Solubilization of Lecithin in Antibiotic Preparations by a Nonionic Surfactant

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A nonionic surfactant (alkyl phenoxy polyethoxy ethanol) solubilizes antibiotic preparations containing up to 1 per cent lecithin. Toxicity studies showed that the surfactant at a concentration of 0.1 per cent (v/v) in 0.1 per cent peptone solution (w/v) permits recovery from a low inoculum of vegetative cells when membrane filtration sterility tests are performed. The addition of the surfactant to peptone diluting fluid makes it possible to perform membrane filtration tests on many antibiotic preparations that otherwise could only be tested by the direct method. The use of surfactant–peptone diluting fluid also makes it possible to use 300 mg. from each of 20 vials instead of 50 mg. when testing oxytetracycline, chlortetracycline, or tetracycline for sterility.

In 1964, the Antibiotic Regulations were amended (1) to permit the testing of the sterility of antibiotics by membrane filtration (2), in addition to the direct test. Membrane filtration is now specified in 51 of the 121 monographs, the direct method in 41, and either in 29.

In the direct method, the antibiotic must be inactivated so that it does not interfere in the test, *e.g.*, penicillin is inactivated by the addition of the enzyme penicillinase. Thus the direct method is unsuitable for preparations containing penicillin combined with streptomycin or dihydrostreptomycin for which there are no suitable inactivators.

The membrane filtration technique, on the other hand, requires that the test material be soluble enough to pass through the pores of the membrane filter without clogging them; therefore, its use is limited to materials that are soluble or can be solubilized in some way. For example, in combination with streptomycin or dihydrostreptomycin, mentioned above, the slightly soluble procaine penicillin G is converted by the addition of penicillinase to soluble penicilloic acid until its concentration is reduced to about 5 mg./ml. At this concentration the antibiotic is dissolved (3). Since streptomycin and dihydrostreptomycin are both in solution, the formulation may then be filtered without difficulty.

However, problems are presented by formulations containing emulsifying agents, such as sodium carboxymethylcellulose or lecithin, a water-dispersible phospholipid with strongly polar groups. These compounds are used as suspending and/or homogenizing agents to produce oil-in-water emulsions of antibiotics that are rather insoluble in water. Although these agents improve the resuspendability and syringeability of the aqueous suspensions of antibiotics, the resulting suspensions are not completely soluble at the concentrations used in the membrane filtration test, and they tend to clog the filter. The clogged membrane will either stop the filtration or greatly extend the filtration time. In either case, a filtration sterility test cannot be performed.

Because of these difficulties, a study was undertaken to devise a reliable method for solubilizing injectable preparations containing lecithin. The first approach involved the use of lecithinase to hydrolyze lecithin, in analogy with the use of penicillinase to convert procaine penicillin G to a soluble substance. However, this approach was unsuccessful.

The authors then explored the use of a polyoxyethylene nonionic surfactant (alkyl phenoxy polyethoxy ethanol).[1] Russomanno and Wollish (4) reported using this surfactant to wash and remove oil from the membrane following a filtration sterility test for oils. Since lecithin is used to produce oil-in-water emulsions of antibiotics, it seemed feasible that the surfactant could be used to solubilize these emulsions so that they would be suitable for membrane filtration sterility testing. This approach proved more successful.

EXPERIMENTAL

In the usual membrane filtration sterility test, the sample is added to a sterile 500-ml. conical flask containing approximately 200 ml. of a 0.1% peptone (w/v) solution. The flask is stoppered and swirled to dissolve the drug. The solution is then filtered aseptically.

Ten different antibiotic formulations containing varying amounts of lecithin were used in the tests with the surfactant (see Table I). When 0.1% peptone alone was used as the diluting fluid, the preparations were insoluble and could not be filtered. Adding the surfactant at 0.1% (v/v) to the peptone diluting fluid solubilized each of the preparations so that they could be filtered through a 0.45-μ membrane filter. The total time required to filter the antibiotic preparation and make the three washings did not exceed 20 min.

The solubility of lecithin in 0.1% surfactant–peptone solution was determined on samples of commercial grade lecithin used by two antibiotic manufacturers. Lecithin was added to the surfactant–peptone diluting fluid at 25° until the fluid became turbid. Additional surfactant–peptone diluting fluid was added until the turbidity disappeared. This titration procedure established that 0.5 mg./ml. of lecithin is completely soluble in 0.1% peptone solution containing the surfactant at a concentration of 0.1%. Apparently the successful use of filtration sterility tests for preparations containing lecithin depends on the final concentration of lecithin in the diluting fluid after the preparation has been pooled as directed in the Antibiotic Regulations (1). If this final concentration of lecithin in surfactant–peptone diluting fluid does not exceed

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[1] Available as Triton X-100 from Rohm and Haas, Philadelphia, Pa.

TABLE I—ANTIBIOTIC PREPARATIONS CONTAINING VARYING AMOUNTS OF LECITHIN SOLUBILIZED BY THE SURFACTANT FOR THE FILTRATION STERILITY TEST

Preparation	% Lecithin in Formulation
Procaine penicillin G in streptomycin sulfate soln. (1.0% monothioglycerol)	0.5
Procaine penicillin G and buffered sodium penicillin G for aqueous suspension	0.1
Procaine penicillin G in dihydrostreptomycin soln.	0.1
Procaine penicillin G in dihydrostreptomycin soln.	0.25
Procaine penicillin G, potassium penicillin G, and streptomycin sulfate for aqueous injection	1.0
Procaine penicillin G and buffered potassium penicillin G for aqueous injection	0.3
Procaine penicillin G, potassium penicillin G, and streptomycin sulfate for aqueous injection	0.2
Procaine penicillin in dihydrostreptomycin with prednisolone	0.28
Procaine penicillin G in aqueous suspension	1.0
Chloroprocaine penicillin O for aqueous injection	1.5

0.5 mg./ml., the solutions filter rapidly through a 0.45-μ membrane, whereas the same amount of lecithin is insoluble in peptone diluting fluid alone, and cannot be filtered satisfactorily. Furthermore, when up to 2 mg./ml. of lecithin is added to surfactant–peptone diluting fluid alone, the solution can be filtered, even though it is cloudy; yet when this concentration of lecithin is combined with actual samples containing antibiotics and other components, results are variable: sometimes the solutions can be filtered, sometimes they cannot.

The surfactant was also found to be useful in the filtration procedures for antibiotics that do not contain lecithin. In the standard filtration procedure, a total of 6 Gm. (300 mg. from each of 20 containers) of the antibiotic is added to 200 ml. of the peptone diluting fluid, resulting in a 3% solution. Some antibiotics, such as the tetracyclines, cannot be filtered at this concentration; only 50 mg. can be taken from each container instead of 300 mg., and therefore only 1 Gm., a 0.5% solution, can be tested. When surfactant was added to the peptone diluting fluid, the usual 3% solution could be filtered and the standard amount of the antibiotic could be tested for sterility.

When the filtration procedures for sterility testing were developed, 0.1% peptone was selected as the diluting fluid because it protected the vulnerable vegetative cells in an antibiotic milieu. Before proposing that the surfactant be added to this diluting fluid, it was necessary to investigate its toxicity to microorganisms. Russomanno and Wollish (4) reported that it was nontoxic when in contact with vegetative forms of bacteria for several hours. Dowben and Koehler (5), in a study on the interaction of the surfactant with bovine plasma, found that no gross structural changes occur in the protein, *i.e.*, proteins are not denatured. The following study on the survival of eight microorganisms in surfactant–peptone solution confirms these findings.

TABLE II—SURVIVAL OF ORGANISMS IN SURFACTANT–PEPTONE SOLUTIONS FOR 30 min. AT 25° AS DETERMINED BY MEMBRANE FILTRATION STERILITY TESTS

Organism	Estimated No. Cells of Inoculum	Day Showing Growth After Incubation: Thioglycollate at 32°	Day Showing Growth After Incubation: Sabouraud at 25°
Bacillus subtilis ATCC 6633	44	3	6
Bacillus cereus ATCC 11778	52	2	3
Staphylococcus aureus ATCC 6538P	132	3	6
Sarcina lutea ATCC 9341	66	3	4
Streptococcus faecalis	160	2	3
Klebsiella pneumoniae ATCC 10031	88	1	3
Pseudomonas sp. ATCC 19146	154	3	3[a]
Saccharomyces cerevisiae ATCC 9763	64	3	2

[a] pH was adjusted to 7.0 since this organism will not grow at pH 5.7 ± 0.1.

Eight organisms representing Gram-positive and negative bacilli, Gram-positive cocci, and yeasts were used. A stock suspension of each organism was prepared, and a viable cell count was made by the plate method. The stock suspensions were further diluted with 0.1% peptone so that 1 ml. contained from 40 to 160 cells. One milliliter of the dilute suspension was added to each of 10 flasks containing 200 ml. of surfactant–peptone solution, and the flasks were held at room temperature. After a 30-min. contact period, the viability of the organisms added to the surfactant–peptone was determined by the membrane filtration sterility tests (1). Table II shows that all microorganisms were recovered from small inocula in both thioglycollate and Sabouraud liquid media after incubation for 1–5 days.

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