Connell, *Arch. Microbiol.*, **108**, 93 (1976).

(4) J. P. Widdowson, *Nature*, **214**, 812 (1967).

(5) A. W. Kotula, M. D. Pierson, B. S. Emswiler, and J. R. Guilfoyle, *Appl. Environ. Microbiol.*, **38**, 789 (1979).

(6) C. O. Yehle and R. H. Doi, *Can. J. Microbiol.*, **11**, 745 (1965).

(7) C. E. Huggins, *Transfusion*, **3**, 483 (1963).

(8) T. Nash, *Nature*, **4898**, 1113 (1963).

(9) G. S. Kalesperis, K. V. Prahlad, and D. L. Lynch, *Can. J. Microbiol.*, **21**, 213 (1975).

(10) V. L. Miller and A. C. Jerstad, *J. Med. Chem.*, **9**, 208 (1966).

(11) A. M. Kligman, *J. Am. Med. Assoc.*, **193**, 923 (1965).

(12) S. W. Jacob, M. Bischel, and R. J. Herschler, *Curr. Ther. Res.*, **6**, 134 (1964).

(13) D. C. Wood and J. Woo, *Ann. N.Y. Acad. Sci.*, **243**, 7 (1975).

(14) G. E. Pottz, J. H. Rampey, and F. J. Benjamin, *ibid.*, **141**, 261 (1967).

(15) F. B. Seibert, F. K. Farrelly, and C. C. Sheperd, *ibid.*, **141**, 175 (1967).

(16) H. Basch and H. H. Gadebusch, *Appl. Microbiol.*, **16**, 1953 (1968).

Tensile Strengths and Hardness of Tablets

PAUL J. JAROSZ * and EUGENE L. PARROTT ˣ

Received June 8, 1981, from the *Division of Pharmaceutics, College of Pharmacy, University of Iowa, Iowa City, IA 52242.* Accepted for publication October 13, 1981. *Present address: Ortho Pharmaceutical Corp., Raritan, NJ 08869.

Abstract □ The axial and radial tensile strengths were compared to the hardness of compressed tablets containing various concentrations of lubricants. Since radial tensile strength measurement considers the thickness of a tablet, and only tensile stress and axial tensile strength express the strength in the direction in which capping may occur, the tensile strengths characterize the strength of a tablet more completely than hardness.

Keyphrases □ Tensile strength—axial and radial tensile strengths compared to the hardness of compressed tablets □ Tablets—axial and radial tensile strengths compared to the hardness of compressed tablets

Although the strength of pharmaceutical tablets, which must be sufficient to withstand handling and shipment, may be expressed in a variety of ways, hardness (the force which, when applied diametrically to the tablet, causes fracture) has been the most common expression of strength. Studies of various hardness testers have shown the variations in fracture strength to be due to inaccuracies of instrumental scale values, zero errors, variations in the method of application of the load, physical dimensions, and shape of the tablet (1–4).

Although hardness has been a convenient and useful parameter for in-process control and quality assurance, it

0022-3549/82/0600-0705$01.00/0