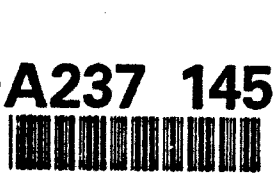

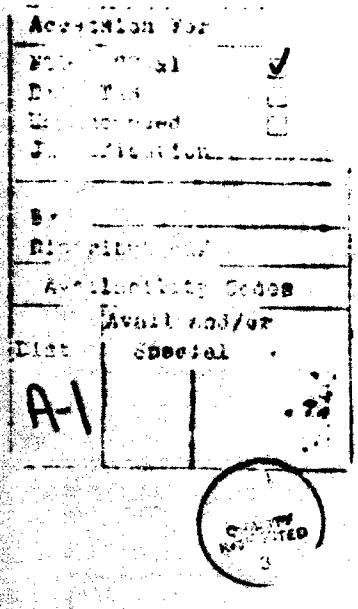

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EXPLORATORY DEVELOPMENT OF AN ULTRA-FAST-CURING WOUND DRESSING

ANNUAL REPORT
November 30, 1990

Contract No. DAMD17-88-C-8012

Kurt Dasse, Donald Dempsey & Ramachandran Thirucote.

Supported By

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

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470 Wildwood Street
Woburn, Massachusetts 01888-1799

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Thermedics Inc. — 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 470 Wildwood Street Woburn, Massachusetts 01888-1799	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Comman — 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-88-C-8012
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62787A — PROJECT NO. 3M1-62787A825 — TASK NO. EA — WORK UNIT ACCESSION NO. DA313891

11. TITLE (Include Security Classification)

(U) Exploratory Development of an Ultra-Fast Curing Wound Dressing

12. PERSONAL AUTHOR(S)

Kurt Dasse, Donald Dempsey, Ramachandran Thirucote

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 11/1/89 TO 10/31/90	1990 November 30	99

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	01		Dermal dressing; Field Wound Dressing
06	15		Drug-dispensing; RA 2
			Controlled Release, sustained release

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

We are developing a drug-dispensing field dermal dressing. The dermal dressing, which can be easily applied by an untrained person, contains antimicrobials to prevent bacterial infection. The medicated dermal dressing is made of an ultra-fast curing polyurethane oligomer which is designed to cure at room temperture and delivers drugs on a controlled, sustained and highly reproducible basis.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b. TELEPHONE (Include Area Code) 301-663-7325 — 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

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Kurt N. Darrow 1/11/11

PI Signature Date

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTD..)

LIST OF FIGURES

LIST OF FIGURES (CONTD..)

LIST OF FIGURES (CONTD..)

FIGURE NO. PAGE

LIST OF TABLES

INTRODUCTION

This report summarizes research conducted over the final year of the contract directed toward developing a second generation Antimicrobial Dermal Dressing (ADD). The dressing is a trilaminate composed of an outer medical grade polyurethane fabric, an acrylic-based pressure sensitive adhesive, and an antimicrobial impregnated polyurethane film which serves as a controlled drug release layer. The objectives in developing this new technology have been to create a dressing that is:

(1) easily applied under adverse climatic conditions,

(2) highly compliant and abrasion resistant, and

(3) allows controlled release of antimicrobial agents over a 72 hour period against a variety of specific microbial organisms.

The new dressing must be capable of incorporating sensitive antimicrobial agents and releasing them in a controlled fashion when in contact with the wound. This has been made possible by developing a room temperature, rapid ultraviolet (UV) curable liquid polyurethane oligomer. The liquid mixture of urethane and drugs is cured under UV lights and the resultant monolithic film provides controlled release of the agents when placed on the wound. This targeted drug delivery minimizes many of the inherent problems associated with conventional systemic drug delivery.

The focus of the research over the third contract year has been to fabricate two types of dressings incorporating:

(1) chlorhexidine gluconate, and

(2) silver sulfadiazine coupled with a second synergistic antimicrobial agent.

Successful completion of the proposed tasks has involved manufacturing the base oligomer, developing reliable fabrication methods, establishing analytical methods to measure the antimicrobial agents, monitoring the elution kinetics and optimizing drug release. Accelerated shelf stability studies were also initiated. USAIDR assumed responsibility for in vivo evaluation of the technology.

This work has resulted in the development of new techniques for drug analyses, improved fabrication methods for sustained release and offers the possibility of enhanced wound healing. Work in the latter portion of the year was devoted to manufacturing and monitoring larger quantities of dressings for shelf stability studies. The following report provides a detailed description of the activities carried out in the performance of this program.

PROGRAM STATUS

The Antimicrobial Dermal Dressing (ADD) under development by Thermedics Inc. according to the terms of the USAIDR research contract DAMD17-88-C-8012 has been shown to be effective. Testing of two formulations was associated with excellent results in vivo when employed prophylactically on inoculated wounds in guinea pigs.

The first test was carried out on a Chlorhexidine Gluconate dressing, and was found to be highly effective against Strep. pyogenes, Staph. aureus and P. aeruginosa under the stringent conditions required by USAIDR. The second test of a Chlorhexidine Gluconate - Silver Sulfadiazine ADD, which is expected to be effective against Candida albicans, was also effective against Staph. aureus and P. aeruginosa. Both of these dressings are currently undergoing accelerated storage stability tests. The start of the storage tests were contingent upon being able to package the dressings in hermetically sealable envelopes. This requirement dictated the sterilization of the product by radiation methods. The irradiation method chosen was Electron Beam; this method showed no tendency to degrade the product.

All tasks are either completed, underway or should be completed by program end.

WORK TO DATE

TASK I

Task I required the preparation of the vinyl terminated silicone oligomer for Year 3 studies. All the ADD's fabricated during Year 3 of the contract were prepared from a single lot of oligomer.

TASK II

Task II called for the preparation of 200 sterile placebo dressings for FY90 User Test. These dressings were fabricated and prepared for delivery to USAIDR.

TASKS III AND IV

Tasks III and IV focussed on optimizing the formulation of the chlorhexidine gluconate dressing. Fabrication of the initial chlorhexidine gluconate dressings was completed by the end of Year 2 (Tasks VIII & IX). An increase in the amount of drug eluted was achieved by the modification of the excipients used in the formulation (1). This, as well as two additional test formulations was submitted to USAIDR for in vivo studies on guinea pigs. Following evaluation, the two final formulations of the ADD's were submitted for testing making a total of five (5) formulations

evaluated by USAIDR.

A. In Vitro Release Kinetics of Chlorhexidine Gluconate ADD's

The initial chlorhexidine gluconate ADD submitted for in vivo testing showed excellent bacterio*static* activity. However, it was subsequently learned that modification of the excipients further increased the release of drug from the matrix, with potential bacterio*cidal* activity. Figure 1 compares the in vitro release rates of formulation 1 with that of formulation 2, incorporating the different excipients.

The total drug content per unit area was shown to be altered by changing the thickness of the dressing. However, as was demonstrated in the animal trials, the 6 mil dressing (Formulation 2) was as effective as the 20 mil dressing (Formulation 3). Two factors limiting maximal thickness of the dressing relate to the flexibility of the ADD and the decrease in the percent elution of the total drug loading. To test flexibility, user tests were conducted with a maximum thickness dressing (20 mil) possible, worn on elbows and wrists. These dressings were found to conform to the uneven contours of the body, and remain adherent for periods up to 3 days. These dressings were also shown to be effective in parallel in vivo trials. However, to reduce cost, while maintaining efficacy, an optimal thickness had to be determined. The objective of this effort was to reduce the thickness to less than 20 mils,

thereby lowering the amount of drug and cost, while maintaining efficacy and handling characteristics. Accordingly, the fourth and fifth formulations incorporating chlorhexidine gluconate, that were submitted for in vivo evaluation were 16 and 12 mils, respectively. All formulations submitted to USAIDR for in vivo trials are summarized in Table I. Figure 2 shows in vitro release kinetics associated with the four formulations of different thicknesses. While significant differences in the kinetics were observed in increasing the thickness of the ADD from 6 - 16 mils, no apparent difference was seen from 16 to 20 mils.

Table I. Formulations Submitted to USAIDR Under Task IV.

USAIDR Sub.No.	Qty	Weight Ratio Drug:Excipient:Matrix	Thickness
Formulation 1	45	30:30:40	6 mils
Formulation 2	45	30:6:24:40	6 mils
Formulation 3	45	30:6:24:40	20 mils
Formulation 4	45	30:6:24:40	16 mils
Formulation 5	45	30:6:24:40	12 mils

Excipients: Propylene glycol 30 parts;
Propylene glycol 6 parts and PEG 300 24 parts.

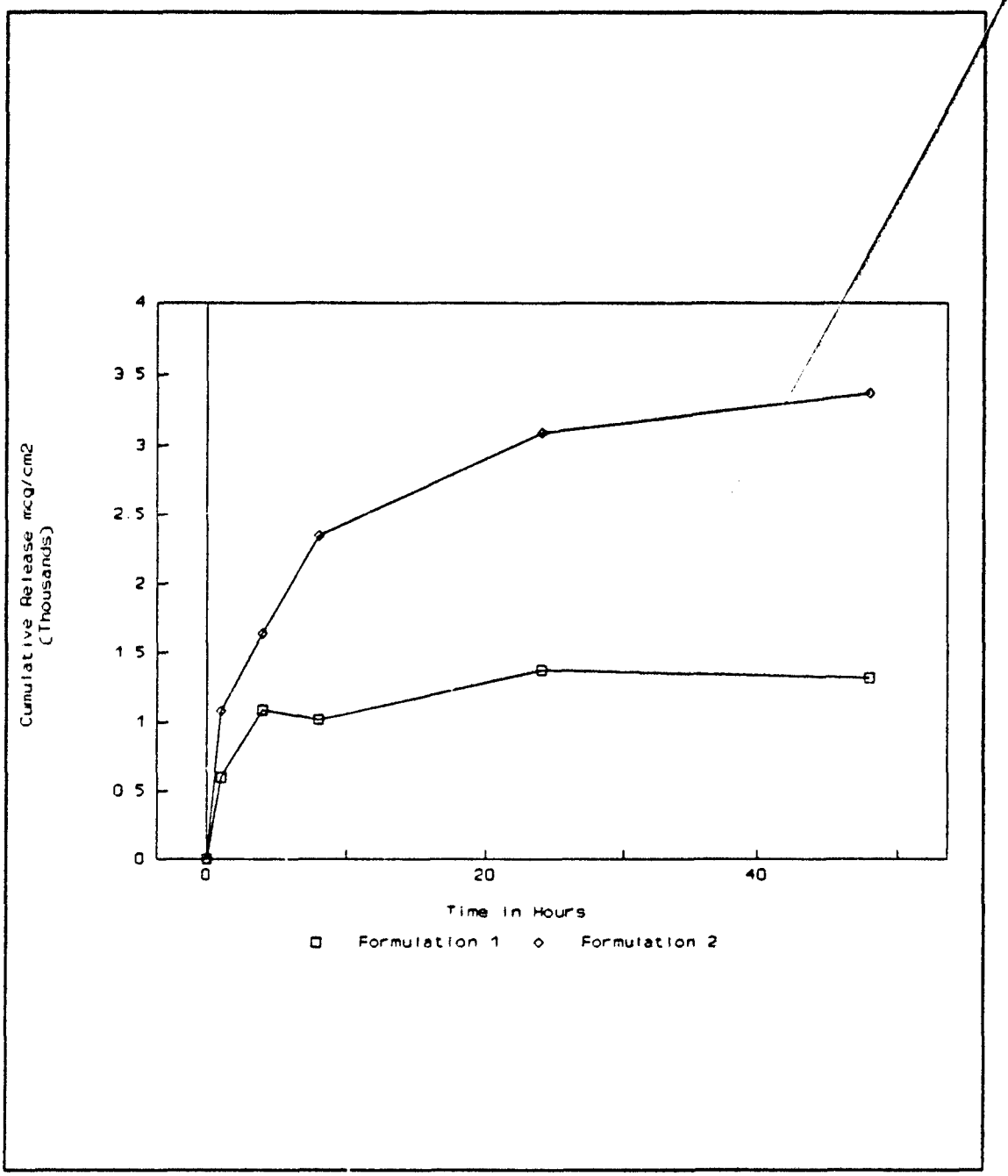


Figure 1. Comparison of the Release Rates of 30% Loaded Chlorhexidine Gluconate ADD's With Different Excipients (Formulation 1 contains 30% propylene glycol as the excipient; Formulation 2 contains 6% propylene glycol and 24% PEG 300).

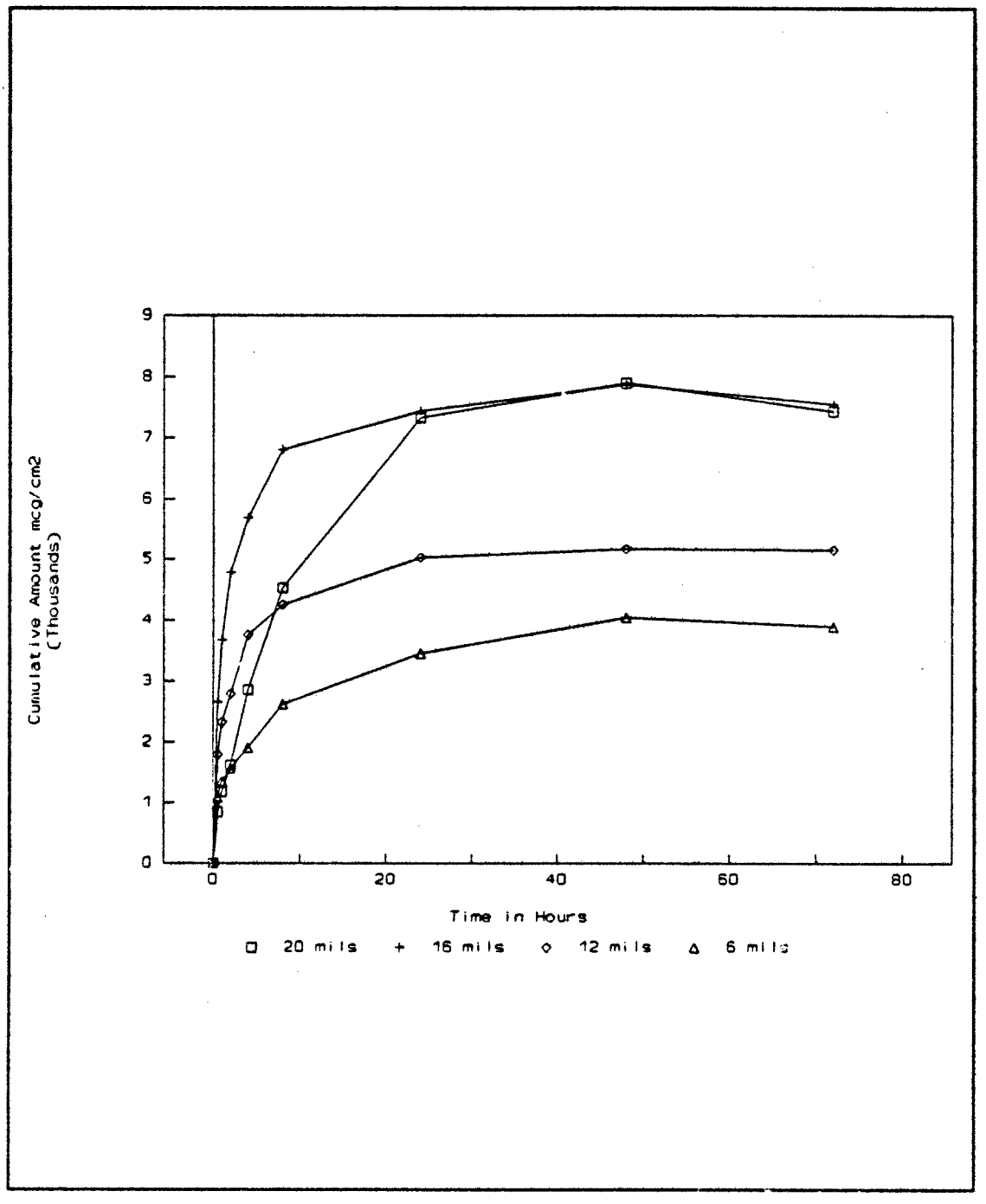


Figure 2. Comparison of the Release Rates of 30% Loaded Chlorhexidine Gluconate ADD's With Different Thicknesses (Excipient Blend 6:24 Propylene glycol to PEG 300).

B. Summary of Animal Test Results

The in vivo tests of the ADD's containing chlorhexidine gluconate were conducted at the laboratory facilities of USAIDR, in Maryland. Five different formulations of chlorhexidine ADD's were tested on inoculated guinea pigs and the results are summarized in Table II. Each dressing was evaluated utilizing the prophylactic protocol. Utilizing the prophylactic protocol, a wound was created, inoculated and the dressing applied immediately (2). The results described in Table II are those obtained utilizing the quantitative assay. Based on these results, formulations 2, 3, 4 and 5 incorporating the same excipient ratio (6:24 parts propylene glycol to PEG 300) were as effective as formulation 1 containing 30 parts of propylene glycol. However, these formulations were easier to fabricate compared to formulation 1. The thicker dressings, formulation 3 (20 mils), 4 (16 mils) and 5 (12 mils) also had superior handling characteristics compared to formulation 2 (6 mils).

USAIDR in vivo results showed that the 30% chlorhexidine gluconate formulations were 100% effective prophylactically. Formulation 5 demonstrated optimal balance between cost and handling characteristics and was chosen for the accelerated stability testing under Task VI.

Table II. Summary of USAIDR In Vivo Tests - Biopsy Results.

USAIDR Gp #	N	Infection None	Subclinical	Clinical
Placebo	4	-	-	4
Formulation 1	6	4	2	0
Formulation 2	7	4	3	0
Formulation 3	6	5	1	0
Formulation 4	5	5	-	-
Formulation 5	5	5	-	-

Formulation 1 = 30% Chlorhexidine gluconate ADD, containing 30% of Propylene glycol, 6 mils thick.

Formulation 2 = 30% Chlorhexidine gluconate ADD, with 6% propylene glycol and 24% PEG, 6 mils thick.

Formulation 3 = 30% Chlorhexidine gluconate ADD, with 6% propylene glycol and 24% PEG, 20 mils thick.

Formulation 4 = 30% Chlorhexidine gluconate ADD, with 6% propylene glycol and 24% PEG, 16 mils thick.

Formulation 5 = 30% Chlorhexidine gluconate ADD, with 6% propylene glycol and 24% PEG, 12 mils thick.

TASKS V AND VI

Tasks V and VI included the fabrication, delivery and accelerated storage stability tests for sterilized chlorhexidine gluconate ADD's. The work statement called for 200 sterile ADD's to be delivered to USAIDR, along with 100 placebos for in vivo tests under Task V. Also an additional 100 ADD's were to be delivered for demonstration purposes. All ADD's had to be manufactured from the same lot as those tested for shelf stability under Task VI. The minimal number of ADD's required under these combined tasks was over 500.

The dressings submitted to USAIDR to this juncture in the program had been sterilized utilizing ethylene oxide. This method was shown to be effective and suitable for materials packaged in heat sealable, permeable pouches. Also, ethylene oxide sterilization permitted rapid processing for screening tests. However, ethylene oxide is a contact sterilant and is not effective on devices packaged in moisture resistant packages. The wound dressings prior to shelf stability testing were required to be packaged in moisture resistant aluminum pouches. Hence ethylene oxide was not the recommended method of terminal sterilization.

A. Effects of Sterilization

Ionizing radiation is a popular and established industrial process for the terminal sterilization of medical devices. The principle advantage of using ionization techniques is that the dressings can be sterilized in hermetically sealed aluminum pouches. Moreover, ionization techniques are generally simpler leaving no residuals and require no post-sterilization treatment.

There are two types of ionizing radiation:

(a) electromagnetic radiation which includes gamma rays and X-rays, and

(b) particulate radiation which includes beta and alpha particles.

Both types of radiation produce bactericidal effects through the interaction of high energy electrons and the substrate. In the former, gamma radiation, the high energy electrons are produced by the interaction of gamma rays with the atomic electrons of the substrate. In the latter, beta particles (electrons) are delivered from an external source in predetermined doses. In both of these types of radiation, it is the high energy electrons which produce chemical changes in the target leading to the destruction of microorganisms (3, 4). The degree of ionization achieved in any device is directly related to the amount of radiation absorbed. When applied to packaged products, both gamma rays as well as electron particles effectively penetrate the packaging and

sterilize the product. However, the degree of penetration, as well as the degree of ionization due to gamma radiation, far exceeds that of electron beam radiation (Figure 3).

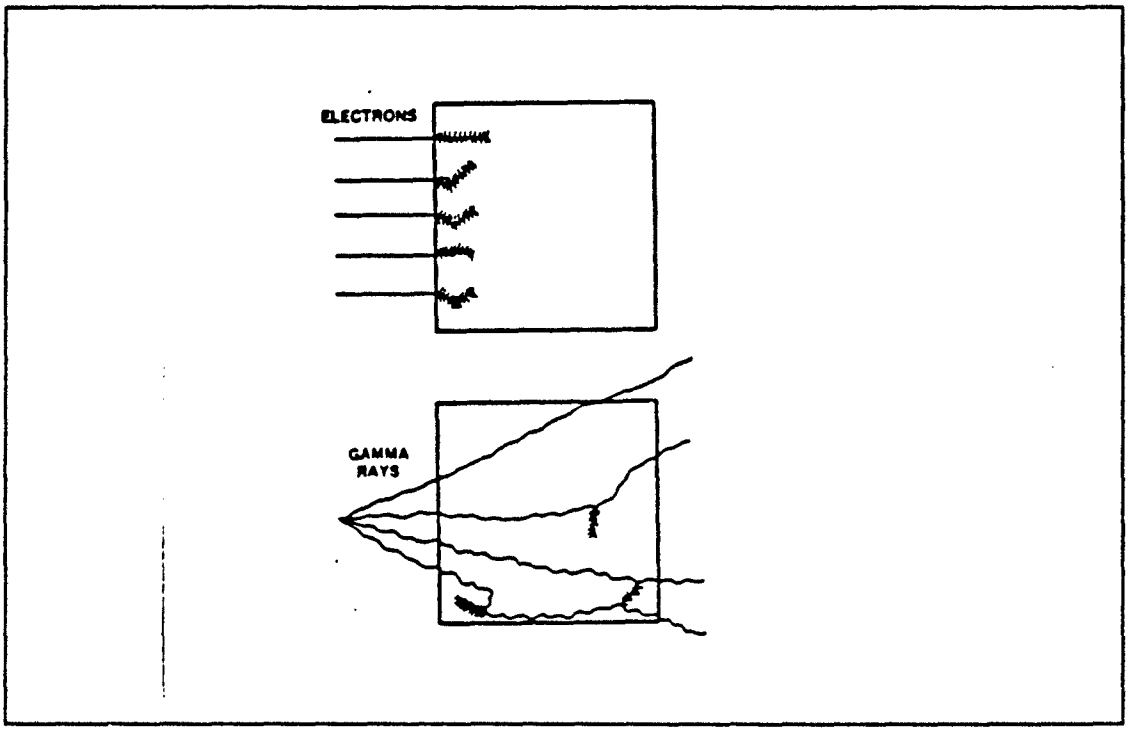


Figure 3. Penetration of Electron Beams compared to Gamma Rays.

Gamma radiation, due to its penetrative, energy transmittance properties, has the potential to cause undesirable side effects with many polymers (5, 6). Electron beam on the other hand, utilizes an external finite dose of electrons. The degree of penetration and ionization within the substrate is controlled by the dose. This method is less likely to cause deleterious effects

on polymer networks such as degradation or crosslinking (7), is highly effective, and widely used for terminal sterilization of hermetically packaged devices. Based on the known mechanisms of each of these forms of sterilization, gamma radiation is less attractive as a means of sterilizing the ADD because it may have a greater effect on the polymer/drug interaction compared to electron beam methods.

The effect of these sterilization techniques, i.e. ethylene oxide, gamma and electron beam radiation, on the release kinetics of the wound dressings were compared using in vitro diffusion methods. Initial tests performed on the packaged ADDs' showed that the elution kinetics of dressings subjected to gamma sterilization exhibited a lower release rate, and reduced the total amount of drug eluted from the polymeric matrix (Figure 4). The dressings subjected to electron beam radiation, on the other hand, exhibited release kinetics comparable to ADDs' subjected to ethylene oxide, as illustrated in figure 5.

Based on the elution kinetics and preliminary results in animals, it was recommended that the dressings be packaged in hermetically sealed aluminum pouches followed by sterilization utilizing electron beam radiation methods.

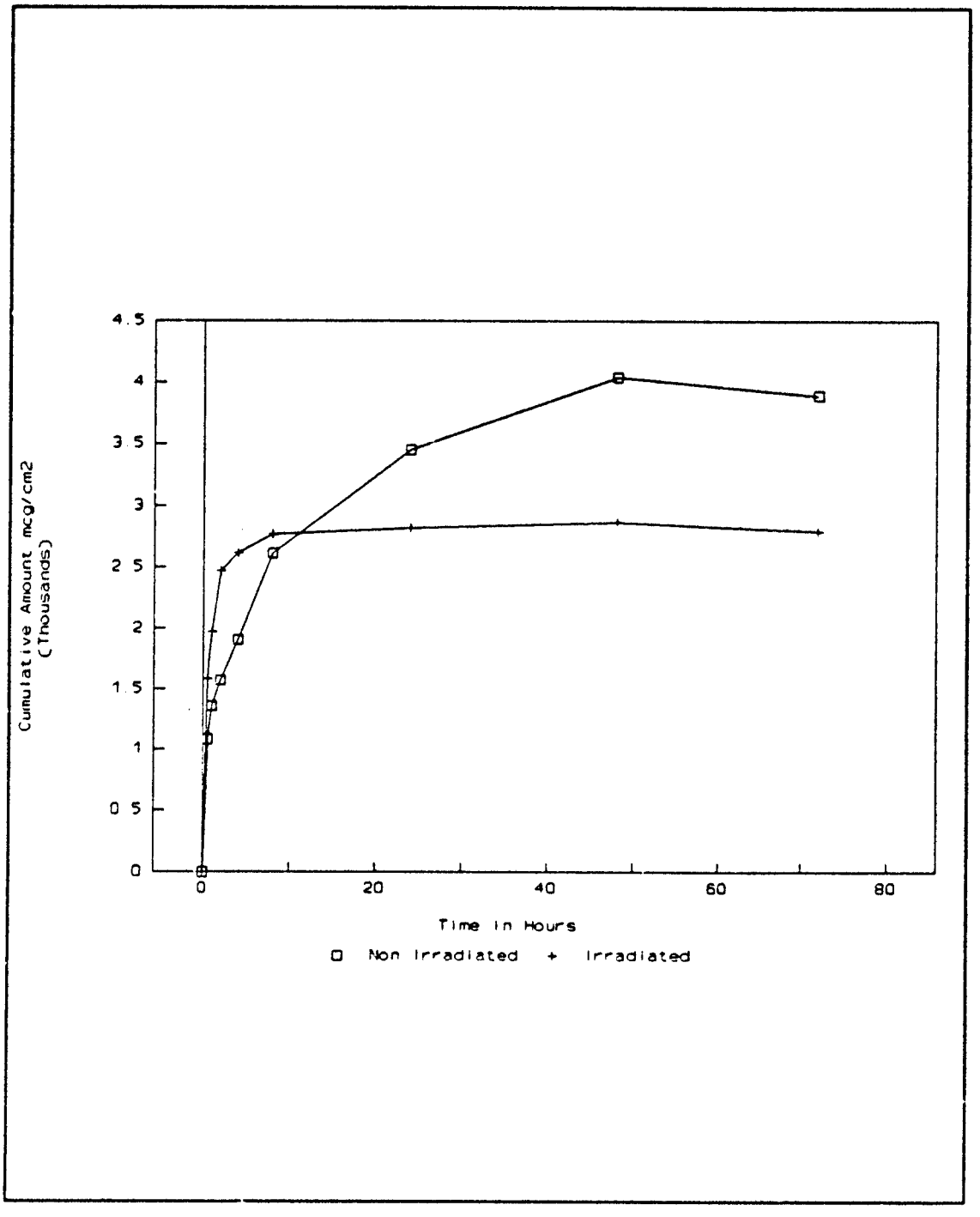


Figure 4. Effect of Gamma Radiation on a 6 Mil Thick Chlorhexidine Gluconate ADD (Note the reduction in drug release in the gamma irradiated sample).

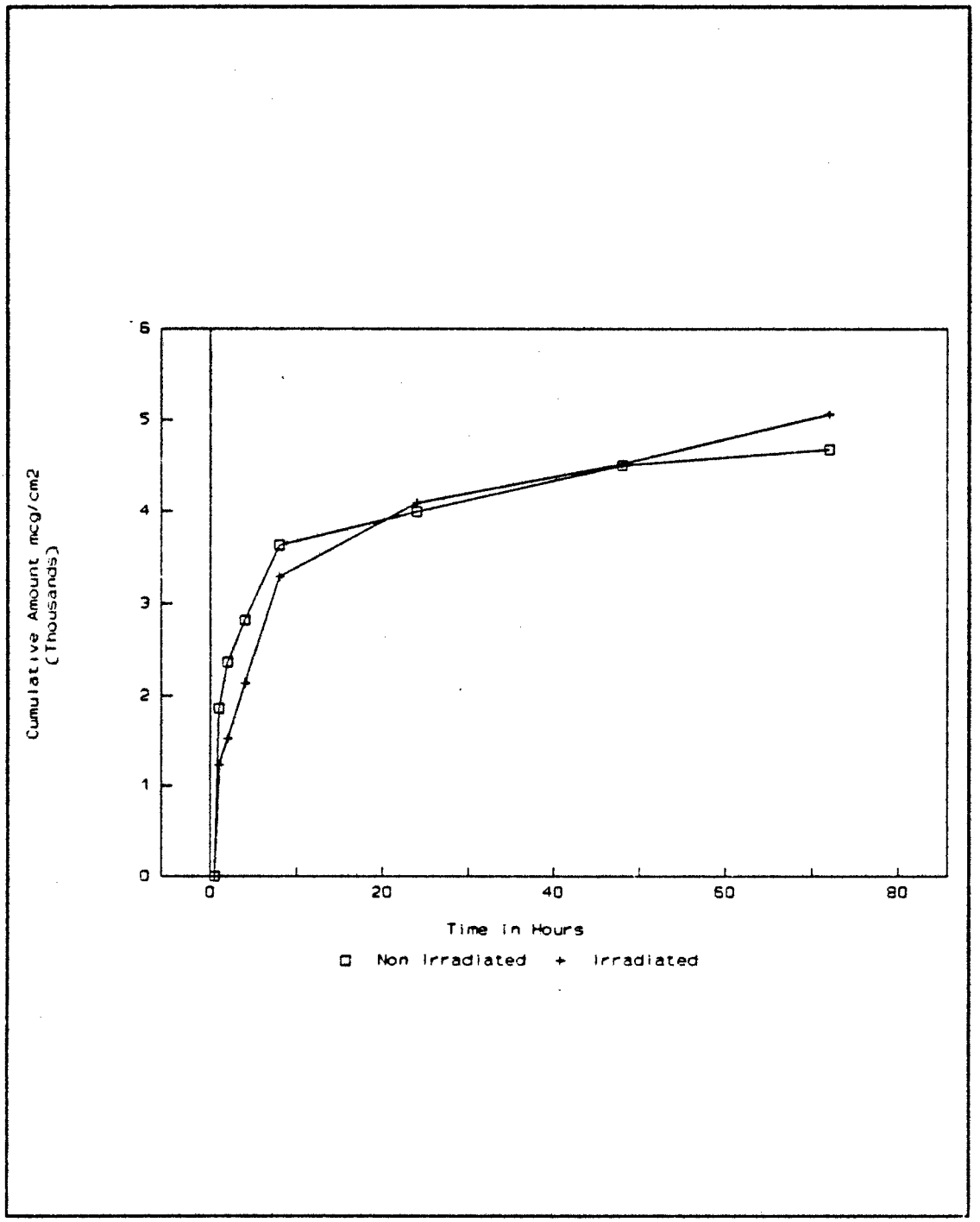


Figure 5. Effect of Electron Beam Radiation on a 12 Mil Thick Chlorhexidine Gluconate ADD.

B. Fabrication of Chlorhexidine Gluconate ADD's for Accelerated Shelf Stability Studies

All prior batches manufactured at Thermedics for delivery to USAIDR, were made utilizing 150 grams of material which yielded 100 to 150 ADD's. The requirements of Tasks V and VI called for the scaling up of the batch sizes 3 to 4 times. A pilot batch was manufactured using a laboratory scale Hockmeyer mixer. The excipients were first mixed and the lyophilized chlorhexidine gluconate slowly dispersed. The oligomer was then added into the drug-excipient mixture. The drug loaded oligomer was cured under UV lights. The resultant roll stock was then die cut, placed on Spandra® and the fabrication completed. The final dressings were sealed in aluminum pouches and sterilized by electron beam irradiation.

Five hundred and forty (540) chlorhexidine gluconate ADD's were fabricated and sterilized. Two hundred 1.5 x 1.5 inch ADD's were delivered to USAIDR, along with 100 placebos for in vivo animal testing. An additional one hundred 1 x 1 inch ADD's were also delivered for demonstration purposes. The remaining ADD's were set aside for shelf stability studies.

C. Shelf Stability Studies

The chlorhexidine gluconate ADD's were subjected to a battery of quality control tests. The elution kinetics were recorded documenting the initial time point (t = 0) for the accelerated shelf stability. The baseline release profile of these ADD's is shown in figure 6. The dressings exhibited effective results when subjected to microbiological zone of inhibition and sterility tests; these results along with the certificate of analysis, are appended (Appendix I).

The ADD's were subjected to various temperatures for the accelerated shelf stability studies. Forty-eight ADD's were tested under five conditions specified below:

(1) 45^0 C, 90% R.H

(2) 38^0 C, 90% R.H,

(3) Room Temperature,

(4) 23^0 C, under water,

(5) -40^0 C.

The stability of chlorhexidine ADD's is being determined using two methods:

1. analyzing the drug for the presence of the degradation product, and
2. measuring the maximum amount of drug eluted from the ADD's over 72 hours.

The major decomposition product of chlorhexidine gluconate is p-chloroaniline (PCA). A concentration of five hundred micrograms per milliliter (mcg/ml) of PCA is considered a safe biological level in animals (8). This value corresponds to a 25% degradation of chlorhexidine gluconate. A tenth of this value was selected as the maximum allowable concentration of PCA: i.e. 50 mcg/ml, which relates to 2.5% PCA or a 97.5% stable product. The assay methodology adopted in our laboratory for chlorhexidine analysis has a sensitivity of less than 10 mcg/ml (<10 ng) of PCA. The accelerated stability tests conducted until now show that chlorhexidine gluconate is stable when stored at 45^0 C for two months. Results of the assay show p-chloroaniline to be below detectable limits; figure 7 represents a chromatogram depicting this result. Figure 8 represents a chromatogram of a 1000 mcg/ml chlorhexidine gluconate standard spiked with p-chloroaniline. A comparison of these two chromatograms confirms that chlorhexidine gluconate stored at 45^0 C for two months does not exhibit any detectable degradation products.

The second method for determining storage stability involves an analysis of elution data acquired from ADD's removed from each of the storage conditions. The tabulated results and elution curves for these five conditions (-40^0 C, 23^0 C under water, ambient, 38^0 C and 45^0 C) are summarized in Table III and figure 9. Figure 10 lists data obtained for these samples compared with those at t = 0 revealing no significant change in elution rates. This series of 6

curves was generated from 1 data point for each time period. Therefore, it was decided to use a "paired t-test", to compare the 2 extremes; -40°C and 45°C. The statistical results summarized below indicate there is no significant decrease in drug elution at the two month period.

Table III. Results of Two Month Shelf Stability

	Cumulative Amount Chlorhexidine Gluconate (mcg/cm^2)					
Time (Hours)	t = 0	45°C	38°C	23°C water	RT	-40°C
0	0	0	0	0	0	0
0.5	1054	945	858	1173	1069	1216
1	1442	1427	1304	1528	1546	1670
2	1935	1507	1683	1950	1785	2037
4	2925	2067	2530	3028	2661	3104
8	3745	2808	3380	4134	3724	4110
24	4300	3765	4037	4823	4639	4616
48	4647	3955	4021	4940	4843	4957
72	4501	4173	4056	5014	4928	4642

Table IV. Results of "Paired t-test" Performed on Samples Stored Under Extreme Conditions.

Two Sample Analysis Results

		Sample 1	Sample 2	Pooled
Sample Statistics :	No. of Obs.	9	9	18
	Average	2294.11	2928	2611.06
	Variance	2.1528E6	3.15158E6	2.65219E6
	Std. Dev.	1467.24	1775.27	1628.56
	Median	2067	3104	2437.5

Conf. Interval for Ratio of Variances : 95 percent

(Equal Vars.)	Sample 1 - Sample 2	-2261.76	993.984	16 D.F.
(Unequal Vars.)	Sample 1 - Sample 2	-2266.47	998.689	15.5 D.F.

Conf. Interval for Ratio of Variances : 0 percent
Sample 1 v Sample 2

Hypothesis Test for HO : Diff = 0 Computed t statistic = -0.82569
vs Alt: NE Sig. Level = 0.421122
at Alpha = 0.05 so do not reject HO.

Sample 1 = 45^0 C Sample 2 = -40^0 C

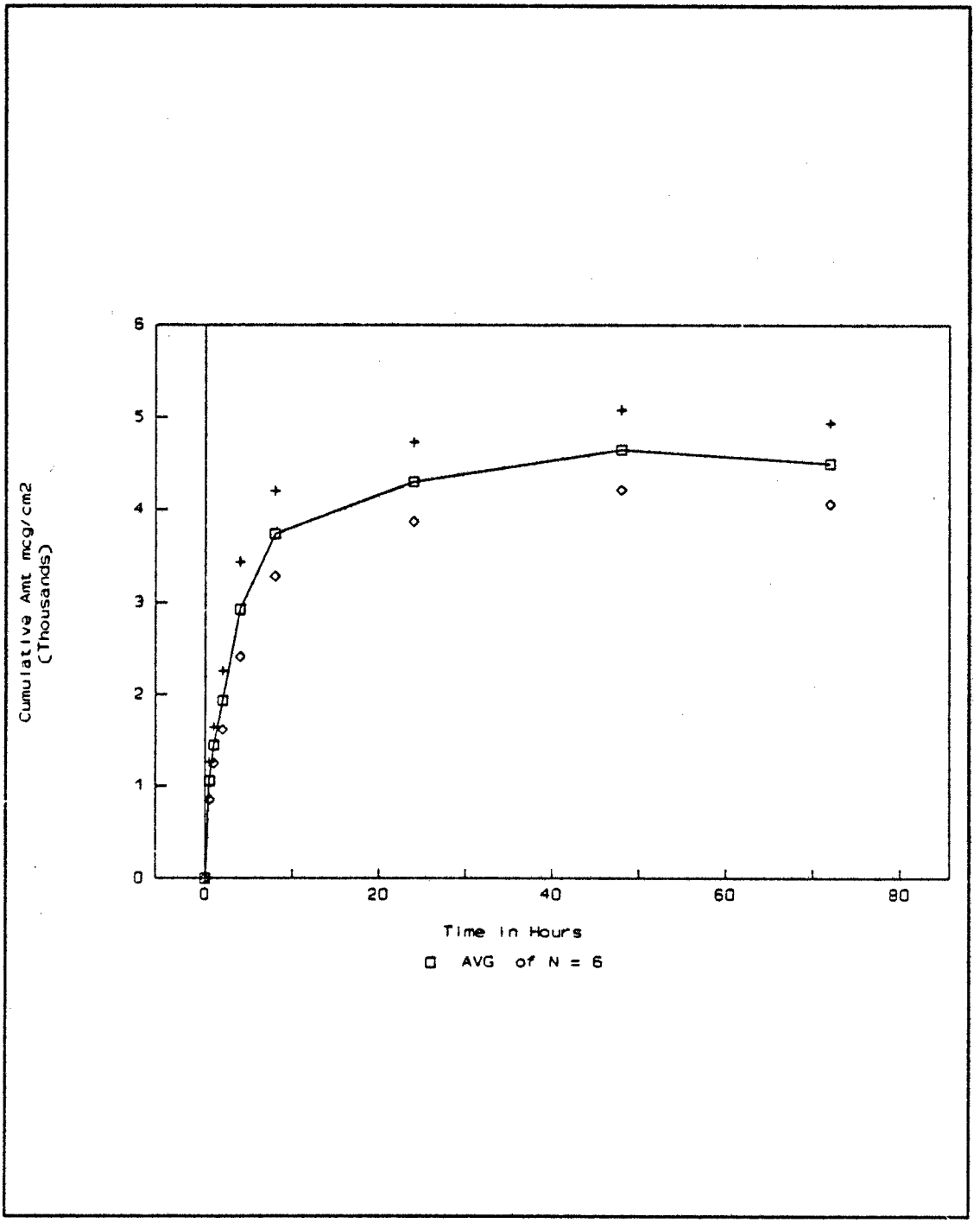


Figure 6. Release Profile of 30% Chlorhexidine Gluconate ADD's After E-beam Sterilization. Stability Samples at Time t = 0.

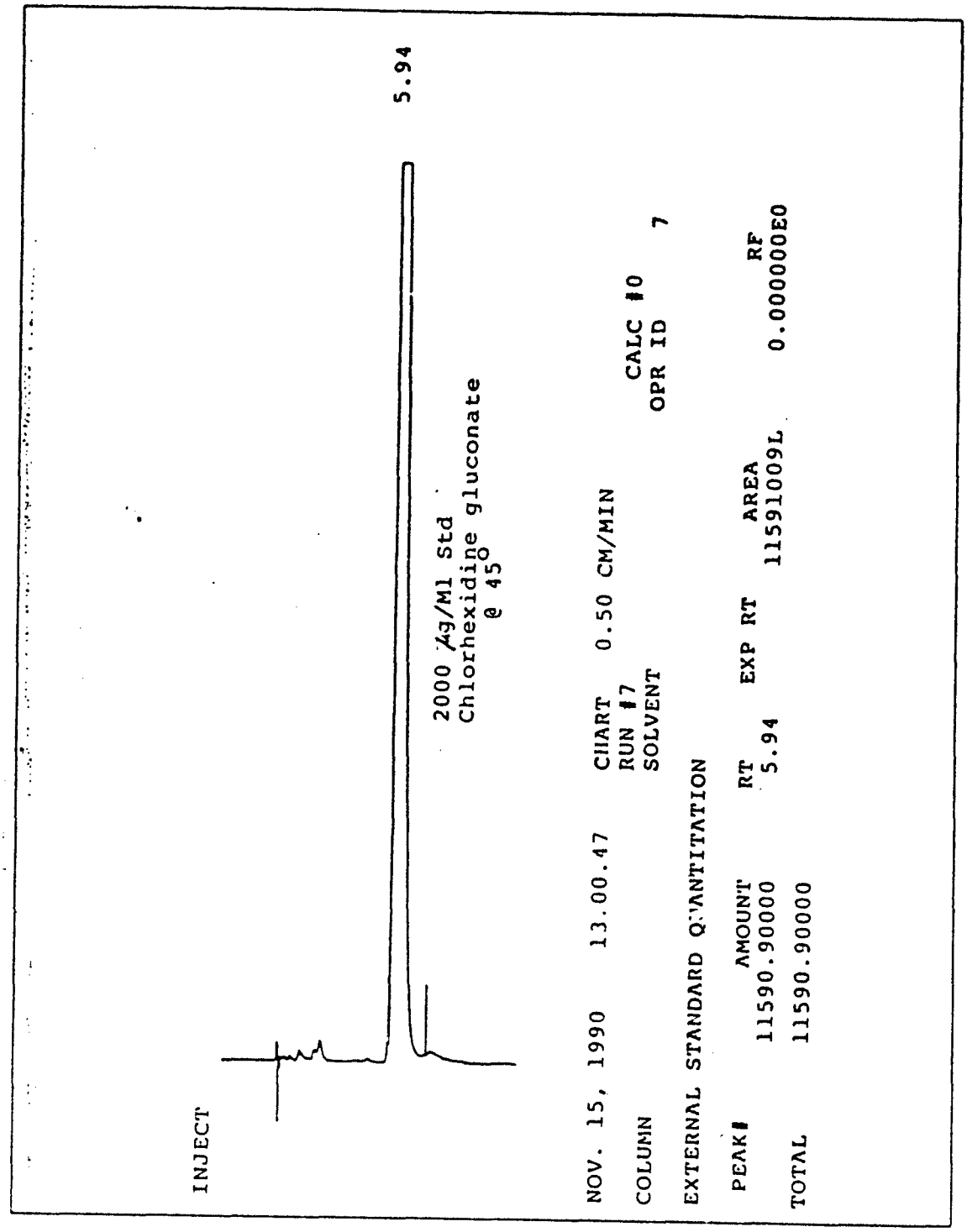


Figure 7. Chromatogram of Lyophilized Chlorhexidine Gluconate Stored at 45° C for Two Months.

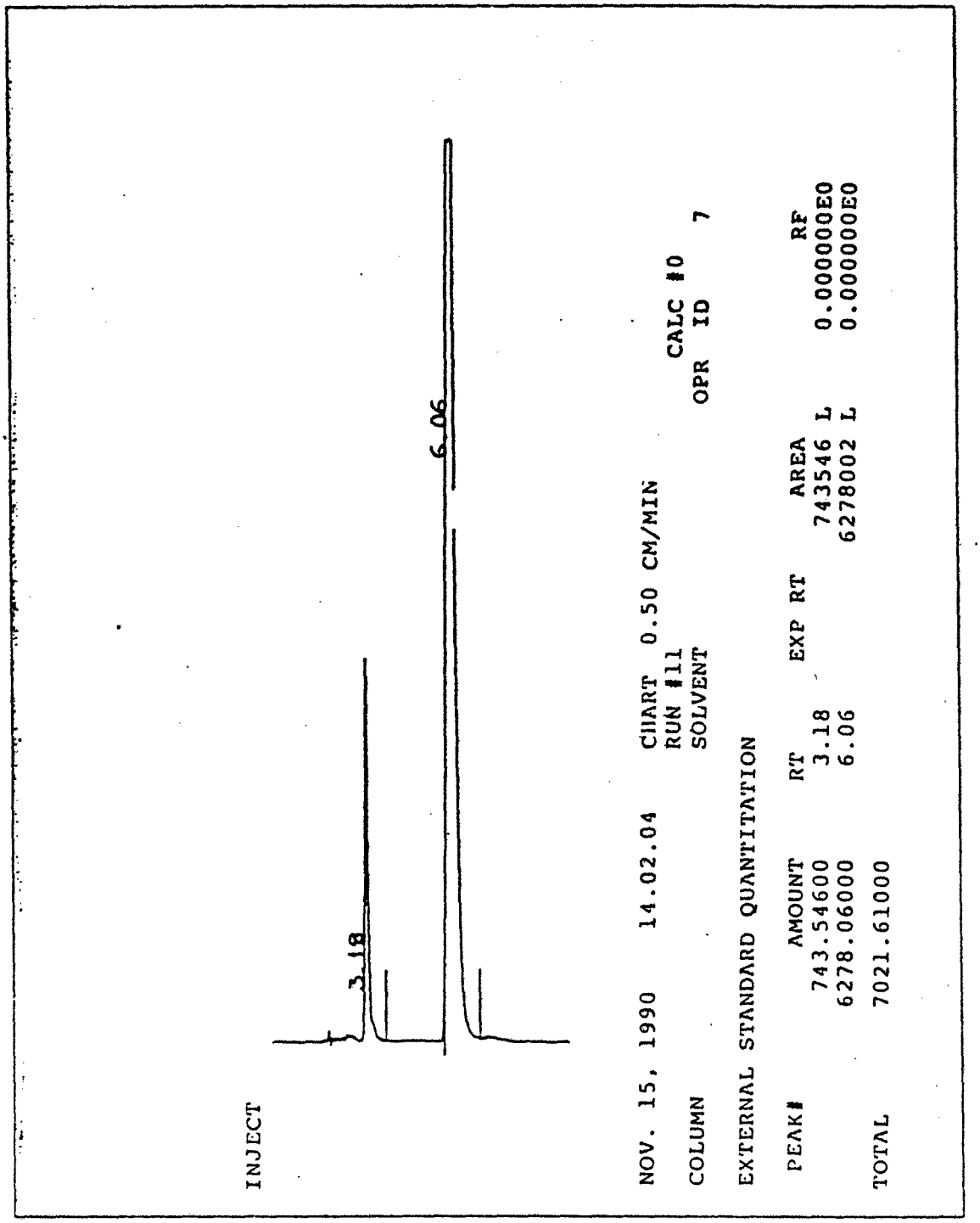


Figure 8. Chromatogram of Chlorhexidine Gluconate Standard (1000 mcg/ml) Spiked with p-chloroaniline (50 mcg/ml).

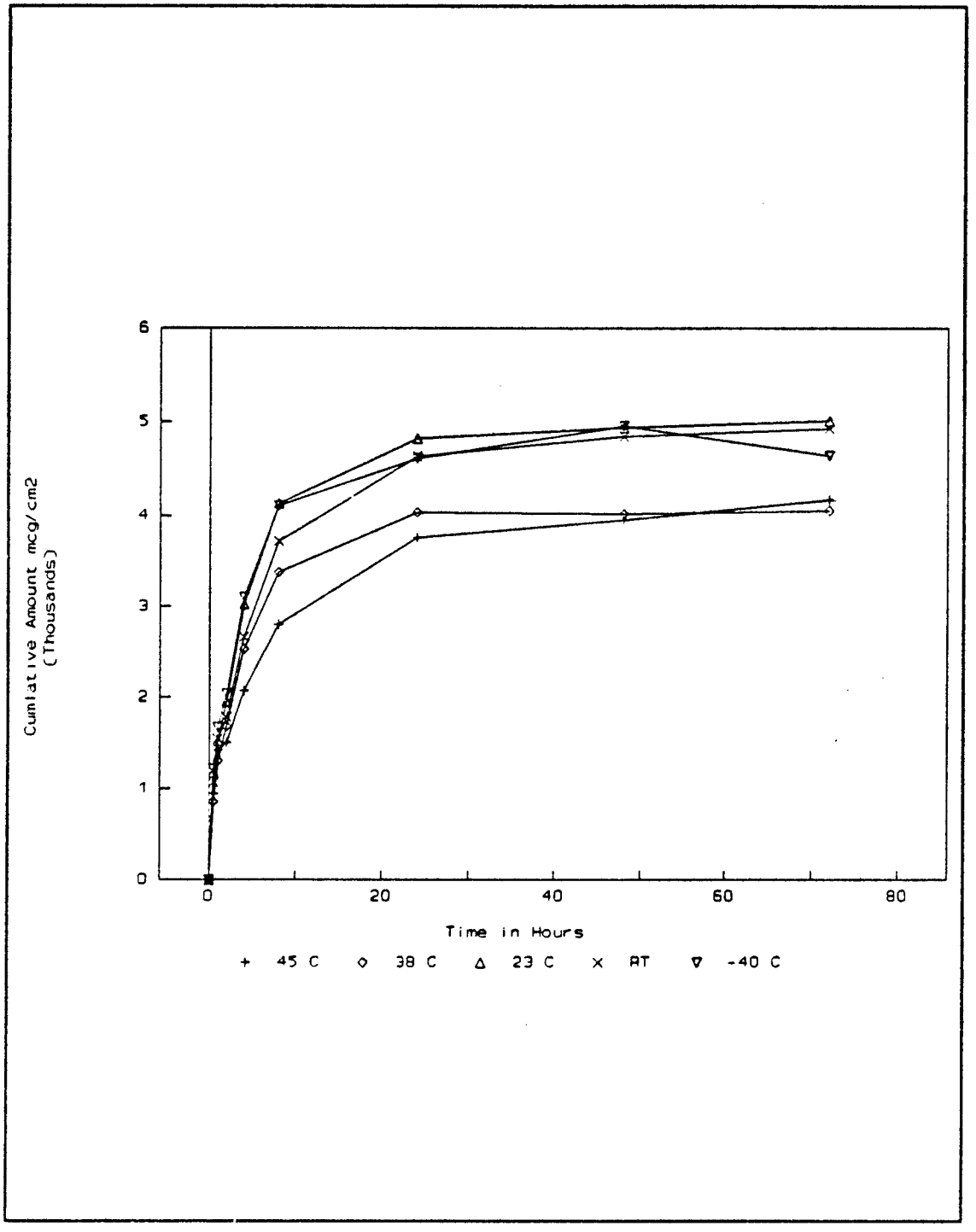


Figure 9. Elution Curves of Two Month Stability Samples of Chlorhexidine Gluconate ADD's.

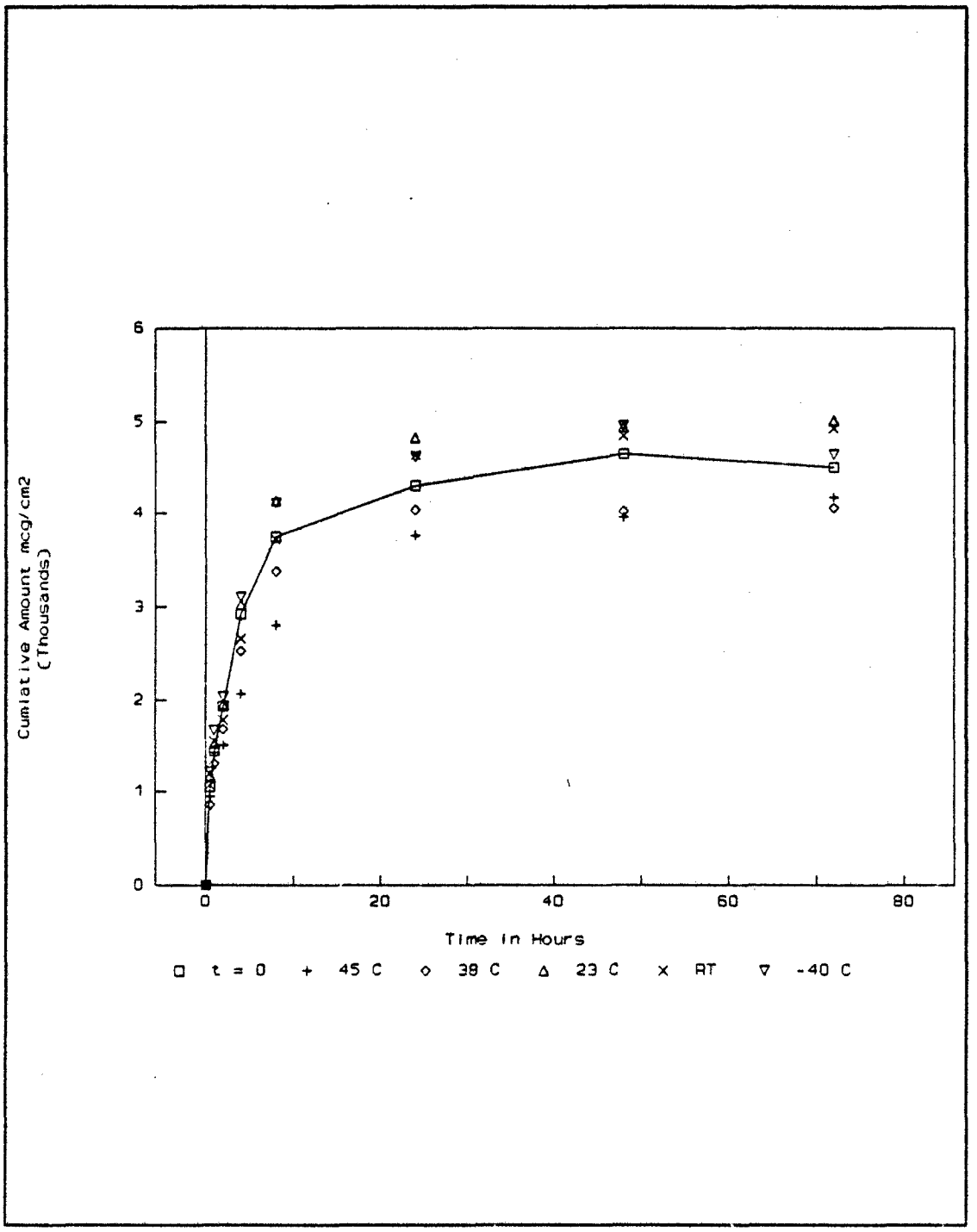


Figure 10. Comparison of the Elution Curves of the Chlorhexidine Gluconate ADD's at Time Periods, t = 0 & t = 2 Months.

TASK VII

The second medicated antimicrobial dermal dressing to be developed had to be effective against a broad spectrum of bacteria: Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, and fungi such as Trichophyton species, Epidermophyton species and Candida albicans. Many antimicrobials were screened for their spectrum of activity to find suitable candidates for incorporation into the UV cure polymer matrix. The most promising candidate, silver sulfadiazine was fabricated into ADD's for in vivo evaluation.

A. Preparation of Formulations Incorporating Photo Opaque Drugs

Initial trials based on microbiological tests, using a two percent silver sulfadiazine loading showed that a higher concentration was necessary in order to be effective. However, incorporation of larger amounts of drug prevented the polymer from curing into a film. Photo opaque powdered drugs such as silver sulfadiazine and nystatin inhibit or interfere with the polymerization of the oligomer by blocking the UV energy needed to dissociate the photoinitiator into free radicals. Comparison of the UV absorbance spectrum for Irgacure 651 and Silver sulfadiazine (Figures 11 and 12) showed that these two compounds had similar maximum absorbance peaks at 260 nanometers.

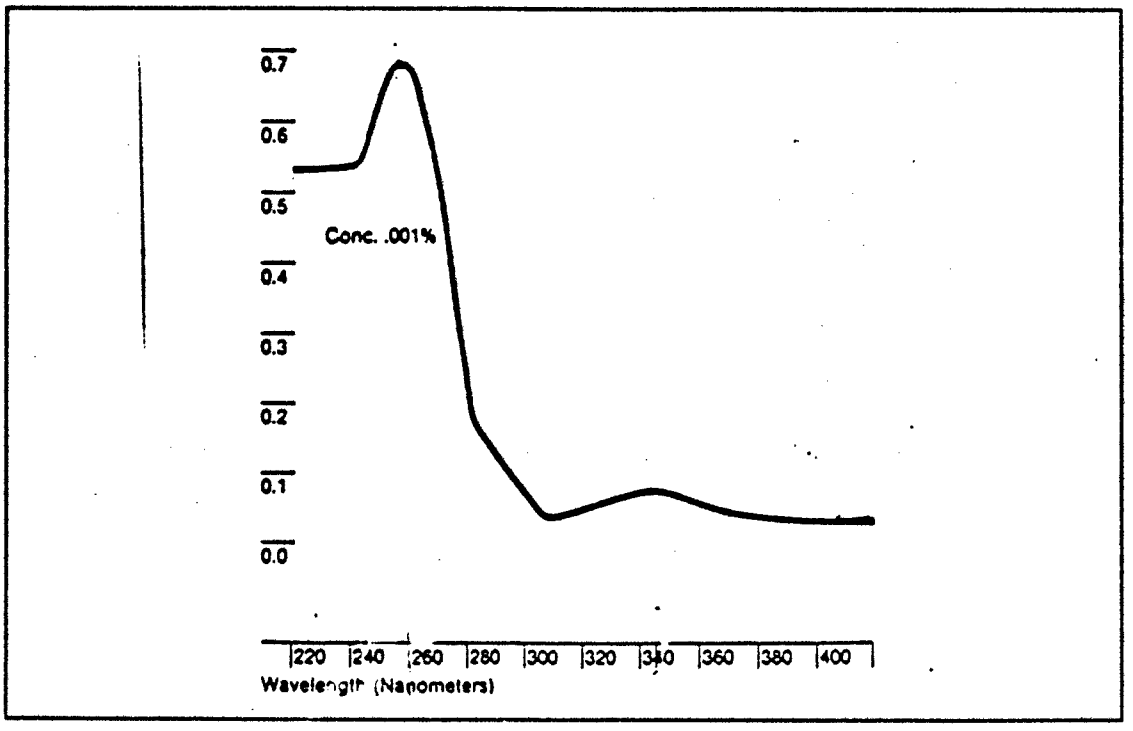


Figure 11. Absorption Characteristics of Irgacure 651.

It became necessary to investigate the use of a photoinitiator which dissociated through the absorption of a longer wavelength energy for incorporating photo-opaque drugs. One such photoinitiator is camphorquinone, which has been medically accepted in the dental industry for curing acrylic fillings (9). This compound has a maximum absorption wavelength of 460 nanometers, well above the interference levels of silver sulfadiazine. Various concentrations of silver sulfadiazine were incorporated into the polymer using the new photoinitiator. Cured films containing silver sulfadiazine showed that drug loadings up to 30% were possible employing this photoinitiator.

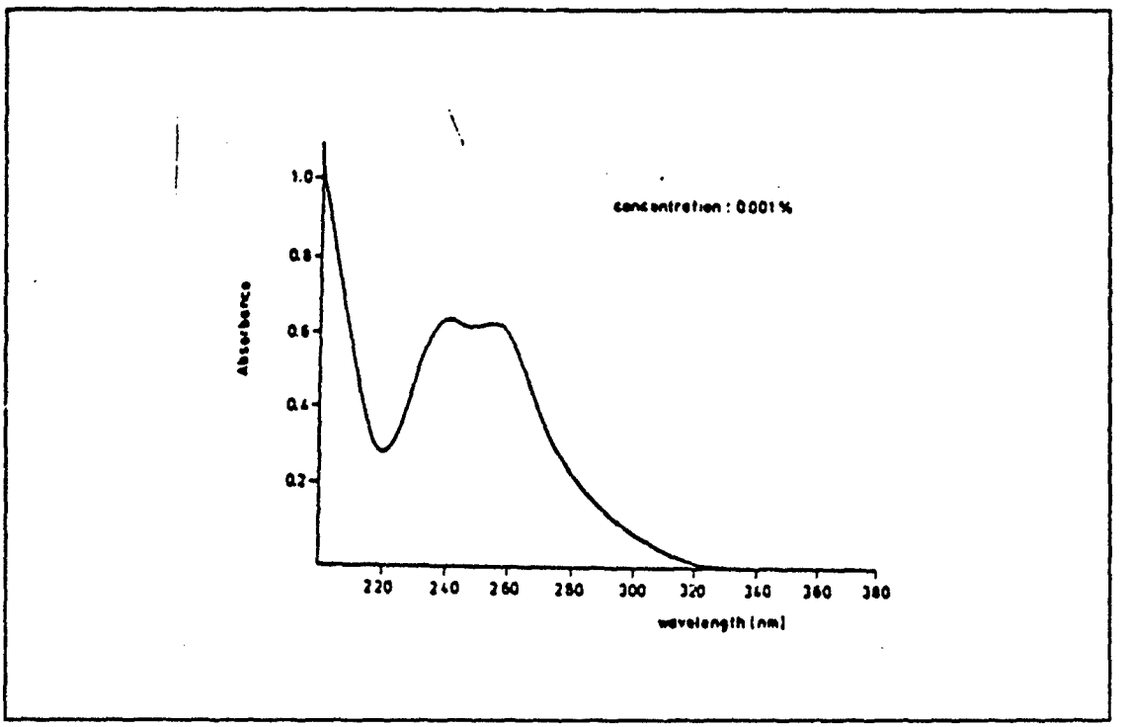


Figure 12. UV Spectrum of Silver Sulfadiazine.

Sample dressings containing nystatin exhibited the same curing problem as the silver sulfadiazine dressings. The inability to cure was eliminated by using the long wave length photoinitiator, camphorquinone. The use of this initiator permitted the fabrication of test sheets for Task XI (Year 2) containing high levels (up to 30%) of nystatin and silver sulfadiazine.

B. Drug Formulation Screening Tests

The activity of antimicrobials was evaluated using plate assay techniques to measure the inhibitory effect of candidate drugs on microorganisms. Utilization of microbiological methods detects a change in antimicrobial activity that may go undetected using standard chemical methods. The plate assay technique depends upon diffusion of a test antibiotic from a saturated disc, through a solidified agar layer in the petri dish, so that growth of the target microorganism is prevented entirely in a circular area (zone) around the disc containing a solution of the antimicrobial. In Task XI (Year 2), assay plates were prepared by pouring a molten mixture, 1 to 10 ratio of Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes inoculum with Trypticase Soy Agar (TSA), or Candida albicans and Trichophytons inoculum with Sabaurouds Dextrose Agar (SDA), into petri dishes and allowing it to harden. The TSA plates were incubated at 30 - 35^{0}C for 72 hours and the SDA plates at 20 - 25^{0}C for 5 days. This procedure was repeated for each test formulation and the controls. Attempts to screen a sixth organism, Epidermophytons failed. This microorganism has an incubation period of 22 - 24 days, which is well beyond the effective duration of activity (72 hours) of the fabricated dressings.

Fourteen different formulations incorporating Silver sulfadiazine, Clindamycin phosphate, Gentamicin sulfate,

Chlorhexidine gluconate or Nystatin, either alone or in combination with one another were tested (Table V). Twelve of these test samples were formulated to a 30 percent loading; i.e. 30 milligrams of drug per 100 milligram patch. The formulation incorporating three antimicrobials; i.e. clindamycin, gentamicin and silver sulfadiazine, was selectively tested for activity against gram negative and gram positive bacteria and fungi. The most promising test results are reported in Table VI.

Table V. List of Formulations Tested Microbiologically.

Antimicrobial	Concentration by weight (%)
Chlorhexidine	30
Chlorhexidine*	30
Chlorhexidine	33
Chlorhexidine Nystatin	15 15
Chlorhexidine Nystatin	20 10
Chlorhexidine Silver sulfadiazine	15 15
Chlorhexidine Silver sulfadiazine	10 20
Silver sulfadiazine	30
Silver sulfadiazine*	10
Silver sulfadiazine Nystatin	20 10
Chlorhexidine Silver sulfadiazine Nystatin	10 10 10
Nystatin	30
Silver sulfadiazine*+	2
Clindamycin phosphate Gentamicin sulfate Silver sulfadiazine	20 25 2

* Irgacure 651 was used as the photoinitiator.
+ Silver sulfadiazine was incorporated in a hydrophilic cream.

Table VI. Summary of Zone of Inhibition Tests.

Test Formulation	Test Organism				
	S.aureus	P.aerug	S.pyog	C.albic	T.mentag
10% Chlor. 10% S.sulfa 10% Nystatin	+	+	+	+	+
15% Chlor. 15% Nystatin	+	+	+	+	+
10% Chlor. 20% S.sulfa.	+	+	+	+	+
20% S.sulfa. 10% Nystatin	+	+	+	+	+
20% Clinda. 25% Genta. 2% S.sulfa.	+	+	N/A	+	N/A

The formulations reported here are those which showed promising results.

TASK VIII

This task required the submission of five formulations to USAIDR for in vivo testing on guinea pigs. The five formulations reported in Table VI appeared equally effective against target organisms based on the zone of inhibition tests. Nystatin is primarily an antifungal agent and exhibits no antibacterial properties, whereas silver sulfadiazine exhibits both antifungal and antibacterial activity. Moreover, sulfadiazine has been shown to have synergistic properties in the presence of chlorhexidine (10). Results of the zone of inhibition screening test demonstrates the microbiological efficacy of this combination. Based on these observations, an initial formulation was prepared incorporating 20% silver sulfadiazine in combination with 10% chlorhexidine gluconate for in vivo testing.

The results of the in vivo test for this initial formulation indicated this choice was effective for prophylactic treatment. However, the dressings were not as effective when tested under therapeutic conditions. The incorporation of previously tested antibiotics was thought necessary to improve therapeutic efficacy. However, an in vitro test showed that combinations of gentamicin sulfate with chlorhexidine gluconate were chemically incompatible. Therefore, the remaining formulations submitted under Task VIII incorporated various combinations of silver sulfadiazine, chlorhexidine gluconate and clindamycin phosphate.

A. Formulations Submitted Under Task VIII.

Forty five dressings of five formulations were fabricated and submitted to USAIDR for in vivo tests on guinea pigs. The formulations consisted of:

(1) 20% Silver sulfadiazine and 10% Chlorhexidine gluconate (B.N. 003281),

(2) 20% Clindamycin phosphate and 10% Chlorhexidine gluconate (B.N. 007121),

(3) 20% Silver sulfadiazine and 10% Clindamycin phosphate (B.N. 007131),

(4) 10% Silver sulfadiazine, 10% Chlorhexidine gluconate and 10% Clindamycin phosphate (B.N. 007132)

and, (5) 12% Silver sulfadiazine, 10% Chlorhexidine gluconate and 12% Clindamycin phosphate (B.N. 009101).

The ADD's containing clindamycin phosphate and chlorhexidine gluconate (B.N. 007121) did not require any modification of the excipients to facilitate UV cure. However, the other three formulations containing silver sulfadiazine required alternative excipients. The use of alternative excipients was necessary because heterogenous immiscible phases formed while using the existing excipient which caused the mixture to separate at the knife over roll station before UV curing. For this reason an excipient which was homogeneous with the polymer was chosen. The propylene glycol and polyethylene glycol (PEG) 300 were replaced by a high molecular

weight glycol (Poloxamer 182), a block copolymer, which formed a nonflocculated homogeneous dispersion, which could be cured by UV radiation. All the dressings had a nominal thickness of 12 mils and were sterilized by EtO.

B. In Vitro Release Kinetics of Silver Sulfadiazine ADDs

The release kinetics for silver sulfadiazine and the water soluble drugs were tested in vitro using established methods employing Franz diffusion cells and water as the dissolution media (11). Quantitative analysis of silver sulfadiazine was performed by HPLC using a UV detector. Chromatography was performed on an Octadecyl Silane (ODS) column using 1% acetic acid-methanol (60:40) as the mobile phase. Silver sulfadiazine was detected at 254 nm. This quantitation was based on the detection of sulfadiazine moiety of the silver sulfadiazine (12).

The elution kinetics for four formulations are reported. Figures 13 and 14 show the release profile of the ADD's containing silver sulfadiazine and chlorhexidine gluconate - Batch No. 003281. Figure 15 shows the release profiles of both clindamycin phosphate and chlorhexidine gluconate - Batch No. 007121. Lastly, figures 16 and 17 are the elution curves for chlorhexidine gluconate from the triple loaded ADD's Batch Nos. 007132 and 009101.

The in vitro analyses of dressings containing any combinations

of clindamycin phosphate and silver sulfadiazine were not carried out for two reasons: (1) the in vivo trials showed that the dressings containing these combinations were no more advantageous than any of the other combinations; and (2) the existing chromatographic methods were not specific enough to distinguish the amount of clindamycin phosphate in the presence of silver sulfadiazine and vice versa. Consequently, no in vitro results of either drug are provided for these formulations (Batch Nos. 007131, 007132 and 009101). However, the release of chlorhexidine gluconate from the triple loaded dressings (Batch Nos. 007132 and 009101) are reported.

The in vitro results for the formulations incorporating silver sulfadiazine indicate that the maximum amount of drug eluted from the dressing was less than 1% of it's loading. Nevertheless, the in vivo results demonstrated this loading to be effective.

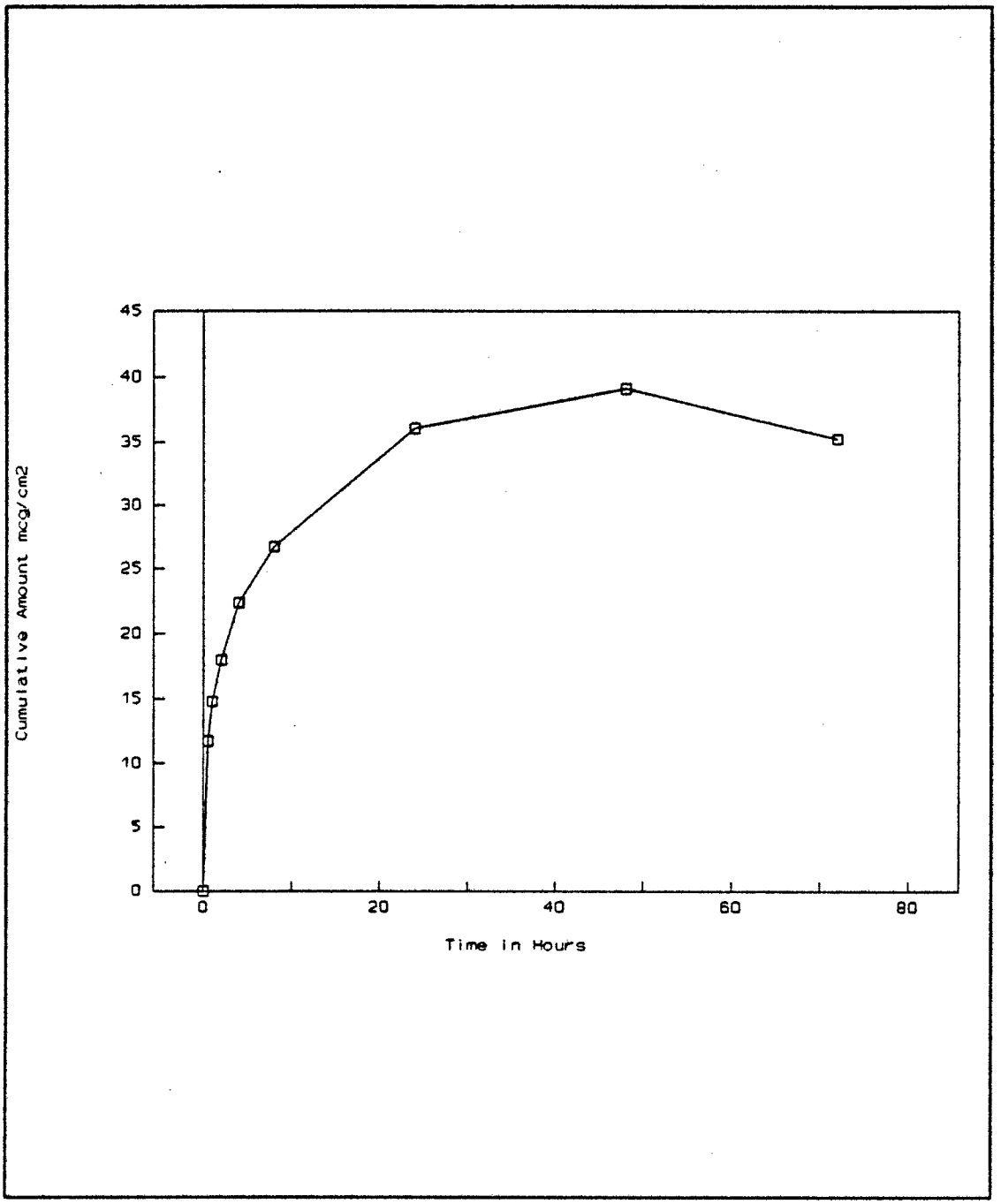


Figure 13. Release Profile of Silver Sulfadiazine from a Dual Loaded ADD containing 20% Silver Sulfadiazine and 10% Chlorhexidine Gluconate - 12 Mils (B.N. 003281)

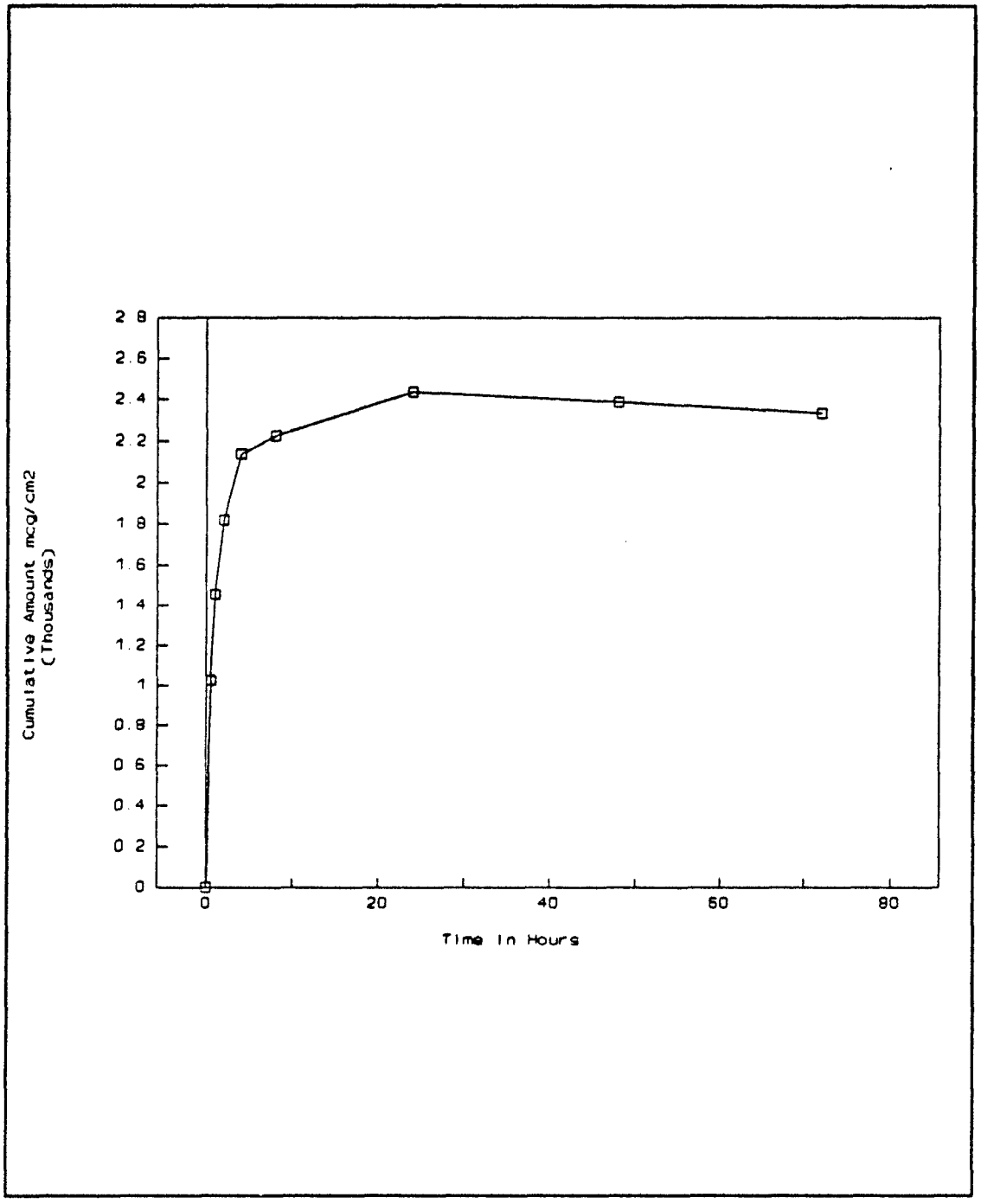


Figure 14. Release Profile of Chlorhexidine Gluconate from a Dual Loaded ADD containing 20% Silver Sulfadiazine and 10% Chlorhexidine Gluconate - 12 Mils (B.N. 003281).

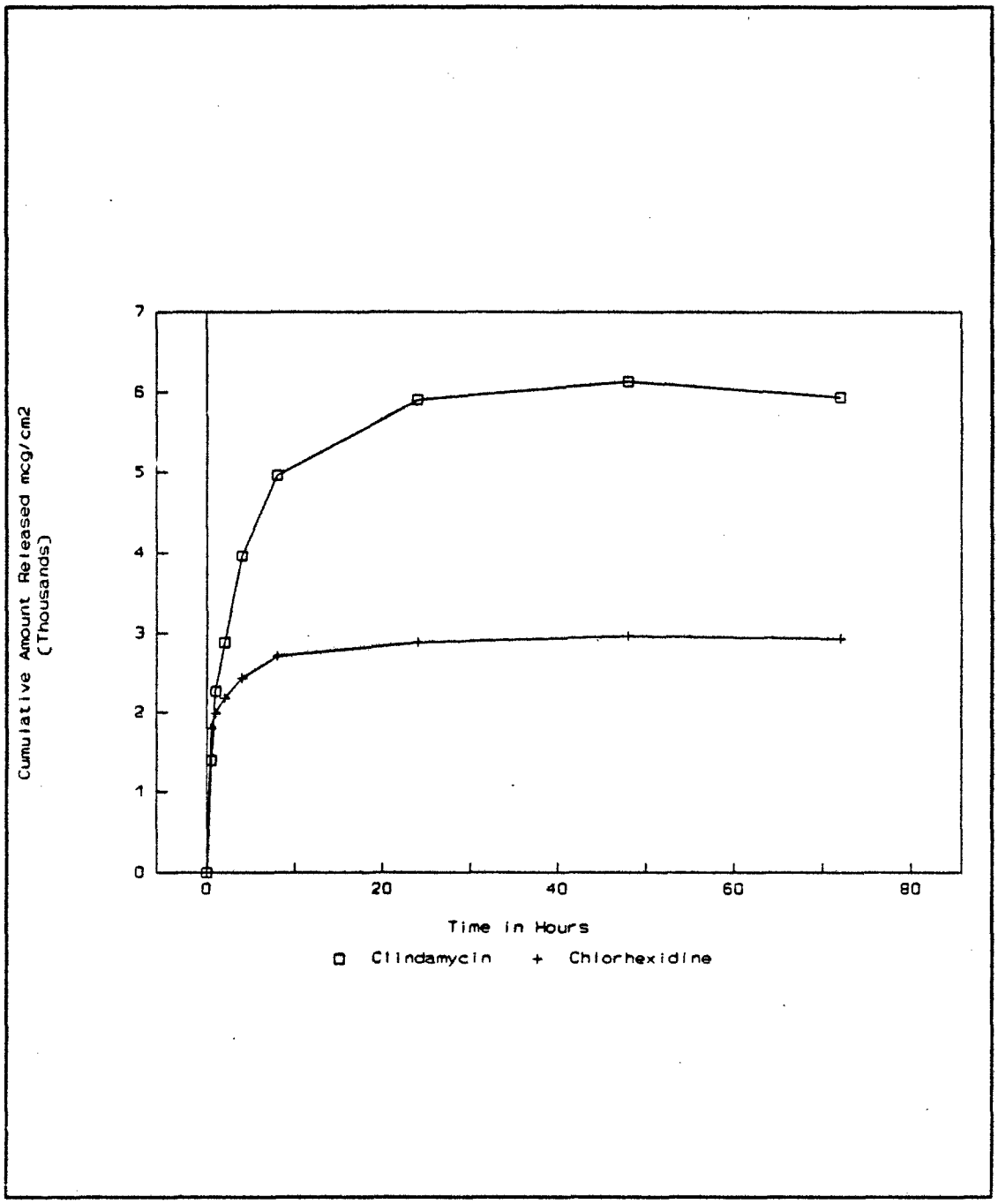


Figure 15. Release Profile of Dual Loaded ADD containing 20% Clindamycin Phosphate and 10% Chlorhexidine Gluconate - 12 Mils (B.N. 007121).

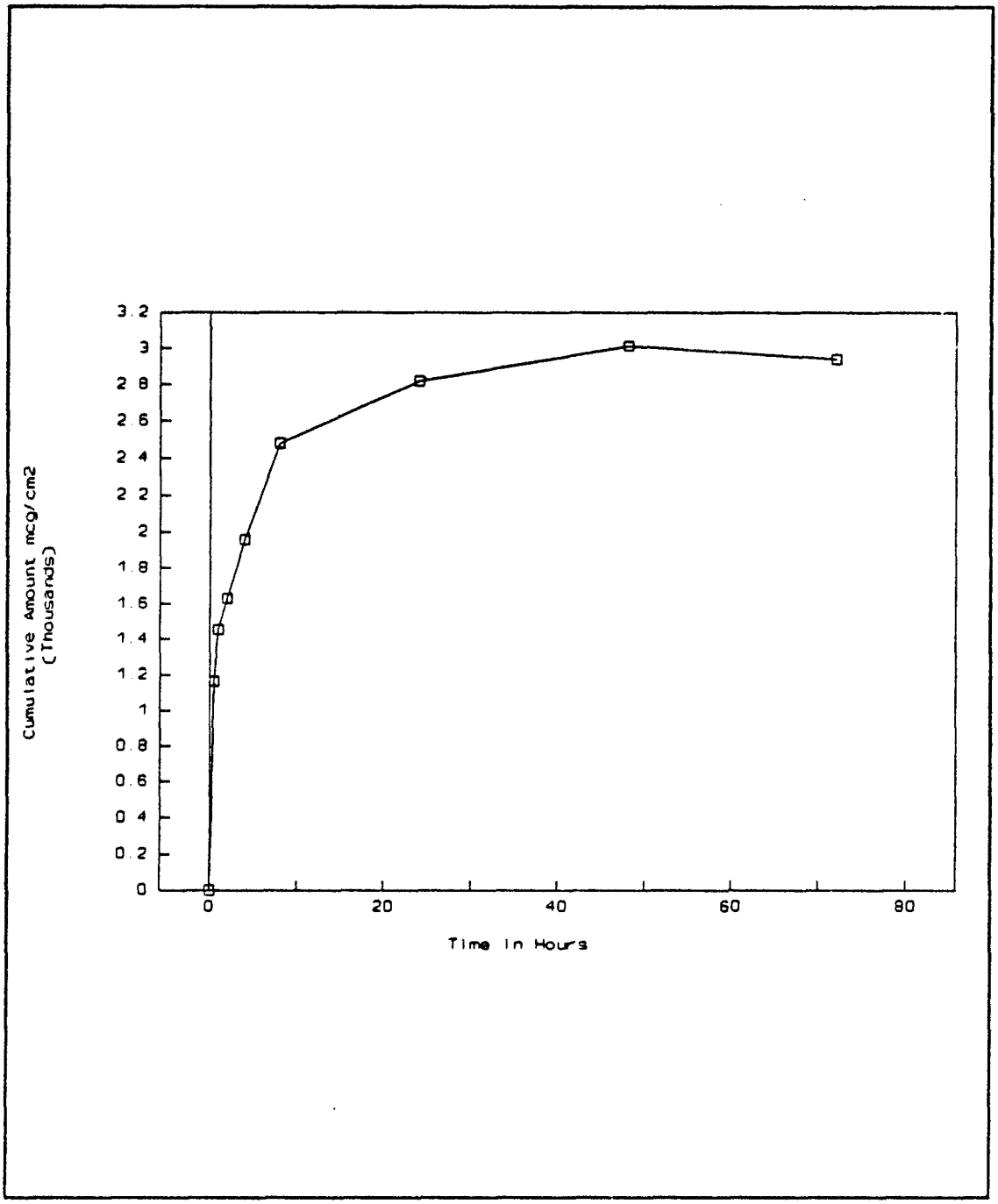


Figure 16. Release Profile of Chlorhexidine Gluconate from a Triple Loaded ADD containing 10% Silver Sulfadiazine, 10% Chlorhexidine Gluconate and 10% Clindamycin Phosphate - 12 Mils (B.N. 007132).

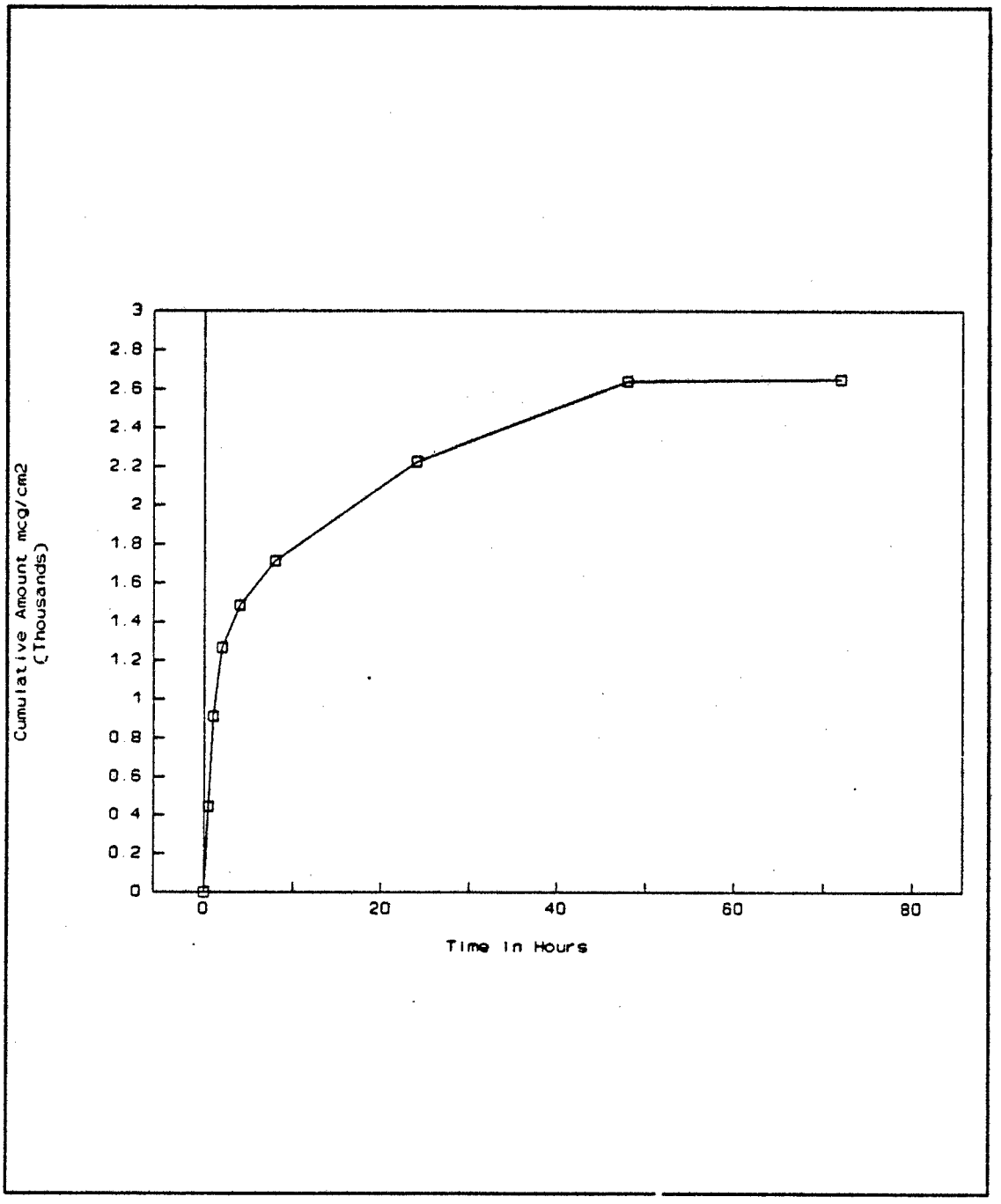


Figure 17. Release Profile of Chlorhexidine Gluconate from a Triple Loaded ADD containing 12% Silver Sulfadiazine, 10% Chlorhexidine Gluconate and 12% Clindamycin Phosphate - 12 Mils (B.N. 009101).

C. Summary of USAIDR In Vivo Results

The in vivo tests of the ADD's submitted under Task VIII were conducted at the laboratory facilities of USAIDR in Maryland. Five different formulations were tested on inoculated guinea pigs and the results summarized in Table VII.

Table VII. Summary of USAIDR In Vivo Tests - Biopsy Results.

USAIDR Gp #	N	Staph. aureus Clin. Infection	N	P. aeruginosa Clin. Infection
Formulation 1	5	0	6	0
Formulation 2	6	0	5	1
Formulation 3	5	2	6	4
Formulation 4	5	0	6	1
Formulation 5	-	-	5	3

Formulation 1 = 20% Silver sulfadiazine & 10% Chlorhexidine gluconate

Formulation 2 = 20% Clindamycin phosphate & 10% Chlorhexidine gluconate

Formulation 3 = 20% Silver sulfadiazine & 10% Clindamycin phosphate

Formulation 4 = 10% Silver sulfadiazine, 10% Chlorhexidine gluconate & 10% Clindamycin phosphate

Formulation 5 = 12% Silver sulfadiazine, 10% Chlorhexidine gluconate & 12% Clindamycin phosphate.

Results of prophylactic tests on animals showed that the formulation containing 20% silver sulfadiazine and 10% chlorhexidine gluconate successfully inhibited clinical infections caused by Staphylococcus aureus and Pseudomonas aeruginosa. These encouraging results led to the recommendation that this formulation was the optimal choice for accelerated stability testing under Task X.

TASKS IX AND X

Tasks IX & X required the preparation, packaging and sterilization of 500 individual ADD's containing 20 parts silver sulfadiazine and 10 parts chlorhexidine gluconate. All the dressings were die cut from sheet stock which was prepared from a one kilogram batch. This batch was mixed in a stainless steel container with a high shear Hockmeyer mixer for uniform drug dispersion. The sheet stock was prepared immediately after proper dispersion and all the ADD's for Tasks IX & X were fabricated from this sheet stock the following day.

Task IX required the preparation 200 sterile ADD's. Each dressing was individually sealed in an aluminum pouch, labelled and sterilized by electron beam irradiation. These were delivered to USAIDR along with 100 sterile placebos. The remaining ADD's were subjected to a battery of quality control tests. The elution kinetics were also determined and recorded for reference as the initial starting value for the storage stability study. Sample dressings were successfully tested for microbiological zone of inhibition and sterility. Results have been included in the appendix.

A. In Vitro Analysis of Silver Sulfadiazine ADD's

The procedures used for the analysis of these ADD's had to be modified because silver sulfadiazine is nearly insoluble in water. Distilled water which was previously used as a dissolution media in the receptor cell was not predicted to be effective in extracting silver sulfadiazine from the ADD s. Therefore, the techniques for the HPLC analyses of these ADDs were tested under two conditions:

(1) utilizing conventional extraction methods previously employed to detect the presence of water soluble drugs, and
(2) the extraction of silver sulfadiazine under ideal conditions.

The first method (Task VIII-B) was employed to compare the elution rate under the identical conditions previously used for the water soluble drugs, whereas the second method was intended for the specific assay of silver sulfadiazine.

Various solvents were substituted for water to determine an optimal extraction media for the dressing (Table VIII). A thirty-five percent ammonia solution was chosen as the dissolution medium since silver sulfadiazine is freely soluble in ammonia. The volatility of this media excluded the use of the Franz diffusion cells because of it's open design. As an alternative, a modification of the USP Rotating Bottle method was used for the total extraction of silver sulfadiazine (13). The dressings were placed in tightly capped amber bottles containing the ammonia

solution and extracted for 24 hours in a sieve shaker. The extracts were then diluted and assayed (Appendix III). This procedure allowed the detection of the maximum amount of silver sulfadiazine that could be extracted in vitro from the ADD's.

Table VIII. Solubility of Silver Sulfadiazine

Solvent	Solubility (mg/100 ml)
Water	0.11
Dimethyl Sulfoxide	> 35
10% w/v Ammonia solution	> 2.10^3
25% w/v Ammonia solution	> 5.10^3
35% w/v Ammonia solution	freely soluble
Propylene glycol	slightly soluble
15% PEG 300 solution	insoluble
40% PEG solution	insoluble
Oleic Acid	slightly soluble
Light Mineral Oil	slightly soluble

Initially, a 1 cm diameter disc of the ADD was placed into a 50 ml amber bottle containing 20 ml of a 25% ammonia solution and extracted for 24 hours using a sieve shaker. Six milliliters of the extract was diluted to 100 ml with distilled water and assayed for silver sulfadiazine. The extraction was performed on three ADD's with a control sample run concurrently. The control sample contained a predetermined amount of silver sulfadiazine. Six milliliters of this solution was diluted to 100 ml like the test samples. The second formulation was also assayed similarly, but to test for content uniformity, thirty 1 cm (diameter) discs, in 3 groups of 10, were extracted. Two sets of controls which bracketed

the amounts of silver sulfadiazine contained in 10 discs were also run.

Two formulations of ADD's containing 20% silver sulfadiazine by weight were assayed utilizing this method. The first formulation (B.N. 003281) contained 20% silver sulfadiazine and 10% chlorhexidine gluconate. The second formulation (B.N. 007131) contained 20% of silver sulfadiazine and 10% clindamycin phosphate.

The results of these assays indicate that about 90% of the silver sulfadiazine in the ADD can be extracted under ideal conditions (Table IX). The above procedure was utilized to determine the total silver sulfadiazine content in those dressings undergoing accelerated storage stability. However, these extraction conditions are not representative of those in vivo. Elution of the drug into the wound is hypothesized to be less than that observed under these conditions, because of the high water content associated with the wound.

Table IX. Results of Silver Sulfadiazine Assay.

Sample	EXPERIMENTAL mg/Disc AgSdz	THEORETICAL mg/Disc AgSdz	RELEASE % AgSdz
	1 Disc/extraction		
Control	5.03	4.68	107
Sample I			
# A	4.18	4.68	89.3
# B	4.30	4.78	90.0
# C	4.17	4.92	84.7
	10 Discs/extraction		
Controls	mg/10 Discs AgSdz		% AgSdz
Set 1	43.3	46.8	92.5
Set 2	109.4	122.1	89.5
Sample II			
# A	50.7	57.2	88.6
# B	51.1	54.2	93.2
# C	55.4	56.8	97.5

AgSdz = Silver sulfadiazine

Sample I = 20% Silver sulfadiazine:10% Clindamycin phosphate (Batch No. 007131)

Sample II = 20% Silver sulfadiazine:10% Chlorhexidine gluconate (Batch No. 003281)

B. Shelf Stability Studies

The ADD's (B.N. 010181 - PDDS2) were tested at five different temperatures for the accelerated shelf stability studies. A group of forty-eight ADD's was placed under each of the five conditions specified below:

(1) 45^0 C, 90% R.H,

(2) 38^0 C, 90% R.H,

(3) Room Temperature

(4) 23^0 C, under water,

(5) -40^0 C.

The elution kinetics of these ADD's were recorded and used as the initial time point (t = 0). These are reported as elution curves, figures 18 and 19.

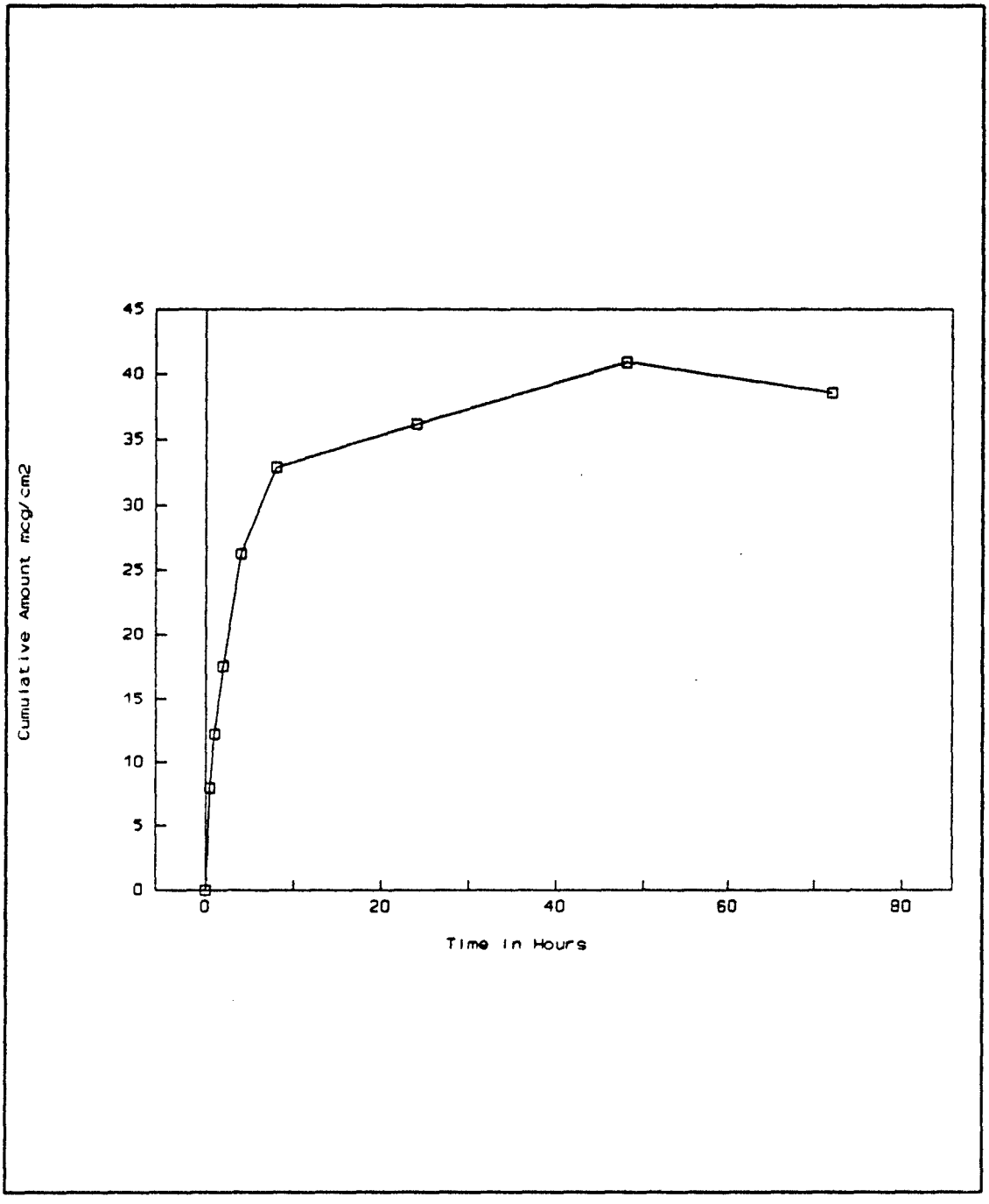


Figure 18. Release Profile of Silver Sulfadiazine from a Dual Loaded ADD containing 20% Silver Sulfadiazine and 10% Chlorhexidine Gluconate - 12 Mils (B.N. 010181 - PDDS2).

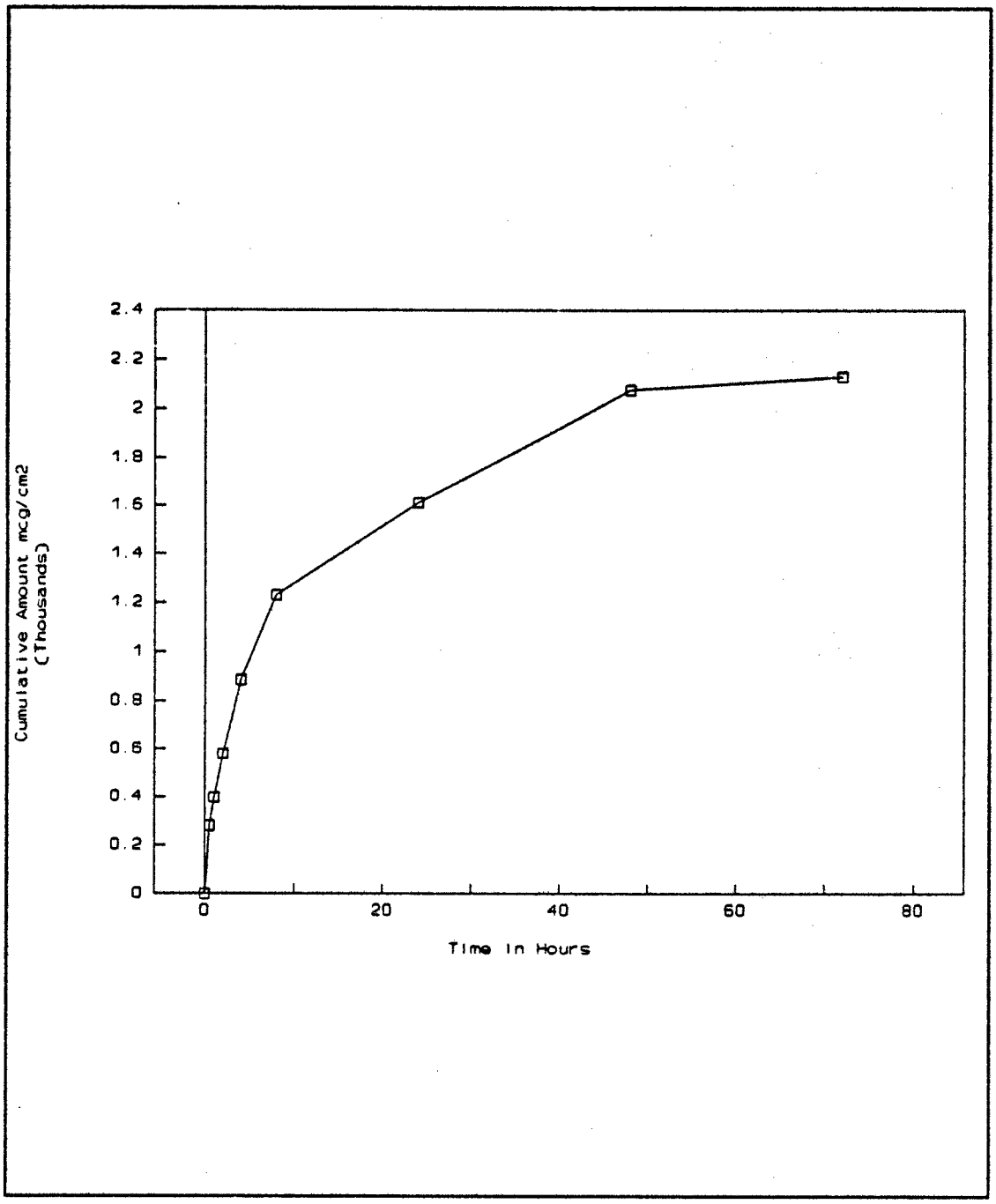


Figure 19. Release Profile of Chlorhexidine Gluconate from a Dual Loaded ADD containing 20% Silver Sulfadiazine and 10% Chlorhexidine Gluconate - 12 Mils (B.N. 010181 - PDDS2).

CONCLUSIONS

Thermedics Inc. has developed two sustained release Antimicrobial Dermal Dressings. Both types of dressings incorporate antimicrobial agents to prevent infection in superficial lesions for periods up to 72 hours.

The first formulation, a chlorhexidine gluconate ADD, was formulated and tested successfully on guinea pigs. This dressing was effective in vivo against Strep. pyogenes, Staph. aureus and P. aeruginosa under prophylactic conditions. A six month accelerated stability task is entering its fourth month of testing. Analysis of the results up to the two month period show no instability in the product under accelerated conditions.

The second formulation, a chlorhexidine gluconate - silver sulfadiazine ADD, was also fabricated and tested on guinea pigs. This too showed promising results against Staph. aureus and P. aeruginosa under prophylactic conditions. The effectiveness of this dressing against Candida remains to be evaluated.

In conclusion, all tasks have been completed or are in progress. Resulting dressings have been shown to meet the design requirements of being easy to apply and effective against selected organisms.

REFERENCES.

1. Dasse, K., Dempsey, D. and Thirucote, R., USAIDR Contract No. DAMD17-88-C-8012, Exploratory Development of an Ultra-Fast-Curing Wound Dressing, Annual Report, Nov 30, 32, (1989).

2. Eisenberg, G. H. G., Personal Communication to Dasse, K., Evaluation of Antimicrobial Dermal Dressing from Thermedics, Inc., Company A, Company B and Company C., September 29, (1990).

3. Hutchinson, F., Molecular Basis for Action of Ionizing Radiation Injury, Science, 134, 533, (1961).

4. Ginosa, N., The Effects of Ionizing Radiation on the Nucleic Acids of Bacteriophages and Bacterial Cells, Ann. Rev. Microbiol., 21, 235, (1967).

5. Liu, D., Zhang, L., Wang, Y. and Chen, W., Effects of Radiation Cross Linking on the Mechanical Properties of Polyethylene Sheets, Radiat. Phys. Chem., V. 29, (3), 175 - 177, (1987).

6. Plester, D., Effects of Radiation Sterilization on Plastics, Ind. Sterilization, Int. Symp., Amsterdam, Neth., Sept. 1972 Pub. Duke Univ. Press, Durham, N.C., (1973).

7. Yoshi, F., Sasaki, T., Makuchi, K. and Tamura, N., Durability of Radiation Sterilized Polymers, 1. Estimation of Oxidative Degradation in Polymers by Chemiluminescence, J. Appl. Polym. Sci., V. 30, (2), 3339 - 3346, (1985).

8. Scott, A. I. and Eccleston, E., Proc. European Soc. for Study of Drug Toxicity, 8, 195, (1966).

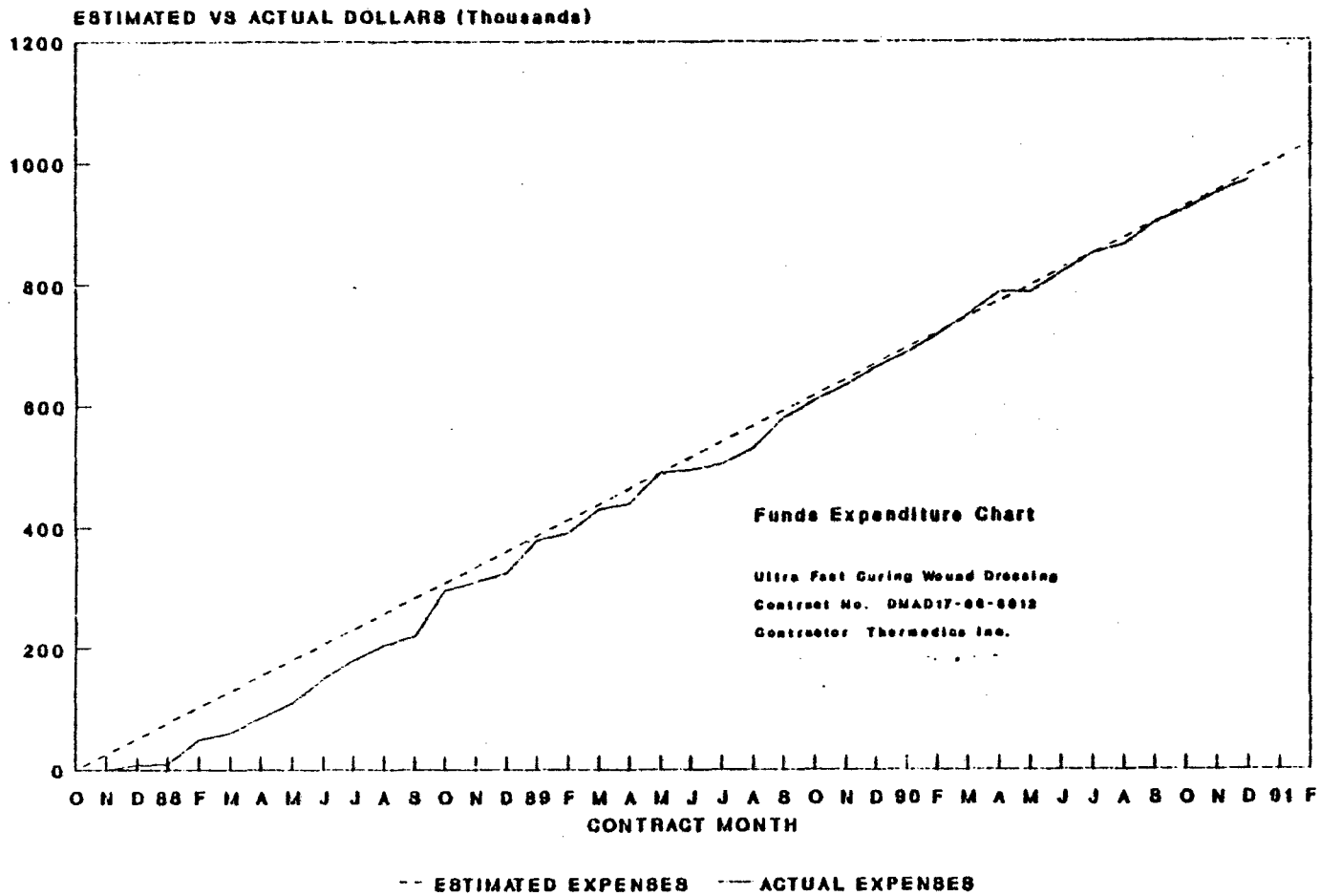
9. Lee, H. L., Orlowski, J. A. and Rogers, B. J., A Comparison of Ultraviolet-curing and Self-curing Polymers in Preventive, Restorative and Orthodontic Dentistry, Int. Dent. J., 26, 134 - 151, (1976).

10. Quesnel, L. B., Al-Najjar, A. R. and Buddhavudhikrai, P., Synergism between Chlorhexidine and Sulphadiazine, J. Appl. Bact., 45, 397-405, (1978).

11. Dasse, K., Dempsey, D. and Thirucote, R., USAIDR Contract No. DAMD17-88-C-8012, Exploratory Development of an Ultra-Fast-Curing Wound Dressing, Annual Report, Nov 30, xiii - xiv, (1989).

12. Bult, A. and Plug, C. M., Silver Sulfadiazine, in Analytical Profiles of Drug Substances, 13, Florey, K. (ed.), Academic Press, N. Y., 553 - 571, (1982).

13. Physical Tests and Determinations, USP/NF XXII, 1578, (1990).



Attachment to Data Item A-5001B

APPENDIX I

CERTIFICATE OF ANALYSIS

REPRODUCED FROM FERMION CERTIFICATE OF COMPLIANCE

Product		
CHLORHEXIDINE GLUCONATE		
Batch No. 89E580	Quantity 20kg	
Date of manufacture:	May 1989	
		BP Requirements
Indentification	Positive (IR)	Positive (test A=IR)
Colour	Almost Colourless	Almost Colourless to pale straw-coloured
Odour	Odourless	Odourless or almost Odourless
Sulphated Ash	0.01%	< 0.1%
pH	6.6	5.5 - 7.0
Assay	20.4 %w/v	19.0 -21.0% w/v
p-Chloroaniline	< 10 ppm	< 500ppm
Related Substances (HPLC)	1.2%	< 3%
Density	1.06 g/ml	1.06 - 1.07 g/ml

THE MATERIAL CORRESPONDS TO THE REQUIREMENTS OF BP

Date of analysis 16.05.1989
Date of expiry May 1992

Signature Espoo, September 1st, 1989

Orion Corporation
fermion

Signature on File
Tuula Hauta-aho M.SC
Quality Control Chemist

002031-FDDS-1

E-BEAM SERVICES, INC.

CERTIFICATE OF IRRADIATION

CUSTOMER: THERMEDICS
CUSTOMER ORDER: 33990
PRODUCT: SURGICAL DRESSINGS
PROCESSING CENTER: CRANBURY, NEW JERSEY
SIDES IRRADIATED: ONE
DATE IRRADIATED: AUGUST 15, 1990
EBS JOB NUMBER: E15286-3
EBS LOT NUMBER: 901506

PRODUCT CODE	LOT #	NUMBER OF UNITS	AVE. SURFACE DOSE IN MRADS
SURGICAL DRESSINGS		1	2.4

Certified by: Denise Cascarett

Approved by:

E-BEAM SERVICES, INC

QEB #900071E
DISK #3-90

Thermedics Inc.

CERTIFICATE OF ANALYSIS

Antimicrobial Dermal Dressings

Date	:	September 4, 1990.
30% Chlorhexidine Gluconate ADD's	:	Batch # 008031 PDDS1

Description	:	A sterile 1.5 x 1.5 inch drug loaded island matrix; reinforced by a 2.5 x 2.5 inch adhesive backing, covered by a removable release liner and packaged in an aluminum pouch.
Color	:	White to Off-white
Thickness		
total	:	0.611 mm ± 0.014 mm
perimeter	:	0.254 mm ± 0.013 mm.
Weight		
total	:	1.440 g ± 0.019 g.
Identification (I.R.)	:	complies.
Dissolution time	:	> 2.5 mg. per cm^2. in 24 hours
Biological Activity	:	USP XXII, 1990
P. aeruginosa		positive.
S. aureus		positive.
Sterility (USP XXII, 1990)	:	passes
Assay	:	135 mg. chlorhexidine gluconate/ADD.

Chemist 9/4/90

Manager 9/5/90

470 Wildwood Street • P.O. Box 2999 • Woburn, MA 01888-1799 • (617) 938-3786 • Telex 7557644 • FAX (617) 933-4476

Fine Chemicals Corporation

(PTY)LTD. Reg.No 85/02851/07

15 HAWKINS AVENUE, EPPING 1, CAPE TOWN
P.O. BOX 253, EPPINDUST 7475 SOUTH AFRICA
TELEPHONE: (021) 531-6471

TELETEX: (0) 53-6470 FINCHEM
FAX NATIONAL: (021) 531-1458
FAX INTERNATIONAL: (2721) 531-1458

ANALYTICAL N° U 1610 BATCH N° SRP 1

DATE 25/07/89 CODE 6002

CERTIFICATE OF ANALYSIS

PRODUCT: SILVER SULPHADIAZINE (MICRONISED)

TEST	SPECIFICATION	RESULT
DESCRIPTION	WHITE OR ALMOST WHITE POWDER	COMPLIES
IDENTIFICATION	I.R. CONCORDANT WITH REFERENCE STD.	COMPLIES
LOSS ON DRYING	MAX. 1,0%	0,10%
NITRATE	MAX. 300 PPM	COMPLIES
SILVER CONTENT	27,2% TO 32,0%	29,8%
SULPHADIAZINE CONTENT	67,2% TO 72,7%	70,9%
PARTICLE SIZE	90% LESS THAN 10 MICRON 99,5% LESS THAN 50 MICRON	COMPLIES COMPLIES
SULPHADIAZINE, FREE	MAX. 0,1%	COMPLIES

APPROVED: .....

QUALITY ASSURANCE MANAGER

Refer Inquiries To:
Ceres Chemical Co. Inc.
5 Teramar Way
White Plains, NY, USA 10605
Phone: 914-949-5337
Fax: 914-997-7042

TOTAL P.01

E-BEAM SERVICES, INC.
32 Melrich Road
Cranbury, New Jersey 08512

CERTIFICATE OF IRRADIATION

CUSTOMER: THERMEDICS INC.
CUSTOMER ORDER: 2466
PRODUCT: WOUND DRESSING SAMPLES
PROCESSING CENTER: CRANBURY, NEW JERSEY
SIDES IRRADIATED: ONE
DATE IRRADIATED: OCTOBER 30, 1990
EBS JOB NUMBER: E15698-3
EBS LOT NUMBER: 903010

PRODUCT	LOT #	NUMBER OF UNITS	AVG. SURFACE DOSE IN MRADS
WOUND DRESSING	2.5 MR	1 CASE	2.5

Certified by: Chad Rhodes

Approved by: Michael S Gray

E-BEAM SERVICES,INC.

QES #9001003
DISK #4-90

APPENDIX II

MICROBIOLOGICAL TEST RESULTS

toxikon

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TEST RESULT CERTIFICATE

Client: Thermedics, Inc.

Address: 470 Wildwood Avenue
P.O. Box 2999
Woburn, MA 01888-1799

Project #: 90-1835

Date of Test: 08/28/90
Test Completion: 09/04/90

P.O. #: 24156-897

Contact: R. Thirucote

TEST ARTICLE DESCRIPTION: Chlorhexidine Gluconate Dressing

Lot# N/A

NAME OF STUDY: Membrane Filtration Sterility

REFERENCE: USP XXII, 1990, Pp. 1483-1488.

GENERAL PROCEDURE: The test articles (2 units) were aseptically pooled with 300 ml of Fluid D. The extract was then decanted into a sterile container and filtered though a sterile membrane filter. The membrane was then removed from the filter holder and cut in half. One half was immersed in 100 ml of Fluid Thioglycollate Medium (FTM) and one half was immersed in 100 ml of Trypticase Soy Broth (TSB). Each vessel was incubated at 30-35°C and 20-25°C respectively. The contents of each vessel were examined for growth during the 7 day incubation period.

RESULTS: There was no growth observed in either media inoculated with the test article during the 7 day observation period.

CONCLUSION: The test article is considered sterile according to the procedures outlined in USP XXII via membrane filtration technique.

AUTHORIZED PERSONNEL:

Steven P. Lynn, Ph.D
Study Director

Susan Yadlon, B.S.
Quality Assurance

TEST ARTICLE DESCRIPTION: Chlorhexidine Gluconate Dressing

LOT #: N/A

NAME OF STUDY: Zone of Inhibition

REFERENCE: Based on the method described in USP XXII, 1990.

GENERAL PROCEDURE: The test article was analyzed for its ability to produce a zone of inhibition against cultures of *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). The test article (three 0.8 cm diameter discs) and placebo discs (three 0.8 cm diameter discs) were placed on the surface of Trypticase Soy Agar containing the test organism. The positive control for *S. aureus* was a mixture of penicillin and streptomycin. The positive control for *P. aeruginosa* was ampicillin. The negative control for both organisms was an untreated filter disc. Three plates were used for each determination. The plates were inverted and incubated at 30-35°C for 72 hours.

RESULTS:

	Zone of Inhibition (in cm)							
	S. aureus				*P. aeruginosa*			
	1	2	3	Ave	1	2	3	Ave
Neg. Control	0	0	0	0	0	0	0	0
Pos. Control	1.40	1.30	1.30	1.33	2.0	2.0	2.0	2.0
Test Article	1.50	1.80	1.70	1.67	1.50	1.60	1.50	1.53

TEST ARTICLE DESCRIPTION: Combination Silver Sulfadiazine/ Chlorhexidine Gluconate Dressing

LOT #: B.N. 010181-PDDS2

NAME OF STUDY: Zone of Inhibition

REFERENCE: Based on the method described in USP XXII, 1990.

GENERAL PROCEDURE: The test article was analyzed for its ability to produce a zone of inhibition against cultures of *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). The test article (three 0.8 cm diameter discs) and placebo discs (three 0.8 cm diameter discs) were placed on the surface of Trypticase Soy Agar containing the test organism. The positive control for *S. aureus* was a mixture of penicillin and streptomycin. The positive control for *P. aeruginosa* was ampicillin. The negative control for both organisms was an untreated filter disc. Three plates were used for each determination. The plates were inverted and incubated at 30-35°C for 72 hours.

RESULTS:

	Zone of Inhibition (in cm)							
	S. aureus				*P. aeruginosa*			
	1	2	3	Ave	1	2	3	Ave
Neg. Control	0	0	0	0	0	0	0	0
Pos. Control	1.2	1.1	1.8	1.37	1.9	1.8	2.0	1.9
Test Article	1.6	1.4	1.7	1.57	1.4	1.5	1.6	1.5

TEST ARTICLE DESCRIPTION: Combination Silver Sulfadiazine/ Chlorhexidine Gluconate Dressing

Lot#: 010181-PDDS2

NAME OF STUDY: Membrane Filtration Sterility

REFERENCE: USP XXII, 1990, Pp. 1483-1488.

GENERAL PROCEDURE: The test articles (2 units) were aseptically pooled with 300 ml of Fluid D. The extract was then decanted into a sterile container and filtered though a sterile membrane filter. The membrane was then removed from the filter holder and cut in half. One half was immersed in 100 ml of Fluid Thioglycollate Medium (FTM) and one half was immersed in 100 ml of Trypticase Soy Broth (TSB). Each vessel was incubated at 30-35°C and 20-25°C respectively. The contents of each vessel were examined for growth during the 7 day incubation period.

RESULTS: There was no growth observed in either media inoculated with the test article during the 7 day observation period.

CONCLUSION: The test article is considered sterile according to the procedures outlined in USP XXII via membrane filtration technique.

APPENDIX III

ASSAY METHODOLOGY FOR

IN VITRO RELEASE KINETICS

HPLC Method for the Analysis of Silver Sulfadiazine In Vitro Using Ultraviolet Detection.

Quantitative analysis of silver sulfadiazine was performed by HPLC using a UV detector. The method was linear and precise and can be used for determining sample concentrations as low as one microgram per milliliter. The chromatographic conditions used for the analysis are outlined below.

Materials

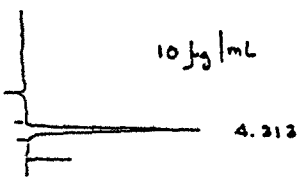
Chromatography was performed on an AllTech OctaDecyl Silane (ODS) column (4.6 mm x 250 mm - 5 u) using 1% acetic acid-methanol (60:40) as the mobile phase. The flow rate was adjusted to 1 ml/min using a Waters Solvent Delivery Module (Model 590). One microliter (1 ul) injections of the sample were introduced through a Waters U6K injector and the sample quantified by means of a Waters 441 UV Absorbance Detector, connected to a Shimadzu Integrator (Model CR601 - Chromatopac). Silver sulfadiazine was detected at 254 nanometers.

Method

This quantitation was based on the detection of sulfadiazine moiety of the silver sulfadiazine (12). This method is useful in determining drug solutions with concentrations of 1 mcg/ml and

above. Example chromatograms for 10 and 25 mcg/ml of silver sulfadiazine are shown in figure A3.1. Silver sulfadiazine standard solutions were prepared and used to generate a standard calibration curve, plotting concentration versus area shown in figure A3.2.

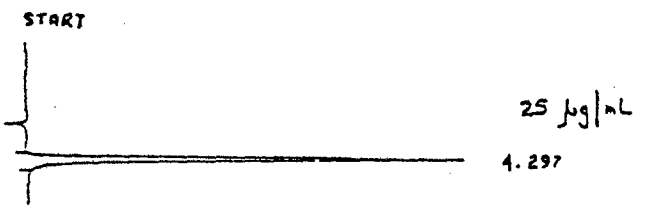
START



CHROMATIOPAC CR601 FILE 0
SAMPLE NO. 0 METHOD 21
REPORT NO. 33

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	4.313	9147			100	
	TOTAL	9147			100	

START



CHROMATOPAC CR601 FILE 0
SAMPLE NO 0 METHOD 21
REPORT NO 30

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	4.297	22851			100	
	TOTAL	22851			100	

Figure A3.1. Typical Chromatograms for Silver Sulfadiazine

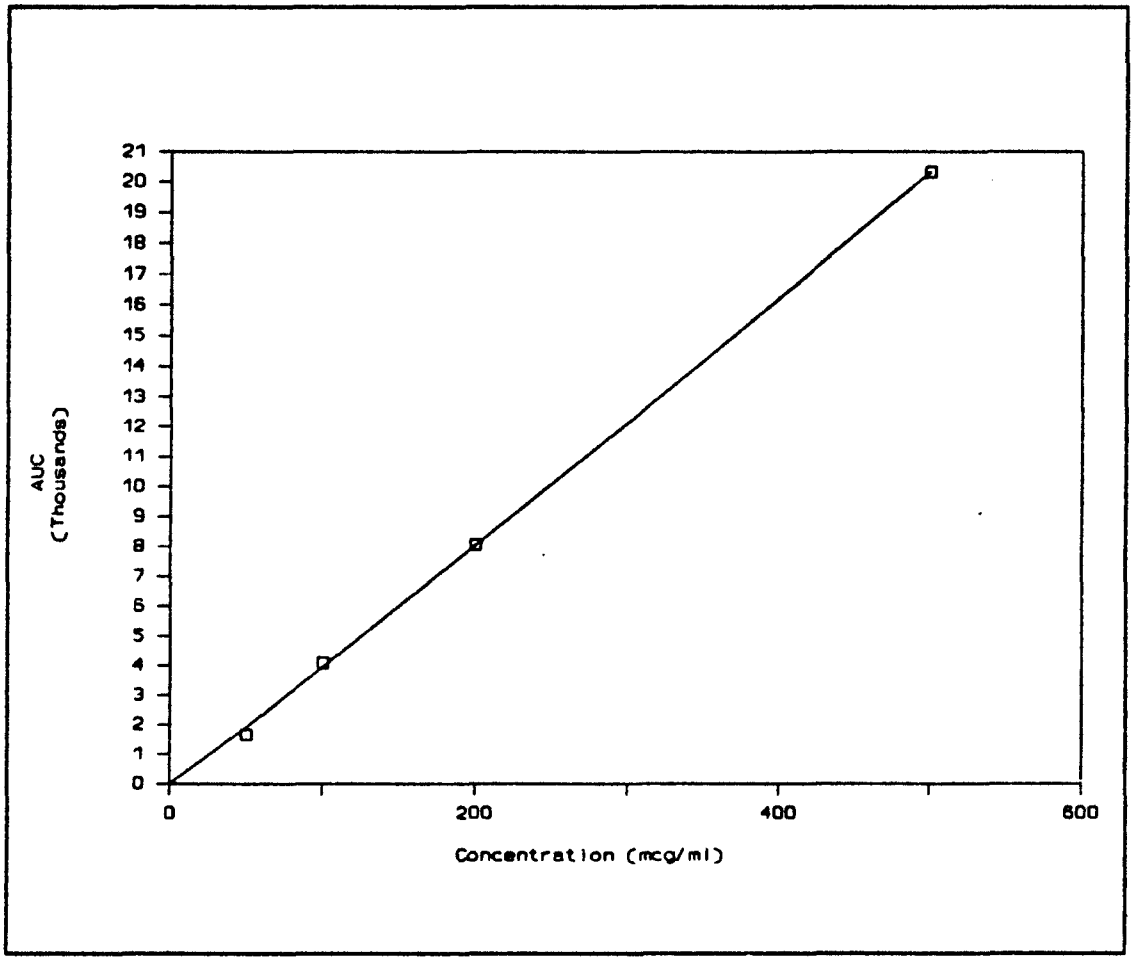


Figure A3.2 Typical Calibration Curve Obtained for Silver Sulfadiazine Assay

APPENDIX IV

IN VITRO DATA SHEETS

TITLE : Formulation 1 - Chlorhexidine gluconate - 30% Excipient 30%

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC	Hr.	Data of Average Values mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0	0	0	0	0.0	0.0	0.0	0.0	0.0
100	419997	474606	447302	1	146.6	146.6	454.4	454.4
200	953607	926238	939923	4	263.7	267.3	828.7	374.3
300	1466489	1411698	1439094	8	243.4	250.0	775.0	-53.7
400	1982141	1912216	1947179	24	330.6	336.7	1043.7	268.7
500	2264835	2137955	2201395	48	314.7	323.0	1001.2	-42.5
600	2895387	2772896	2834142	72	304.4	312.2	968.0	-33.3
800	3207704	3187629	3197667					
900	3747448		3747448					
1000	4008633	3986294	3997464					

Regression Output:

Constant	160605
Std Err of Y Est	161794.8
R Squared	0.987688
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	3988.658
Std Err of Coef.	157.4464

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
1	818991	798989	781430	802816	645912	623280	745236.3	79260.3
4	1231903		1210578		1194239		1212240	15421.11
8	1105076		1220024		1069403		1131501	64267.26
24	1620899		1307438		1509503		1479280	129742.1
48	1731935		1191389		1324270		1415865	229985
72	1569226		1199845		1354928		1374666	151443.7

Formulation	Wt.%
Chlorhexidine	30
Propylene glycol	30
Oligomer	40

Date: 01/04/90
File CHG3030.WK1

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ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Formulation 2 - Chlorhexidine gluconate 30% PG 6% PEG 24% (6 Mil Thick)

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	651553	635565	643559
500	2514236	2497240	2505738
1000	5739827	5778169	5758998
2000	11708183	11925306	11816745

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	382.4	382.4	1082.1	1082.1
1	469.1	478.7	1354.7	272.6
2	542.2	553.9	1567.6	212.9
4	658.9	672.5	1903.2	335.6
8	906.6	923.1	2612.4	709.2
24	1196.8	1219.4	3451.0	838.6
48	1398.0	1427.9	4040.9	589.9
72	1340.5	1375.5	3892.6	-148.3

Regression Output:

Constant	-115536
Std Err of Y Est	235621.5
R Squared	0.99822
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	5917.423
Std Err of Coef.	144.2521

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	1973020	2799643	1668525	2147063	477894.8
1	2620418	2870018	2490857	2660431	157356.4
2	3299509	3454345	2524517	3092790	406771.4
4	4385335	3985082	2980627	3783681	590887.9
8	5521057	6586390	3640809	5249419	1217772
24	6885551	8071294	5941804	6966216	871229.8
48	7539842	10411254	6519321	8156806	1647679
72	7648551	8775144	7026920	7816872	723565.6

Formulation	Wt.%
Chlorhexidine	30
Propylene glycol	6
PEG 300	24
Oligomer	40

Date: 01/04/90
File FORM2

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Formulation 3 - Chlorhexidine gluconate 30% PG 6% PEG 24% (20 Mil Thick)

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	651553	635565	643559
500	2514236	2497240	2505738
1000	5739827	5778169	5758998
2000	11708183	11925306	11816745

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	298.2	298.2	843.8	843.8
1	409.0	416.4	1178.5	334.7
2	561.7	571.9	1618.6	440.0
4	993.8	1007.9	2852.3	1233.8
8	1574.6	1599.4	4526.4	1674.0
24	2549.3	2588.7	7325.9	2799.6
48	2725.9	2789.6	7894.6	568.7
72	2557.5	2625.6	7430.5	-464.1

Regression Output:

Constant	-115536
Std Err of Y Est	235621.5
R Squared	0.99822
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	5917.423
Std Err of Coef.	144.2521

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	1577230	1770000	1599073	1648768	86136.77
1	2133259	2495282	2285272	2304604	148426.1
2	2678531	4144504	2801945	3208327	663891.3
4	5226736	7641917	4427834	5765462	1366282
8	9556287	11281685	6767740	9201504	1859770
24	14175602	17875577	12858062	14969747	2123969
48	16012158	17583839	14447317	16014605	1280277
72	15717876	14444287	14892344	15018169	527499

Formulation	Wt.%
Chlorhexidine	30
Propylene glycol	6
PEG 300	24
Oligomer	40

Date: 01/04/90
File CHT30524.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Formulation 4 Chlorheridine Gluconate 30:6:24 (16 Mil Thick)

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	703326	682639	692983
500	2954195	3074364	3014280
800	5010641	4889083	4949862
1000	6573677	6496506	6535092
2000	12303808	12315480	12309644

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	935.8	935.8	2648.4	2648.4
1	1274.3	1297.7	3672.5	1024.1
2	1655.4	1687.3	4775.0	1102.5
4	1969.5	2010.9	5690.8	915.8
8	2355.7	2405.0	6806.1	1115.4
24	2569.0	2627.9	7437.0	630.9
48	2718.0	2782.2	7873.6	436.6
72	2597.8	2665.8	7544.1	-329.5

Regression Output:

Constant	51238.14
Std Err of Y Est	176077.4
R Squared	0.998787
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	6180.552
Std Err of Coef.	107.6904

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	6162364	5844701	5047972	5148581	6396019	6411232	5835145	554471.4
1	8502389	8017478	7277155	7176790	8090149	8498521	7927080	528776.3
2	10917151	10434510	9804288	9301672	10876334	10361538	10282582	573800.9
4	13028399	12147710	10957414	10951975	13246477	13010390	12223728	960818.5
8	15075334	14767630	13264084	12681579	16488668	15389080	14611063	1285044
24	17731750	17421828	14579678	13964830	16488668	15389080	15929306	1400108
48	17807891	18194299	14970653		16426199		16849761	1268488
72	17278600		13927516		17115546		16107221	1542721

Formulation	Wt.%
Chlorheridine	30
Propylene Glycol	6
PEG 300	24
Oligomer	40

Date: 1/31/90
File FORM4

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Formulation 15 Chlorhexidine gluconate 30:6:24 (12 Mil Thick)

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	561973	582007	571990
500	2904176	2735056	2819616
800	5015976	4838407	4927192
1000	5758511	5959114	5858813
2000	12353230		12353230

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	657.4	657.4	1860.3	1860.3
1	818.4	834.8	2362.5	502.2
2	975.8	996.3	2819.5	457.0
4	1260.2	1284.6	3635.5	816.0
8	1381.0	1412.5	3997.4	362.0
24	1558.1	1592.7	4507.2	509.8
48	1612.6	1651.6	4674.0	166.8
72	1590.5	1630.8	4615.2	-58.8

Regression Output:

Constant	-109338
Std Err of Y Est	162676.9
R Squared	0.998964
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	9178.834
Std Err of Coef.	99.49458

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	4383009	4335936	3261997	3223369	4355315	4154770	3952399	507273.9
1	5826618	5539882	4124541	3926614	5083896	5181840	4947222	697227
2	6440575	6636586	5196536	5119882	6113629	6008724	5920155	576687.1
4	8779358	8546321	7270268	7182172	7212876	7073021	7677336	702542.3
8	9323445		8633315		7314363		8423708	833488.3
24	11124611		10153656		7276191		9518153	1634112
48	11366454		10437533		7760853		9854947	1528538
72	11390722		10307253		7456343		9718106	1659348

Formulation	Wt.%		
Chlorhexidine	30	"Machine, 12 Mils"	
Propylene glycol	6		
PEG 300	24		
Oligomer	40		

Date: 02/13/90
File: FORM15.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Formulation 11 Chlorhexidine Gluconate 30:6:24 (6 Mil Thick) Gamma Sterilized

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STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	681894	650123	666009
500	2995526	3094110	3044818
800	4726613	4823270	4774942
1000	6020085	6501856	6260971

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	559.6	559.6	1583.8	1583.8
1	682.3	696.3	1970.6	386.8
2	855.7	872.8	2470.0	499.4
4	902.6	924.0	2614.9	144.9
8	956.2	978.8	2769.9	154.9
24	972.1	996.0	2818.7	48.9
48	988.1	1012.4	2865.0	46.3
72	962.3	987.0	2793.1	-71.9

Regression Output:

Constant	2931.184
Std Err of Y Est	110653.9
R Squared	0.998698
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	6138.368
Std Err of Coef.	127.9428

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	3612056	3559875	2585731	2559203	4197235	4115444	3438257	655637.5
1	4950850		2990349		4632875		4191358	859105.7
2	6474852		3699925		5592414		5255730	1157604
4	6531353		4416323		5682742		5543473	869055
8	6875060		4780450		5961560		5872357	857444.1
24	7746381		4837630		5326343		5970118	1271755
48	6747297		5320714		6136267		6068093	584391.7
72	6804034		5161434		5763556		5909675	678501.6

Formulation	Wt.%
Chlorhexidine	30
Propylene Glycol	6
PEG 300	24
Oligomer	40

Date: 11/17/89
File Form11

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Chlorhexidine Gluconate ADD's B.N. 008031E-PDDS1 (Set 1)

STANDARD CALIBRATION CURVE

= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	628592	586368	607480
500	2907409	2872586	2889998
800	4823876	4834027	4828952
1000	6348941	6321570	6335256
2000	13401626	12638172	13044899

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	434.5	434.5	1229.7	1229.7
1	528.0	538.9	1525.1	295.3
2	740.8	754.0	2133.8	608.7
4	1143.6	1162.1	3288.9	1155.1
8	1418.7	1447.3	4095.9	807.0
24	1562.4	1597.9	4521.9	426.0
48	1749.1	1788.1	5060.4	538.5
72	1652.4	1696.1	4800.0	-260.5

Regression Output:

Constant	-176096
Std Err of Y Est	200957.2
R Squared	0.998588
No. of Observations	5
Degrees of Freedom	4
X Coefficient(s)	6537.082
Std Err of Coef.	122.9071

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	2977017	2603097	2413290	2664468	234196.3
1	3493647	3103185	3230110	3275647	162525.1
2	4959537	4219846	4820048	4666477	320908.8
4	7994378	6431314	7474029	7299907	649887.7
8	9772500	8217787	9304384	9098224	651234.6
24	10647790	8783515	10680735	10037347	886694.9
48	11437887	10949407	11386142	11257912	219096.1
72	10966207	9232918	11677629	10625585	1025700

Formulation	Wt.%		Date:	08/23/90
Chlorhexidine	30	"After E-Beam sterilization"	File:	B0090031E
Propylene Glycol	6	Set 1		
PEG 300	24			
Matrix	40			

STD
101.387
72.41959
140.4347
284.7783
288.9585
390.5752
104.3922
446.6739

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Chlorhexidine Gluconate ADD's B.N. 008031E-PDDS1 Set 2

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	512785	518900	515843
500	2898234	2867061	2882648
800	4991913	4998578	4995246
1000	5603535	5656796	5630166
2000	12071584	12878013	12474799

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	319.6	319.6	904.4	904.4
1	472.4	480.4	1359.5	455.0
2	601.7	613.5	1736.2	376.8
4	889.3	904.3	2559.3	823.1
8	1176.8	1199.1	3393.4	834.1
24	1446.8	1476.2	4177.6	784.2
48	1459.9	1496.0	4233.8	56.2
72	1448.2	1484.7	4201.6	-32.2

Regression Output:

Constant	-147175
Std Err of Y Est	267615.1
R Squared	0.997241
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	6223.125
Std Err of Coef.	163.6756

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	1927035	2027821	1876734	1887554	1489223		1841673	184115.8
1	3541438		2557354		2278756		2792516	541644
2	4585628		3276397		2929749		3597258	713067.4
4	6519381		5354612		4287108		5387034	911610
8	8056236		6956756		6516252		7176415	647598.2
24	9508963		8772552		8286903		8856139	502392.8
48	9385385		8640956		8786748		8937696	322110.4
72	9169720		8651421		8773646		8864929	221220.7

Formulation	Wt.%		Date:	08/27/90
Chlorhexidine	30	"After E-Beam sterilization"	File:	EC080312.WK1
Propylene Glycol	6	Set 2		
PEG 300	24			
Matrix	40			

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Chlorhexidine Gluconate ADD's B.N. 008031-PDDS1 2 Month Sample @ 45 C/ 90% RH

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	467687	448195	457941
500	3040010	2886689	2963350
800	4782207	4784922	4783565
1000	5675335	5459266	5567301
2000	12726955	12585654	12656305

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	334.0	334.0	945.2	945.2
1	496.0	504.4	1427.3	482.2
2	520.1	532.5	1506.9	79.6
4	717.3	730.3	2066.8	559.9
8	974.5	992.4	2808.5	741.7
24	1305.9	1330.3	3764.7	956.2
48	1364.7	1397.4	3954.6	189.9
72	1440.4	1474.5	4172.8	218.2

Regression Output:

Constant	-218373 "2 Month Sample"
Std Err of Y Est	313113.3
R Squared	0.996323
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	6304.249
Std Err of Coef.	191.5027

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	1776137	1767078	1752691	1655524	2206288	2164971	1887115	215059.9
1	2501919		2605821		3618084		2908608	503465.4
2	2601741		3002645		3576465		3060284	400011.2
4	3260297		4336471		5314697		4303822	839023
8	5348750		5605877		6820239		5924955	641705.1
24	7974299		7664704		8404695		8014566	303438.9
48	8425247		7619909		9110626		8385261	609239.1
72	8438032		7722994		10425168		8862065	1143179

Formulation	Wt.%
Chlorhexidine	30
Propylene Glycol	6
PEG 300	24
Matrix	40

Date: 10/29/90
File: 2MSTA-45

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Chlorhexidine Gluconate ADD's B.N. 008031-PDDS1 2 Month Sample @ 38 C/90% RH

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	626401	622783	624592
500	3037933	3021214	3029574
800	5340031	5204862	5272447
1000	6333264	6046154	6189709
2000	11743334	11517463	11630399

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	303.2	303.2	858.0	858.0
1	453.1	460.7	1303.8	445.8
2	583.1	594.5	1682.3	378.5
4	879.3	893.8	2529.6	847.3
8	1172.3	1194.3	3379.9	850.3
24	1397.1	1426.4	4036.7	656.9
48	1386.0	1420.9	4021.3	-15.4
72	1398.4	1433.1	4055.7	34.4

Regression Output:

Constant	169858.2	"2 Month Sample"
Std Err of Y Est	280538.4	
R Squared	0.996568	
No. of Observations	6	
Degrees of Freedom	4	
X Coefficient(s)	5847.175	
Std Err of Coef.	171.5797	

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	2125830	2117694	1848705	1728560	1911143	1923725	1942610	141542.9
1	2620294		3031225		2806785		2819435	168000.2
2	3363007		3721117		3654393		3579506	155492.3
4	4953856		5162431		5816949		5311079	367699.6
8	6677589		6931111		7465099		7024600	328225.6
24	8017622		8383899		8615243		8338921	246041.9
48	8108139		9615774		8098531		8274148	241597.9
72	8296233		8274380		8469823		8346812	87438.24

Formulation	Wt.%
Chlorhexidine	30
Propylene Glycol	6
PEG 300	24
Matrix	40

Date: 10/31/90
File: 2MSTA-38.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Chlorhexidine Gluconate ADD's B.N. 003031-PDDS1 2 Month Sample @ RT

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
500	3226522	3096717	3161620
800	5019617	5236543	5128080
1000	6157377	6295124	6226251
2000	12265064	12565648	12415356

Data of Average Values
dil adj

Hr.	mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	377.6	377.6	1068.6	1068.6
1	537.0	546.5	1546.5	477.9
2	617.4	630.8	1785.3	238.8
4	924.8	940.3	2661.0	875.7
8	1292.7	1315.8	3723.7	1062.7
24	1606.9	1639.2	4638.9	915.2
48	1671.0	1711.2	4842.6	203.7
72	1699.7	1741.5	4928.4	85.8

Regression Output:

Constant	58610.99	"2 Month Sample"
Std Err of Y Est	77878.71	
R Squared	0.999784	
No. of Observations	5	
Degrees of Freedom	3	
X Coefficient(s)	6194.942	
Std Err of Coef.	52.60154	

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	2279151	2276466	2357840	2254422	2663956	2554740	2397763	156217.1
1	3026535		3761713		3368010		3385419	300387.5
2	3594926		4425371		3630063		3883453	383462.1
4	5079486		6632755		5651642		5787961	641403.8
8	7827790		9056229		7315886		8066635	730289.3
24	10302011		10737379		8999529		10013006	738280.2
48	9549833		10528555		11152494		10410294	659605.9
72	12435010		10246774		8982704		10588163	1419696

Formulation	Wt.%
Chlorhexidine	30
Propylene Glycol	6
PEG 300	24
Matrix	40

Date: 11/1/90
File: 2MSTA-RT.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Chlorhexidine Gluconate ADD's B.N. 008031-PDDS1 2 Month Sample @ RT Under Water

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	626401	622783	624592
500	3037933	3021214	3029574
800	5340031	5204862	5272447
1000	6333264	6046154	6189709
2000	11743334	11517463	11630399

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	414.5	414.5	1173.0	1173.0
1	529.7	540.1	1528.5	355.5
2	676.0	689.2	1950.5	422.0
4	1053.2	1070.1	3028.3	1077.7
8	1434.4	1460.8	4133.9	1105.6
24	1668.4	1704.3	4823.1	689.2
48	1704.0	1745.7	4940.4	117.3
72	1729.1	1771.7	5013.9	73.5

Regression Outputs

Constant	169858.2	"2 Month Sample"
Std Err of Y Est	280538.4	
R Squared	0.996568	
No. of Observations	6	
Degrees of Freedom	4	
X Coefficient(s)	5847.175	
Std Err of Coef.	171.5797	

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	2424855	2323061	3118499	3246501	2276840	2171426	2593530	424662.9
1	3080080		3597024		3125047		3267384	233812.7
2	3994260		4393172		3980116		4122516	191469.8
4	6684914		5969763		6328969		6327882	291960.2
8	9068202		9059535		8543785		8557174	411895.4
24	10040597		9885297		9850336		9925410	92690.59
48	10597998		9603919		10198670		10133529	408436.7
72	10488406		10328725		10023552		10280228	192849.3

Formulation	Wt.%
Chlorhexidine	30
Propylene Glycol	6
PEG 300	24
Matrix	40

Date: 10/31/90
File: 2MSTR-WT.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Chlorhexidine Gluconate ADD's B.N. 008031-PDDS1 2 Month Sample @ -40 C

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	626401	622783	624592
500	3037933	3021214	3029574
800	5340031	5204862	5272447
1000	6333261	6046154	6189709
2000	11974791	12439009	12206900

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	429.6	429.8	1216.3	1216.3
1	579.5	590.3	1670.5	454.3
2	705.4	719.9	2037.3	366.8
4	1079.2	1096.8	3104.1	1066.7
8	1425.4	1452.4	4110.2	1006.1
24	1595.5	1631.1	4616.1	505.9
48	1711.7	1751.6	4957.0	340.9
72	1597.5	1640.3	4642.1	-314.9

Regression Output:

Constant	65627.83 "2 Month Sample"
Std Err of Y Est	175155.4
R Squared	0.998776
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	6120.33
Std Err of Coef.	107.1265

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	3005261	3001797	2907659	2908428	2188405	2164476	2696004	369515.4
1	3747506		3869550		3220932		3612663	281440.8
2	4208834		4393063		4546986		4382961	138234.7
4	7330400		6343106		6338632		6670713	466473
8	8921695		9107754		8338855		8789435	327537.3
24	10259998		9456351		9775574		9830641	330390.1
48	11104808		9901390		10619253		10541817	494335.2
72	10357488		9353494		9817988		9842957	410262

Formulation	Wt.%
Chlorhexidine	30
Propylene Glycol	6
PEG 300	24
Matrix	40

Date: 10/30/90
File: 2MSTB-40.WK1

ELUTION RATE WORKSHEET FOR SILVER SULFADIAZINE "A"

TITLE : 20:10 Silver SulfadiazineChlorhexidine Gluconate ADD's 9. N. 003281

STANDARD CALIBRATION CURVE

= 254 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
1	46372	44364	45368
5	273436	281165	277301
10	549823	553172	551498
15	801711	819580	810646

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	4.1	4.1	11.7	11.7
1	5.1	5.2	14.8	3.0
2	6.2	6.4	18.0	3.2
4	7.7	7.9	22.4	4.4
8	9.2	9.4	26.7	4.3
24	12.5	12.8	36.1	9.4
48	13.5	13.8	39.1	3.0
72	12.1	12.5	35.2	-3.9

Regression Output:

Constant	-1175.73
Std Err of Y Est	7979.336
R Squared	0.999596
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	54538.39
Std Err of Coef.	633.2009

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	214448	255063	205355	224955.3	21610.56
1	262974	320957	249534	277821.7	30990.87
2	323430	390370	301459	338419.7	37813.66
4	423496	479151	361092	421246.3	48223.63
8	519375	574044	415254	502891	65865.31
24	740342	713776	590990	681702.7	65053.97
48	759709	670700	776738	735715.7	46495.69
72	668635	674797	635459	659630.3	17275.85

Formulation	Wt.%
Silver sulfadiazine	20
Chlorhexidine gluconate	10
PEG 300	16
Propylene glycol	4
Matrix	50

Date: 12/12/90

File: 9003281s.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : 20:10 Silver Sulfadiazine:Chlorhexidine Gluconate ADD's B. N. 003281

STANDARD CALIBRATION CURVE

= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	619520	600716	610118
500	3117956	3120785	3119371
800	4985128	4794929	4890029
1000	6071163	6017421	6044292

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	362.2	362.2	1025.1	1025.1
1	504.7	513.8	1454.1	429.0
2	630.2	642.8	1819.2	365.2
4	740.8	756.6	2141.1	321.9
8	767.4	785.9	2224.1	83.0
24	841.8	861.0	2436.5	212.4
48	822.4	843.5	2387.1	-49.4
72	804.2	824.8	2334.2	-52.9

Regression Output:

Constant	20430.12
Std Err of Y Est	48668.43
R Squared	0.999742
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	6067.358
Std Err of Coef.	56.27254

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	2402738	2341651	2378828	2227049	1987059	1971724	2218175	177647
1	3636005		2922089		2690685		3082926	402334
2	4315594		3604970		3612031		3844198	333339.5
4	4891292		4199381		4455056		4515243	285659.5
8	5113108		4327639		4588378		4676375	326647.6
24	5490682		4823725		5068778		5127728	275456.3
48	5525816		4624003		4881577		5010465	379276.3
72	5012750		4805380		4882066		4900055	85609.82

Formulation	Wt.%
Silver sulfadiazine	20
Chlorhexidine gluconate	10
PEG 300	16
Propylene glycol	4
Matrix	50

Date: 09/05/90

File: B003281.WK1

ELUTION RATE WORKSHEET FOR CLINDAMYCIN PHOSPHATE "A"

TITLE : Batch No. 007121 - 20% Clindamycin 10% Chlorhexidine

STANDARD CALIBRATION CURVE

= 194 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	3400978	3384284	3392631
500	18201675	18505687	18353681
800	31495347	31354622	31424985
1000	38863552	36557727	37710640
2000	73198389	76087102	74642746

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	492.3	492.3	1393.2	1393.2
1	788.0	800.3	2264.9	871.7
2	998.4	1018.1	2881.2	616.2
4	1376.1	1401.0	3965.0	1083.6
8	1720.4	1754.8	4966.0	1001.1
24	2043.9	2086.9	5905.9	939.8
48	2115.4	2166.5	6131.3	225.5
72	2045.6	2098.4	5938.6	-192.7

Regression Output:

Constant	82658.69
Std Err of Y Est	781243.6
R Squared	0.999351
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	37506.53
Std Err of Coef.	477.8151

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	14872928	14893514	20090508	19959203	20696656	20772325	18547522	2607572
1	22972114		32958402		32985843		29638786	4714063
2	30781299		39285106		42518951		37528452	4950252
4	49291109		53034363		52758859		51694777	1703367
8	68344013		63626434		61853754		64608067	2739047
24	83044821		73066549		74111343		76740904	4477903
48	83528541		75184481		79563532		79425518	3407846
72	82061854		71589171		76762730		76804585	4275557

Formulation	Wt.%
Clindamycin	20
Chlorhexidine	10
Propylene glycol	6
PEG 300	24
Oligomer	40

Date: 08/31/90

File: B007121.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Dual Loaded ADD's - E. N. 007121

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	495049	498858	496954
500	2818823	2851914	2835369
800	4877314	4745178	4811246
1000	5910123	5765662	5837893

Data of Average Values
dil adj

Hr.	mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	635.3	635.3	1797.9	1797.9
1	685.0	700.9	1983.6	185.7
2	754.2	771.4	2183.0	199.4
4	840.9	859.7	2433.1	250.1
8	936.9	957.9	2710.8	277.8
24	995.4	1018.8	2883.2	172.3
48	1019.4	1044.3	2955.3	72.2
72	1009.1	1034.6	2927.9	-27.4

Regression Output:

Constant	-54938.6
Std Err of Y Est	93817.74
R Squared	0.999001
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	5940.054
Std Err of Coef.	108.4761

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	4256829	4303754	3553819	3457800	3365412	3375282	3718816	402051.8
1	3924889		4056274		4061488		4014217	63200.29
2	4190742		4517753		4567359		4425295	167078.6
4	4943758		4951045		4925181		4939995	10989.1
8	5705545		5451328		5373549		5510141	141773.4
24	6057370		5797642		5717821		5857611	144961.1
48	6185209		5701277		6114642		6000376	213448
72	5917217		5866702		6034015		5939311	70059.15

Formulation	Wt.%		Date:	03/16/90
Chlorhexidine gluconate	10	12 Mls 1.5 sq.in.	File:	9007121.WK1
Clindamycin PO4	20			
Propylene glycol	6			
PEG 300	24			
Matrix	40			

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : Triple Loaded ADD's - B. N. 007132

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	495049	498858	496954
500	2818823	2851914	2835369
800	4877314	4745178	4811246
1000	5910123	5765662	5837893

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	411.7	411.7	1165.2	1165.2
1	502.6	512.9	1451.4	286.2
2	563.0	575.6	1628.5	177.5
4	678.1	692.1	1958.7	329.8
8	859.1	876.1	2479.2	520.5
24	973.9	995.4	2816.9	337.7
48	1040.4	1064.7	3013.1	196.2
72	1012.6	1038.6	2939.3	-73.8

Regression Output:

Constant	-54938.6
Std Err of Y Est	93817.74
R Squared	0.999001
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	5940.064
Std Err of Coef.	108.4761

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	2592736	2556690	2289575	2283873	2384224	2237471	2390762	137555.7
1	3194542		2833810		2762829		2930394	189015.6
2	3546674		3097431		3224257		3289454	189108.1
4	4204192		3760579		3953607		3972793	181611.7
8	5331058		4903182		4910395		5048212	200024.2
24	6007229		5614689		5568386		5730101	196868.5
48	6469892		6035336		5869309		6124846	253224.5
72	6464653		5528767		5886536		5959985	385587.7

Formulation	Wt.%		Date:	08/16/90
Chlorhexidine gluconate	10	12 Mils 1.5 sq.in.	File:	B007132.WK1
Clindamycin PO4	10			
Silver sulfadiazine	10			
Pluronic L-62	30			
Matrix	40			

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : 12:Silver sulfadiazine 10:Chlorhexidine gluconate 12:Clindamycin phosphate ADD's B. N. 009101

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	543954	582546	563250
500	3043792	3021822	3032807
800	5037611	4945711	4991661
1000	6440266	6038695	6239481
2000			ERR

Data of Average Values

Hr.	mcg/ml dil adj	mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	157.3	157.3	445.1	445.1
1	318.1	322.0	911.2	466.2
2	439.5	447.5	1266.4	355.2
4	514.0	525.0	1485.8	219.4
8	592.4	605.3	1712.9	227.1
24	771.4	786.2	2225.0	512.0
48	913.5	932.7	2639.7	414.7
72	913.3	936.1	2649.2	9.5

Regression Output:

Constant	-43212.2
Std Err of Y Est	45744.65
R Squared	0.999786
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	6268.025
Std Err of Coef.	52.89194

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	1123941	1199616	924679	775435	845810	785822	942550.5	163387.5
1	1780202		1975496		2095435		1950378	129913.2
2	2619822		2629462		2885695		2711793	123035.6
4	2212764		3017220		3306309		3178764	120443.9
8	3872845		3459905		3677736		3670162	168667.1
24	5485600		4406463		4483875		4791979	491481
48	6107414		5293579		5646170		5682388	333232.3
72	5879506		5523354		5640527		5681129	148205.8

Formulation	Wt.%
Silver sulfadiazine	12
Chlorhexidine	10
Clindamycin phos.	12
Pluronic L-62	28
Matrix	38

Date: 10/11/90
File hm009101

ELUTION RATE WORKSHEET FOR SILVER SULFADIAZINE "A"

TITLE : 20:10 Silver sulfadiazine:Chlorhexidine Gluconate ADD's B.N. 010181-PDDS2 Set 1

STANDARD CALIBRATION CURVE
= 254 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
1	47448	49780	48614
5	281594	286471	284033
10	568117	576598	572358
15	833426	847205	840316

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	2.9	2.9	8.3	8.3
1	4.2	4.3	12.1	3.8
2	6.4	6.5	18.3	6.1
4	9.2	9.4	26.6	8.3
8	11.2	11.5	32.5	5.9
24	12.2	12.4	35.2	2.8
48	14.0	14.3	40.5	5.3
72	13.8	14.2	40.0	-0.5

Regression Output:

Constant	-1388.49
Std Err of Y Est	7360.373
R Squared	0.99968
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	56524.58
Std Err of Coef.	584.1306

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	172125	162597	160724	165148.7	4991.924
1	232959	251906	226010	236958.3	10943.7
2	354554	360935	358230	357906.3	2615.067
4	523156	510231	526826	520071	7117.416
8	649196	675252	577233	633893.7	41453.2
24	694514	703979	659998	686163.7	18901.11
48	837370	741313	792565	790416	39244.54
72	759865	815255	760980	778700	25852.3

Formulation	Wt.%
Silver sulfadiazine	20
Chlorhexidine gluconate	10
Pluronic L-62	20
Matrix	50

Date: 11/26/90
File: S0101811.WK1

ELUTION RATE WORKSHEET FOR SILVER SULFADIAZINE "A"

TITLE : 20:10 Silver sulfadiazine:Chlorhexidine Gluconate ADD's B.N. 010181-PDDS2 Set 2

STANDARD CALIBRATION CURVE
= 254 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
1	47448	49780	48614
5	281594	286471	284033
10	568117	576598	572358
15	833426	847205	840316

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	2.7	2.7	7.6	7.6
1	4.3	4.3	12.3	4.7
2	5.8	5.9	16.8	4.5
4	9.0	9.2	26.0	9.2
8	11.6	11.8	33.4	7.4
24	12.8	13.1	37.2	3.8
48	14.3	14.6	41.4	4.2
72	12.8	13.1	37.2	-4.2

Regression Output:

Constant	-1388.49
Std Err of Y Est	7360.973
R Squared	0.99968
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	56524.58
Std Err of Coef.	584.1306

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	156481	149835	144629	150305	4851.459
1	256043	230484	233125	239884	13476.9
2	335883	313159	333666	327569.3	10229.76
4	521096	501791	505004	509297	8445.636
8	668402	657800	631563	652588.3	15484.38
24	865599	703376	704010	724328.3	108011.9
48	888498	747036	785088	806874	59770.93
72	785088	667302	710541	720977	48648.96

Formulation	Wt.%
Silver sulfadiazine	20
Chlorhexidine gluconate	10
Pluronic L-62	20
Matrix	50

Date: 11/26/90
File: S0101812.WK1

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : 20:10 Silver Sulfadiazine:Chlorhexidine Gluconate ADD's B.N. 010181-PPDS2 Set 1

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	655604	501975	578790
500	2989292	3056439	3022866
800	4953288	4817120	4885204
1000	5927591	5857809	5892700
2000	12004674	12281809	12143242

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	dif u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	110.1	110.1	311.6	311.6
1	147.6	150.4	425.6	114.0
2	213.4	217.1	614.5	188.9
4	320.3	325.6	921.5	307.0
8	441.0	449.1	1270.8	349.3
24	508.8	519.9	1471.2	200.4
48	747.5	760.2	2151.4	680.2
72	762.1	780.8	2209.7	58.3

Regression Output:

Constant	-25235.2
Std Err of Y Est	82715.82
R Squared	0.999722
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	5062.321
Std Err of Coef.	50.58969

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	779832	754990	522647	565381	628037	598822	642318.2	94262.71
1	1008822		795491		805256		869856.3	98344.4
2	1522727		1140880		1142635		1268747	179592.2
4	2177481		1766784		1805256		1916507	185203.7
8	2875241		2532709		2537589		2648513	160333.3
24	4070036		2577678		2530707		3059474	714832.7
48	4568508		4689061		4261474		4506348	180010.4
72	4662512		4695151		4427337		4595000	119302.1

Formulation	Wt.%	Date:	11/12/90
Silver sulfadiazine	20	File:	B0101811.WK1
Chlorhexidine gluconate	10		
Pluronic L-62	20		
Matrix	50		

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

TITLE : 20:10 Silver Sulfadiazine:Chlorhexidine Gluconate ADD's B.N. 010181-PPDS2 Set 2

STANDARD CALIBRATION CURVE
= 238 nm

mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	655604	501975	578790
500	2989292	3056439	3022866
800	4953288	4817120	4885204
1000	5927591	5857809	5892700
2000	12004674	12281809	12143242

Data of Average Values

Hr.	mcg/ml	dil adj mcg/ml	mcg/cm2	diff u/cm2
0.0	0.0	0.0	0.0	0.0
0.5	87.6	87.6	247.8	247.8
1	127.8	129.9	367.7	119.9
2	188.9	192.0	543.5	175.8
4	295.9	300.6	850.7	307.2
8	413.3	420.7	1190.4	339.8
24	608.4	618.7	1751.0	560.6
48	691.8	707.0	2000.9	249.9
72	707.5	724.8	2051.3	50.4

Regression Output:

Constant	-25235.2
Std Err of Y Est	82715.82
R Squared	0.999722
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	6062.321
Std Err of Coef.	50.58969

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	409141	464261	634469	637809	450776	437715	505695.2	93731.6
1	840500		792349		614833		749227.3	97042.97
2	1121776		1176662		1060535		1119658	47432.31
4	1792132		1730964		1782181		1768426	26799.1
8	2517837		2444906		2477399		2480047	29832.79
24	3495559		3706013		3787610		3663060	123037.4
48	3993441		4271955		4241134		4168843	124664.8
72	4160022		4405738		4226442		4264067	103781.3

Formulation	Wt.%
Silver sulfadiazine	20
Chlorhexidine gluconate	10
Pluronic L-62	20
Matrix	50

Date: 11/12/90
File: BO101812.WK1

APPENDIX V

IN VIVO DATA SHEETS

EFFICACY OF ANTIMICROBIAL DERMAL DRESSINGS FOR PREVENTING INFECTION OF CONTAMINATED FULL THICKNESS SKIN EXCISION WOUNDS ON GUINEA PIGS

	BACTERIAL CONTAMINANT					
	STREP. PYOGENES		*STAPH. AUREUS*		*P. AERUGINOSA*	
FORMULATION[1]	COUNT[2]	SUCCESS RATE[3]	COUNT	SUCCESS RATE	COUNT	SUCCESS RATE
30ChlHx	1.0×10^2	5/5	$\leq 5.9 \times 10^1$	4/4	$\leq 5.5 \times 10^2$	5/5
10ChlHx/20AgSdz			$\leq 1.2 \times 10^3$	5/5	$\leq 1.4 \times 10^2$	6/6
10ChlHx/20Clind			$\leq 2.4 \times 10^2$	6/6	6.8×10^3	4/5
10Chlx/10AgSdz/10Clind	$\leq 4.0 \times 10^2$	5/5	$\leq 1.0 \times 10^2$	5/5	$\leq 2.8 \times 10^3$	5/6
20AgSdz/10Clind			2.2×10^4	3/5	1.5×10^6	2/6
27Gent/20Clind[4]			$\leq 2.2 \times 10^2$	4/4		

EFFICACY OF ANTIMICROBIAL DERMAL DRESSINGS FOR TREATING INFECTED FULL THICKNESS SKIN EXCISION WOUNDS ON GUINEA PIGS

	INFECTING BACTERIA					
	STREP. PYOGENES		*STAPH. AUREUS*		*P. AERUGINOSA*	
FORMULATION[1]	COUNT[2]	SUCCESS RATE[3]	COUNT	SUCCESS RATE	COUNT	SUCCESS RATE
30ChlHx	$\leq 1.1 \times 10^2$	5/5	$\leq 2.5 \times 10^2$	5/5	8.9×10^7	0/5
10ChlHx/20AgSdz			2.3×10^5	2/4	2.1×10^7	0/5
10ChlHx/20Clind			$\leq 1.3 \times 10^2$	5/5		
10Chlx/10AgSdz/10Clind	$\leq 1.2 \times 10^2$	5/5	1.8×10^4	3/4	1.5×10^7	0/5
20AgSdz/10Clind			6.1×10^4	4/6		
27Gent/20Clind[4]			9.6×10^2	4/5		

1. Expressed as % w/v with drug abbreviation (e.g., 30ChlHx is 30% chlorhexidine gluconate. AgSdz is silver sulfadiazine, Clind is clindamycin phosphate and Gent is gentamicin sulfate.
2. Count is expressed as colony forming units of bacteria per gram of tissue (CFU/g) and was calculated as the antilog of the mean of the log counts.
3. Success rate is the number of wounds with ?<10^3 CFU/g per total number of wounds that were treated.
4. Results from experiments done in 1989 (i.e., not with the new batch of 12 mil thick dressings.

APPENDIX VI

TABLE OF DELIVERIES

Year 3

No.	Formulation	Delivered	Date
1	Chlorhexidine 30/30/40	25	11-17-89
2	Chlorhexidine 30/6/24/40 (6 mils)	45	11-17-89
3	Chlorhexidine 30/6/24/40 (20 mils)	45	11-17-89
4	Chlorhexidine 30/6/24/40 (16 mils)	45	12-15-89
5	Chlorhexidine 30/6/24/40 (12 mils)	45	02-15-90
6	Chlorhexidine/ 10/20/30/40 Silver sulfadiazine	45	04-04-90
7	Placebos	45	04-04-90
8	Chlorhexidine/ 10/10/10 Silver sulfadiazine/Clindamycin	45	07-20-90
9	Silver sulfadiazine/ 20/10 Clindamycin	45	07-20-90
10	Chlorhexidine/ 10/20 Clindamycin	45	07-20-90
11	Chlorhexidine 2.5" x 2.5"	200	08-16-90
12	Chlorhexidine 1" x 1"	100	08-16-90
13	Placebos	100	08-16-90
14	Chlorhexidine/ 10/12/12 Silver sulfadiazine/Clindamycin	45	09-12-90
15	Chlorhexidine/ 10/20 Silver sulfadiazine	200	11-01-90
16	Placebos	100	11-01-90
17	Chlorhexidine/ 10/10/10	25	11-09-90
18	Adhesive dressings	200	N/D