Acceleration of Peritoneal Dialysis with Minimal *N*-Myristyl-β-aminopropionate

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Abstract □ A single short-term exposure of the peritoneal membrane to *N*-myristyl-β-aminopropionic acid solution (0.25%) gave an accelerating effect on the peritoneal clearance of salicylate, barbiturate, and diphenylhydantoin, which lasted throughout several subsequent exchanges with standard dialysis fluid. A brief kinetic study on *N*-myristyl-β-aminopropionate was done, and its intraperitoneal absorption and urinary and peritoneal clearances are reported. The LD_{50} of this wetting agent in mice and its hemolytic threshold were also estimated.

Keyphrases □ Peritoneal dialysis of salicylate, barbiturates, and diphenylhydantoin—acceleration with *N*-myristyl-β-aminopropionate □ Salicylate, peritoneal clearance—acceleration with *N*-myristyl-β-aminopropionate □ Barbiturates, peritoneal clearance—acceleration with *N*-myristyl-β-aminopropionate □ Diphenylhydantoin, peritoneal clearance—acceleration with *N*-myristyl-β-aminopropionate □ *N*-Myristyl-β-aminopropionate—intraperitoneal absorption, urinary and peritoneal clearances, used to accelerate peritoneal dialysis of salicylate, barbiturates, and diphenylhydantoin

Previous work in this laboratory (1, 2) showed that the wetting agent *N*-myristyl-β-aminopropionic acid[1], when added to a standard dialysis fluid, accelerated the peritoneal removal of barbiturates, diphenylhydantoin, and salicylate. Since absorption of *N*-myristyl-β-aminopropionate from the peritoneum might occur during its use in dialysis and a hazard of added toxicity from this chemical might arise, an effort was made to attain its beneficial effects with minimum exposure. A priming lavage technique, devised in this laboratory and proven successful in accelerating peritoneal removal of urea (3), was used.

EXPERIMENTAL

Materials—Radioactive drugs were used to facilitate measurement of drug concentration. ^{14}C-Diphenylhydantoin was synthesized and purified by a published method (4). Solutions for injection were freshly prepared to contain 10 mg. diphenylhydantoin as the sodium salt and 1 μc./ml. and were injected at the level of 10 mg./kg. ^{14}C-Phenobarbital was obtained commercially[2], and the injection was prepared to contain 50 mg. phenobarbital as the sodium salt and 0.1 μc./ml. It was injected at the level of 50 mg./kg. ^{14}C-Pentobarbital was obtained commercially[2], and the injection contained 18 mg. pentobarbital as the sodium salt and 4 μc./ml. The pentobarbital dose was 18 mg./kg. ^{14}C-Amobarbital and ^{14}C-butabarbital were synthesized from ^{14}C-urea and the appropriate malonic esters (5). Their identity and purity were checked by IR spectrometry and paper chromatography, and their injections were prepared and used in the same manner as pentobarbital. ^{14}C-Salicylic acid was obtained commercially[2], and the injection contained 50 mg. salicylic acid as the sodium salt and 1 μc./ml. It was injected at the level of 100 mg./kg.

The control dialysis fluid was a typical formula with 377 milliosmoles/l. It contained dextrose (1.5%), sodium lactate (0.5%), sodium chloride (0.56%), calcium chloride (0.025%), and magnesium chloride hexahydrate (0.015%). *N*-Myristyl-β-aminopropionic acid dialysis fluid was prepared simply by dissolving 0.25% of the commercial solution in the control fluid. The only exception was in phenobarbital experiments where the concentration of the surfactant was increased to 0.5%. This change came as a result of preliminary work in which weaker solutions were used and the clearances obtained showed high variations.

Intermittent Dialysis—Healthy, mature, male albino rabbits were used as the experimental animals. They were administered ^{14}C-tagged drug intravenously *via* the marginal ear vein, and 1 hr. was allowed for drug distribution in the body before beginning dialysis. At that time a blood sample was taken and 60 ml./kg. of dialysis fluid was rapidly injected intraperitoneally *via* a pediatric-size catheter. At the end of the prescribed dwell time, the dialysate was drained by gravity, over 10 min., through the same catheter into a graduated cylinder. At the end of drainage, another blood sample was taken and the next exchange of fluid was rapidly injected. The volume of return dialysate was measured for each exchange, it was mixed thoroughly, and a sample was taken for measurement of drug concentration.

Preliminary work showed that a single short-term exposure of the peritoneum to a 0.25% *N*-myristyl-β-aminopropionate solution gave an accelerated dialysis effect which lasted throughout several subsequent exchanges with ordinary control fluid. A similar short-term exposure to control fluid had no significant effect on subsequent exchanges. On this basis, the test animals were given a priming lavage with *N*-myristyl-β-aminopropionate fluid, in which the dialysis fluid was allowed to remain in the peritoneum for 5 min. and then was drained for 10 min., giving a 15-min. first cycle. Then five 30-min. exchanges were run with control fluid, each consisting of a 20-min. dwell period and a 10-min. drainage. Control tests consisted of the same procedure except for elimination of the 15-min. exposure to *N*-myristyl-β-aminopropionate fluid.

Measurement of Drug Concentration—For salicylate and phenobarbital, since the amount of drug metabolized in the 3-hr. experiment is negligible (1, 2), 0.5-ml. samples of plasma and dialysate were added directly to the counting fluid [0.01% 1,4-bis-2-(phenyloxazolyl)benzene and 0.4% 2,5-diphenyloxazole in a solvent of 33% nonionic surfactant[3] and 67% toluene]. Samples thus prepared were counted in a liquid scintillation spectrometer[4] using the channels ratio method for quench correction.

A new procedure was developed for pentobarbital assay in which a sample of 1.0 ml. plasma or 2.0 ml. dialysate was transferred to a centrifuge tube, acidified with 0.4 ml. of 0.5 *N* HCl, and extracted four times with 3-ml. portions of ether. The ether extracts, containing all the unchanged pentobarbital and small amounts of metabolites, were evaporated to dryness. The residue was dissolved in about 0.05 ml. of ether and quantitatively spotted on a 20 × 20-cm. sheet of Whatman No. 2 chromatography paper. The chromatogram was developed in a chromatography chamber[5] with *n*-amyl alcohol–concentrated ammonium hydroxide (90:10 v/v). This system gave average R_f values of 0.90 for pentobarbital and 0.10 for metabolites. After drying, the chromatograms were viewed under UV light to locate the pentobarbital spots. The spots were cut out, placed in counting vials containing 10 ml. scintillation cocktail, shaken well, and counted. Application of this method to samples of known concentrations gave recoveries of 97% or better.

[1] Deriphat 170-C, a mixture of 70% *N*-myristyl- and 30% *N*-lauryl-β-aminopropionic acids as their sodium salts, marketed in 50% solution by General Mills, Kankakee, Ill. Percentages used in this work are of 50% solution as supplied unless otherwise indicated.

[2] Tracerlab, Waltham, Mass.

[3] Triton X-100, Rohm & Haas, Philadelphia, Pa.

[4] Packard 3320 scintillation spectrometer, Packard Instrument Co., LaGrange, Ill.

[5] Eastman.

Table I—Effect of 15-min. Lavage with *N*-Myristyl-β-aminopropionic Acid on Peritoneal Clearances

Drug	Clearance[a], ml./min. Without Lavage	With Lavage	Drug	Clearance, ml./min. Without Lavage	With Lavage
Pentobarbital	0.94[b]	1.65	Amobarbital	0.70[b]	2.31
	0.77[b]	1.60		0.48[b]	1.67
	1.07[b]	2.17		1.06[b]	1.26
	0.98[b]	2.08		1.25[b]	Av. 1.75 ± 0.31
	1.16[b]	2.19		1.05[b]	
	1.09[b]	1.91		Av. 0.91 ± 0.14	
	1.10	Av. 1.93 ± 0.11	Salicylate	0.36[d]	0.84
	Av.[c] 1.02 ± 0.05			0.43[d]	1.01
Phenobarbital	0.86[b]	1.25		0.37[d]	1.05
	1.37[b]	2.05		Av. 0.39 ± 0.02	1.15
	1.06[b]	1.76			1.00
	0.93[b]	1.60			Av. 1.01 ± 0.05
	1.12[b]	Av. 1.67 ± 0.17	Diphenylhydantoin	0.33	1.00
	1.01[b]			0.34	0.70
	Av. 1.06 ± 0.07			0.47[b]	0.68
Butabarbital	1.66[b]	2.58		0.39[b]	Av. 0.79 ± 0.10
	1.94[b]	2.74		0.89[b]	
	1.79	1.92		0.36[b]	
	1.01	1.83		Av. 0.46 ± 0.09	
	Av. 1.60 ± 0.20	Av. 2.27 ± 0.23			

[a] Each entry represents the average clearance for five exchanges on one animal. [b] From previous work (1). [c] Average ± standard error of the mean. [d] From previous work (2).

Amobarbital and butabarbital were extracted completely from acidified samples with 4 × 5 ml. ether. Contamination by metabolites was less than 3%, as determined by paper chromatography. Extracts were evaporated in counting vials, scintillation fluid was added, and they were then counted.

For diphenylhydantoin, the samples were acidified with 0.5 *N* HCl, extracted with 2 × 5 ml. chloroform, which was evaporated in counting vials, and counted in the usual manner.

In each case, an exact dilution of radioactive injection was counted with the samples as a standard.

Studies on *N*-Myristyl-β-aminopropionic Acid—Absorption of the wetting agent *N*-myristyl-β-aminopropionate from the peritoneum might occur during its use in dialysis. High blood levels coupled with low excretion rate might prove hazardous to the health of the patient undergoing dialysis as a result of accumulation of this chemical in the body. Therefore, it was thought pertinent to determine whether this agent is absorbed during its passage across the peritoneum and, if so, to what degree and how rapidly it would be removed in subsequent exchanges. From these data, it would be possible to estimate the likelihood of toxicity.

Since a reliable method for quantitative determination of *N*-myristyl-β-aminopropionic acid could not be found, ^{14}C-tagged compound was used to measure its concentration in body fluids. The synthesis was performed in this laboratory. The first step involved the preparation of ^{14}C-tetradecanitrile by the method of Friedman and Shechter (6), using 1-bromotridecane and ^{14}C-sodium cyanide in dimethyl sulfoxide. The nitrile was reduced to tetradecylamine-1-^{14}C by sodium butoxide in toluene (7). *N*-Myristyl-β-aminopropionate was then prepared by reacting the amine with 20% excess of methyl acrylate. The product was saponified with an equivalent amount of 50% NaOH solution after the excess methyl acrylate was removed at 100° under vacuum (8). The identity and purity of each intermediate compound and the final product were checked with TLC and IR spectrometry. The overall yield of *N*-myristyl-β-aminopropionate was 42%, with a specific activity of 0.373 μc./mg.

To estimate the amount of wetting agent that might be absorbed in a single peritoneal lavage, absorption was measured over a longer period than just a 15-min. cycle. This afforded measurements useful even if lavages of different cycle times were used. The rabbits were prepared for the experiment in the same manner as mentioned previously. The dialysis fluid, containing 0.125% radioactive *N*-myristyl-β-aminopropionate (equivalent in concentration to 0.25% of the commercial solution) was injected at the same level and using the same procedure as before, except that the fluid was left in the peritoneum for 3 hr. Samples of blood and dialysate were taken 5 min. after injection, at 10-min. intervals for the 1st hr., at 20-min. intervals for the 2nd hr., and at 30-min. intervals for the 3rd hr. The samples were counted directly for total radioactivity.

To determine the rate of disappearance from plasma and the urinary clearance of *N*-myristyl-β-aminopropionate, two rabbits were given an intravenous dose of 30 mg. radioactive compound in normal saline. Plasma and urine samples were collected every 10 min. for the 1st hr. and every 20 min. for the following 2 hr. The samples were counted directly.

To determine the peritoneal clearance of *N*-myristyl-β-aminopropionate, a rabbit was injected intravenously with ^{14}C-labeled compound at the level of 10 mg./kg. A 30-min. period was allowed for distribution in the body before commencing dialysis. Five dialysis cycles with control fluid were carried out, each consisting of a 20-min. dwell time and a 10-min. draining period. Plasma, dialysate, and urine samples were collected and directly counted in the liquid scintillation spectrometer.

To determine the hemolytic threshold and the concentration of *N*-myristyl-β-aminopropionate producing 50% hemolysis, a series of dilutions of the surfactant in normal saline, ranging from 2 to 74 mcg./ml., was prepared. Normal saline was used as the negative control (no hemolysis), and a 1% solution of sodium carbonate in saline was used as a positive control (100% hemolysis). Ten milliliters of each solution was placed in a centrifuge tube and kept in a water bath at 37° for 30 min. to provide temperature equilibration. Then 0.1 ml. of heparinized rabbit whole blood was added to each tube and incubated at the same temperature. At the end of 1 hr., the tubes were centrifuged for 10 min. at 60,000 r.p.m. and the supernatant solutions were carefully removed and measured in a spectrophotometer at 540 nm.

To obtain an estimate of the LD_{50} of *N*-myristyl-β-aminopropionate in mice, young, healthy, male albino animals (15–28 g.) were used. They were divided into five groups, each containing five mice.

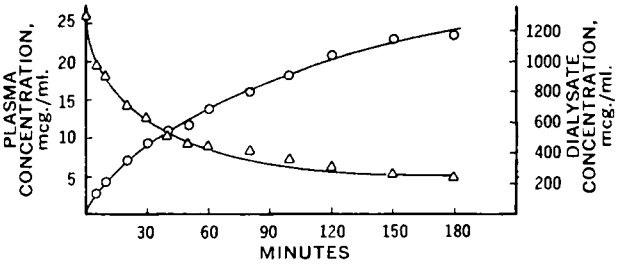


Figure 1—*Intraperitoneal absorption of* N-*myristyl-β-aminopropionate. Key:* ○, *plasma; and* △, *dialysate.*

Table II—Dialysis of *N*-Myristyl-β-aminopropionic Acid with Control Fluid[a]

Average Plasma Concentration, mcg./ml.	Dialysate Concentration, mcg./ml.	Amount Removed, % Dose	Clearance, ml./min.	Accumulated Amount in Urine, mcg.
14.32	0.53	0.23	0.14	77.53
12.77	0.51	0.32	0.21	292.81
14.11	0.45	0.25	0.15	646.87
13.76	0.47	0.25	0.15	1089.17
13.72	0.44	0.25	0.15	1367.87
	Total	1.30	Av. 0.16	

[a] Experiment on Animal E-27, which weighed 2.30 kg. The dialysis cycle time was 30 min.

Four doses of commercial *N*-myristyl-β-aminopropionate, ranging from 250 to 1000 mg./kg., were selected due to a pilot experiment. These doses were administered intraperitoneally as a 1% solution in normal saline to four test groups, and the control group received 2 ml. normal saline. The animals were put under observation for 24 hr.

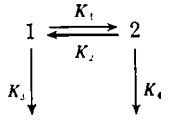
RESULTS

Results of intermittent dialysis of the drugs tested are shown in Table I, where each entry represents the average clearance of five exchanges on one animal. Previously reported results for control fluid were added for comparison. It can be seen that a 15-min. lavage with *N*-myristyl-β-aminopropionate fluid accelerated the peritoneal removal of all drugs tested. The highest increases in clearance were observed with salicylate and diphenylhydantoin, where clearance values about 2.5 times control were obtained. With the barbiturates, clearances ranged between 1.4 and 1.9 times control.

The high affinity of diphenylhydantoin for plasma protein appears to be the underlying cause for its low clearance. If the unbound diphenylhydantoin is considered to be 14% of the total plasma concentration over the range studied (1), the calculated average dialysate–plasma ratio of free drug is found to be 0.50 with control dialysis and 0.99 with the priming lavage technique. In this case, the protein binding of diphenylhydantoin seems to be the rate-limiting step during dialysis and equilibrium between drug in plasma and dialysate is rapidly attained. Therefore, peritoneal removal of diphenylhydantoin could be further accelerated by much shorter dwell periods.

Typical plasma and peritoneal fluid curves demonstrating intraperitoneal absorption of *N*-myristyl-β-aminopropionate are illustrated in Fig. 1. The plasma level did not exceed 2.7 mcg./ml. in the first 5 min., corresponding to the dwell period in a lavage. After 15 min., a period that would allow absorption to continue during drainage, the level did not exceed 7 mcg./ml. The absorption of wetting agents was quite rapid, as reflected in the dialysate curve, with about 30–50 mg./kg. being absorbed in a 15-min. period.

The plasma curves following intravenous injection were biexponential, indicating a two-compartment model. Of several two-compartment models possible, only one gave an acceptable fit to the urine curve as shown in Scheme I, where 1 is the central compartment including the plasma, 2 is a peripheral tissue compartment (possibly largely the liver), K_1 and K_2 are distribution constants between the two compartments, K_3 is the urinary excretion constant, and K_4 represents direct elimination from the tissue compartment (possibly elimination *via* the bile). The constants may be found by solving a biexponential equation obtained from the plasma curve alone, but it was considered preferable to solve it with an analog computer which would utilize the cumulative urine curve as well



Scheme I

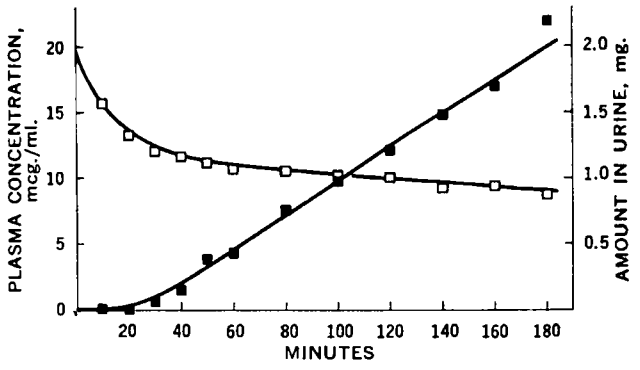


Figure 2—*Plasma and urine curves following intravenous administration of* N-*myristyl-β-aminopropionate to Animal E-24. The lines are computer drawn. Key:* □, *plasma; and* ■, *urine.*

as plasma data. This was done with the data of Animal E-24, as illustrated in Fig. 2, and the rate constants were estimated to be as follows: $K_1 = 1.60 \times 10^{-2}$ min.$^{-1}$, $K_2 = 2.08 \times 10^{-2}$ min.$^{-1}$, $K_3 = 1 \times 10^{-5}$ min.$^{-1}$, and $K_4 = 1.25 \times 10^{-3}$ min.$^{-1}$. These constants demonstrate the relatively low rate of urinary excretion as compared to the presumed biliary excretion. Also, the high distribution volumes, 152% for the central compartment and 380% for the equilibrium distribution volume, explain the relatively low plasma concentrations found in these experiments. The model fitted to these data should not be accepted as an established one for this compound, since it is based on limited data, but it was the only one that satisfactorily fit the data available.

The removal of *N*-myristyl-β-aminopropionate from the body by peritoneal dialysis with control fluid is summarized in Table II. Once absorbed, the wetting agent is slowly removed by peritoneal dialysis. The peritoneal clearance was only 0.16 ml./min., as compared to a urinary clearance of 0.58 ml./min. measured in the same experiment.

The data for the hemolysis experiment are illustrated in Fig. 3, where the results, as percentage hemolysis, were plotted against the concentrations of *N*-myristyl-β-aminopropionate used, to give a dose-response curve. The concentration giving 50% hemolysis was estimated to be 63.2 mcg./ml. It was also found that below 15 mcg./ml. no hemolysis occurred. The plasma level of *N*-myristyl-β-aminopropionate remained below 7 mcg./ml. for the first 15 min. during the intraperitoneal absorption experiment, thus being well below the hemolytic threshold.

The results of the LD_{50} experiment were plotted (Fig. 4) as the logarithms of the doses administered against the probit effects according to the method of Miller and Tainter (9). The best fitting line was calculated by the least-squares method, and the LD_{50} of *N*-myristyl-β-aminopropionate in mice was estimated to be 620 mg./kg. with a standard error of ±136.

DISCUSSION

The data obtained in this study clearly confirm the earlier findings in this laboratory (1, 2) that *N*-myristyl-β-aminopropionic acid has an accelerating effect on the peritoneal clearance of salicylate, barbiturates, and diphenylhydantoin. Of particular interest is the

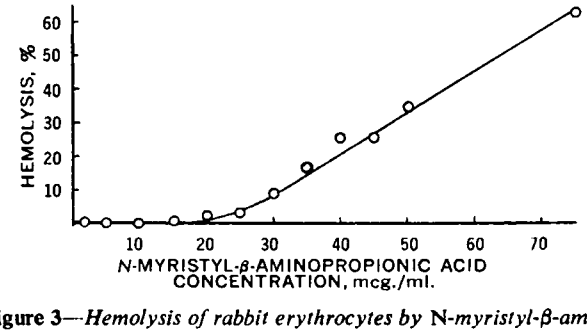


Figure 3—*Hemolysis of rabbit erythrocytes by* N-*myristyl-β-aminopropionate.*

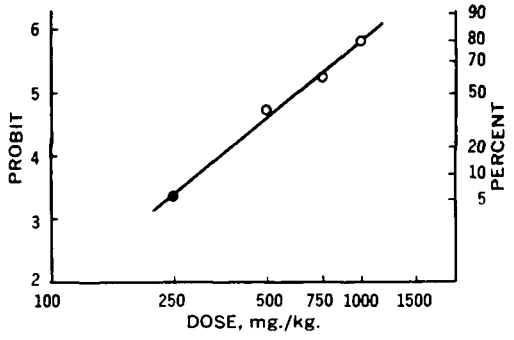


Figure 4—*LD_{50} of N-myristyl-β-aminopropionate in mice.*

finding that a single short-term exposure of the peritoneal membrane to *N*-myristyl-β-aminopropionate solution gave an accelerated dialysis effect which lasted throughout at least five subsequent exchanges with standard dialysis fluid. This would make possible the effective use of this accelerator with minimum exposure to the patient. The absorption tests also demonstrated that, although *N*-myristyl-β-aminopropionate is rapidly taken up from the dialysis fluid, the resulting plasma levels are quite low and appear to be well below concentrations that would result in acute toxicity. With its comparatively short 5-hr. biological half-life (as calculated from the terminal phase of the blood curves), practically all surfactant absorbed into the circulation should be eliminated from the blood in less than 48 hr. These findings should enhance the potential for the clinical use of *N*-myristyl-β-aminopropionate as an accelerator during peritoneal dialysis. Further toxicological studies are essential, of course, before this substance might be tested in man.

The mechanism of action of the surfactant is not yet known. Studies to determine the mechanism are being conducted but are not yet conclusive.

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Survival of *Staphylococcus aureus* on Pharmaceutical Oral Solid Dosage Forms

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Abstract □ The relationship of temperature, relative humidity, size of inoculum, and duration of storage on survival of *Staphylococcus aureus* inoculated onto surfaces of 17 commercial tablets and one gelatin capsule was determined. The data were analyzed using a two-way factorial analysis of variance. Decreased survival was associated with an increase in each of the variables, within limits of the experiment; within those limits, size of inoculum and storage time were the least significant factors and storage environment appeared to be the most significant.

Keyphrases □ *Staphylococcus aureus* inoculation—surface of commercial tablets and capsules, effects of temperature, relative humidity, size of inoculum, and storage time on survival □ Tablet and capsule surface inoculated with *Staphylococcus aureus*—effects of temperature, relative humidity, size of inoculum, and storage time on survival □ Sterility testing, tablets and capsules inoculated with *Staphylococcus aureus*—effects of size of inoculum, storage time, and environmental conditions on survival

The necessity for sterility of parenteral products has long been recognized. NF V was the first official compendium in the United States to require sterility for such products (1). With respect to distribution and use of nonsterile pharmaceuticals, it is now recognized that microbial contamination must be minimal; with respect to certain species, it is totally unacceptable. Kallings *et al.* (2) focused attention on microbial content of nonsterile products when they reported infections resulting from the use of contaminated medications. The Swedish National Board of Health is restricting levels of microbes that may be present in liquid and tableted preparations (3), and a policy of not allowing an excess of 100 colonies/g. for practically all medical preparations was adopted (4). Czechoslovakia adopted statutory microbiological standards for tablets (5).

Numerous studies (6–12) confirmed existence of microbial contamination in some products. Hirsch *et al.* (13) reported that 82% of 57 oral liquid products tested contained microorganisms, and other researchers (14) found more than 100 microorganisms/ml. in 40 of 489 samples of such preparations. USP XVIII now includes a statement regarding microbial contamination of nonsterile pharmaceutical products (15).

The purposes of this study were to: (*a*) determine the ability of solid pharmaceutical dosage forms to carry bacteria; and (*b*) investigate the effect of temperature, relative humidity, size of inoculum, and storage time on