Batch-Swirl Method for Detoxification of Isopropyl Myristate Used for Sterility Testing of Oils and Ointments: Membrane Selection

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Abstract □ A 1-hr batch-swirl method was developed for the preparation of isopropyl myristate to be used in the sterility testing of oils and ointments. The method is simpler and faster than that in *USP XX*. Flow rates of various membrane filters were tested. Cellulose acetate filters increased the filtration rate of oils and ointments and, thus, reduced the exposure time of possible microbial contaminants to the toxicity of isopropyl myristate.

Keyphrases □ Isopropryl myristate—batch-swirl method for detoxification, sterility testing of oils and ointments □ Microbial contamination—batch-swirl method for detoxification of isopropyl myristate used for sterility testing of oils and ointments □ Toxicity—batch-swirl method for detoxification of isopropyl myristate, sterility testing of oils and ointments

Problems are encountered when the *United States Pharmacopeia XX* (USP XX) method is used for the sterility testing of ointments and oils soluble in isopropyl myristate (1). Commercially prepared isopropyl myristate, which is used to dissolve the specimen, is toxic to microorganisms. It was found that the toxicity of isopropyl myristate is partially due to its pH variability, *i.e.*, the higher the pH of isopropyl myristate, the higher the decimal reduction (D value) of *Pseudomonas aeruginosa* (2, 3).

Heated isopropyl myristate has also been shown to be toxic to vegetative microorganisms during prolonged holding temperatures at 47° (3–5). Detoxification of isopropyl myristate by the USP method, which takes more than 8 hr to complete, makes the analysis time-consuming.

USP XX requires rinsing of the membrane with fluids A and D after a sample is filtered. However, after filtration of isopropyl myristate, the mixed cellulose acetate–pyroxylin membrane becomes hydrophobic and the water-based fluids, A and D, are difficult to filter.

Studies have indicated that the D value of *P. aeruginosa* is lower if the microorganisms are filtered through a 0.2-μm membrane rather than through a 0.45-μm membrane. The slower filtration rate of the 0.2-μm filter exposes the microorganisms to the isopropyl myristate for a longer time. Other authors have indicated that the use of the conventional membrane filtration technique may be the cause of low recovery rates of *P. aeruginosa* from oils or ointments (3, 6).

The present report describes a 1-hr batch-swirl method for the detoxification of isopropyl myristate and compares membrane filters used in the analysis of oils and ointments.

EXPERIMENTAL

Three methods for preparation of isopropyl myristate were compared:

Batch-Swirl Method—A 50-g amount of basic alumina (Brockman Activity 1, 80–200 mesh[1]) was weighed in a 1-liter glass beaker, and 500 ml of isopropyl myristate (detyl NF extra-isopropyl myristate NF[2]) was added. The beaker was covered with aluminum foil, and the mixture was swirled at high speed for 1 hr with a magnetic stirrer. The mixture was filter-sterilized through a 0.22-μm filter membrane (mixed cellulose acetate–pyroxylin, 47-mm diameter) and a disk prefilter[3]. Aliquots of 100 ml of filtrate were transferred to sterile 38 × 200-mm screw-capped glass test tubes for toxicity determinations.

USP XX Column-Filtered Method—Basic alumina was added to a 20 mm × 20-cm glass column to a height of 15 cm (54 g), 500 ml of isopropyl myristate was passed through the column, and the eluate was filter-sterilized as in the batch-swirl method.

Untreated Isopropyl Myristate—A 500-ml sample of isopropyl myristate obtained directly from the manufacturer was filter-sterilized as in previous methods.

pH Determination of Isopropyl Myristate—The pH of isopropyl myristate prepared for the three methods was determined after sterilization by the USP XX method, which uses the water rinse technique. A sample containing 100 ml of isopropyl myristate and 10 ml of double distilled water was transferred to a 250-ml container. The mixture was shaken vigorously for 1 hr by a mechanical shaker, then centrifuged for 20 min at 1800 rpm (529×*g*). The water layer was used to determine the pH.

Bacterial Culture Preparation—*Pseudomonas aeruginosa* (ATCC 10145) was used as a test microorganism because of its sensitivity to isopropyl myristate (4, 7). The culture was transferred to a trypticase soy agar slant and incubated for 24 hr at 35°. The slant was washed with sterile phosphate buffer and the final concentration of 10^9 organisms/ml was used as the stock solution for inoculation of isopropyl myristate.

D Value Determinations—Two test tubes, each containing 100 ml of isopropyl myristate, were prepared according to the previously described methods. A control tube of phosphate-buffered water was used to determine the initial bacterial concentration. Tubes were inoculated with 0.1 ml of stock culture solution, and all tubes were vigorously hand-shaken. One tube of isopropyl myristate from each preparation method was placed in a 47° water bath. Ten-milliliter samples were taken from each tube of isopropyl myristate at intervals of 10, 20, 30, 45, and 60 min and at 0-time from the buffered water control tube.

Aliquots were filtered through a 0.22-μm membrane[3], and membranes were transferred to 100 ml of fluid D before sonification for 6 min at 55 kHz. Dilutions were plated on trypticase soy agar and incubated at 35° for 48 hr.

Membrane Filter Evaluation—Time rates to filter the isopropyl myristate and the rinsing fluids, A and D, through various membranes (47-mm diameter, 0.2- and 0.45-μm pore sizes) were determined by placing the membrane in a membrane filtration assembly unit and filtering 100 ml of isopropyl myristate, followed by 200 ml of rinsing fluid D and 100 ml of fluid A.

[1] Fisher Scientific Co., Fair Lawn, N.J.
[2] Givaudan Corp., Clifton, N.J.
[3] Type AP 25 Millipore Corp., Bedford, Mass.

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Table I—pH Levels of Isopropyl Myristate Prepared by Three Methods and Corresponding D Values of *Pseudomonas aeruginosa*

Method	pH	D Value, min: Room Temperature	D Value, min: 47°
USP XX Column-filtered			
A	6.7	>60.0	8.0
B	6.6	>60.0	9.3
Batch-Swirl			
A	6.7	>60.0	12.1
B	6.3	>60.0	11.6
Untreated			
A	4.0	11.4	<10.0
B	3.7	10.0	<10.0

Table II—Comparison of Flow Rates for Membrane Filters Used in the Filtration of Oils and Ointments

Membranes	Flow Rate, min[a]: Isopropyl Myristate 0.2 μm[b]	Isopropyl Myristate 0.45 μm[b]	Fluids A and D 0.2 μm[b]	Fluids A and D 0.45 μm[b]
Acetate Cellulose	2	1	3	1
Cellulose Nitrate	3	1	>120	20
Polyester	2	1	5	2
Mixed Esters of Cellulose	3	1	83	2
Polycarbonate	4	1	14	3
Mixed Cellulose Acetate–Pyroxylin	3	1	>120	19

[a] Approximate time. [b] Pore size of membrane.

RESULTS

The pH levels of the untreated isopropyl myristate, the USP XX column isopropyl myristate, and the batch-swirled isopropyl myristate are shown in Table I. The D values of *P. aeruginosa* obtained at room temperature and at 47°, which correspond to the three methods of preparation of isopropyl myristate, are also shown.

The approximate time required to filter isopropyl myristate and the rinsing fluids, A and D, through various membranes are given in Table II. The acetate cellulose membrane[4] and the polyester membrane[5] showed the fastest flow rates for the 0.2-μm membrane: 2 min for isopropyl myristate with both filters and 3 and 5 min, respectively, for rinsing fluids A and D. An oily film was noted on the polyester membrane after filtration of the isopropyl myristate and rinsing fluids A and D, regardless of pore size.

The flow rates of both the 0.2-μm mixed cellulose acetate–pyroxylin and the pyroxylin membranes exceeded 2 hr for the rinsing fluids. All of the 0.45-μm membranes had acceptable flow rates for isopropyl myristate. Flow rates for the rinsing fluids (pyroxylin, 20 min and cellulose acetate-pyroxylin, 19 min) were not acceptable.

DISCUSSION

The batch-swirl method for detoxification of isopropyl myristate used in the analysis of oils and ointments was simpler and faster than the USP XX method, *i.e.*, 1 hr *versus* 8 hr for the detoxification process. In the batch-swirl method, filter sterilization immediately followed the stirring, with no need for centrifugation. The pH of the isopropyl myristate prepared by the batch-swirl method met the requirements of the USP XX, and D values of the test organisms were comparable to those obtained by the USP XX method.

Use of cellulose acetate filters rather than 0.22- and 0.45-μm mixed cellulose acetate-pyroxylin membranes reduced the analysis time. Rapid filtration reduced the exposure time of microbial contaminants to the toxicity of isopropyl myristate and, thus, provided greater probability of their detection.

[4] Sartorius Filters, Inc., Hayward, Calif.
[5] Nucleopore, Pleasanton, Calif.

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Synthesis and Antitumor Testing of 3-Methenylthiochroman-4-one-1,1-dioxide

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Abstract □ Treatment of thiochroman-4-one-1,1-dioxide (II) with paraformaldehyde and dimethylamine hydrochloride in isopropyl alcohol at reflux afforded directly in 89% yield the dimeric dihydropyran (IV), corresponding to the dimerization of the target compound 3-methenylthiochroman-4-one-1,1-dioxide (III). Neither the monomer III nor the expected Mannich base, 3-dimethylaminomethylthiochroman-4-one-1,1-dioxide, were isolated under conditions of the reaction. The monomer III could be prepared in 55% yield by sublimation of the dimer IV at 230–250°; however, redimerization slowly occurred at room temperature. The dimer IV was also prepared by the use of paraformaldehyde and *N*-methylanilinium trifluoroacetate. The monomer III was found to be marginally active at 10 mg/kg/day *versus* Ehrlich ascites tumor growth in mice.

Keyphrases □ Antitumor agents—3-methenylthiochroman-4-one-1,1-dioxide, synthesis, testing, dimerization, mice □ α-Methenyl carbonyl function—alkylating moiety in some antitumor agents, synthesis of 3-methenylthiochroman-4-one-1,1-dioxide

The presence of a ring system containing an α-methenyl carbonyl function is important as an alkylating moiety in some antitumor agents (1–5). So called quinone methides (1–3), α-methenyl derivatives of quinones, are thought to be active metabolic intermediates in alkylation mechanisms. In general, the stability of the α-methenyl carbonyl

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