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EXPLORATORY DEVELOPMENT of an
ULTRA-FAST-CURING WOUND DRESSING

ANNUAL REPORT

November 30, 1989

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 20701-5012

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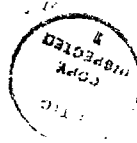


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INTRODUCTION

This report summarizes research conducted over the past year directed toward developing a second generation antimicrobial dermal dressing (ADD). The dressing consists of a trilaminate composed of an outer medical grade polyurethane fabric, an acrylic-based pressure sensitive adhesive, and an antimicrobial impregnated polyurethane laminate which serves as a controlled drug release layer. The objectives in developing this new technology have been to create a dressing that: 1) is easily applicable under adverse climatic conditions, 2) is 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 second contract year has been to develop two types of dressings; 1) a dually loaded gentamicin sulfate, clindamycin phosphate dressing followed by

2) development of a chlorhexidine gluconate dressing. Successful completion of the proposed tasks has involved making the base oligomer, developing fabrication methods, developing methods to measure the antimicrobial agents, monitoring elution kinetics and optimizing drug release. USAIDR assumed responsibility for in vivo evaluation of the technology.

The work resulted in the development of new techniques for drug analyses, improved fabrication methods for sustained release and better management of wound healing. Work in the latter portion of the year was initiated to incorporate additional agents such as silver sulfadiazine and nystatin for inhibition of infection against a wider spectrum of fungi and bacteria. The following report provides a detailed description of the studies 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 DAMD-17-88-C-8012 has shown promising results; however, the in vivo trials demonstrated that further work was required for an optimal formulation. Also, work was directed towards incorporating a non-prescription antiseptic, chlorhexidine gluconate into the ADD's.

The dual loaded ADD's incorporating gentamicin sulfate and clindamycin phosphate were shown to be effective in controlling bacterial proliferation for days. However, there were instances in Year 1 when the dressings failed to completely inhibit growth. The work conducted during the first quarter of Year 2 focussed on optimizing the release from these dual loaded dressings. The second quarter was directed toward the quantitation of the release kinetics from these dressings, as well as the delivery and the subsequent in vivo testing of the optimal formulation^{1,2}.

The incorporation of chlorhexidine gluconate as an antimicrobial agent was a major breakthrough in the third quarter. A modified method for the quantitation of the release kinetics of this agent was developed and validated³. In vivo testing of the initial chlorhexidine formulation using guinea pigs showed favorable results.

All dressings developed in Year 2 were found to release the antimicrobial agents in a controlled fashion and to be effective against the target bacterial organisms. However, during the course of Year 2, the scope of the contract was modified. It was determined that the ADD's must also be effective against fungi. In vitro testing of new antimicrobial agents was initiated. The most promising candidate will be selected early in Year 3 for final in vivo evaluation.

WORK TO DATE

TASK I

Task I focused on optimizing the release of the antibiotics from the dressing and adhesion to the skin for its intended duration of use. The various methods for this undertaking are enumerated as follows:

A. Optimize Dispersion of the Drugs

Various methods were investigated to improve dispersion and to automate mixing. A four fold increase in batch processing was attained, by utilizing a mechanical mixer (Banby Hand Homogenizer). This automated procedure results in a finer dispersion which is easily reproduced and hence the preferred method of manufacture. Figure 1 illustrates the release kinetics and figure 2 compares the photomicrographs of the dispersed solids within the matrices processed manually and through automation.

B. Utilize More Potent Drugs

The use of drugs with high microbiological activity (potency) enhanced the efficacy of the antimicrobial dermal dressings. The stricter limits specified on the purchased antibiotic(s) made this possible. Gentamicin sulfate USP having not less than 675 mcg/ mg

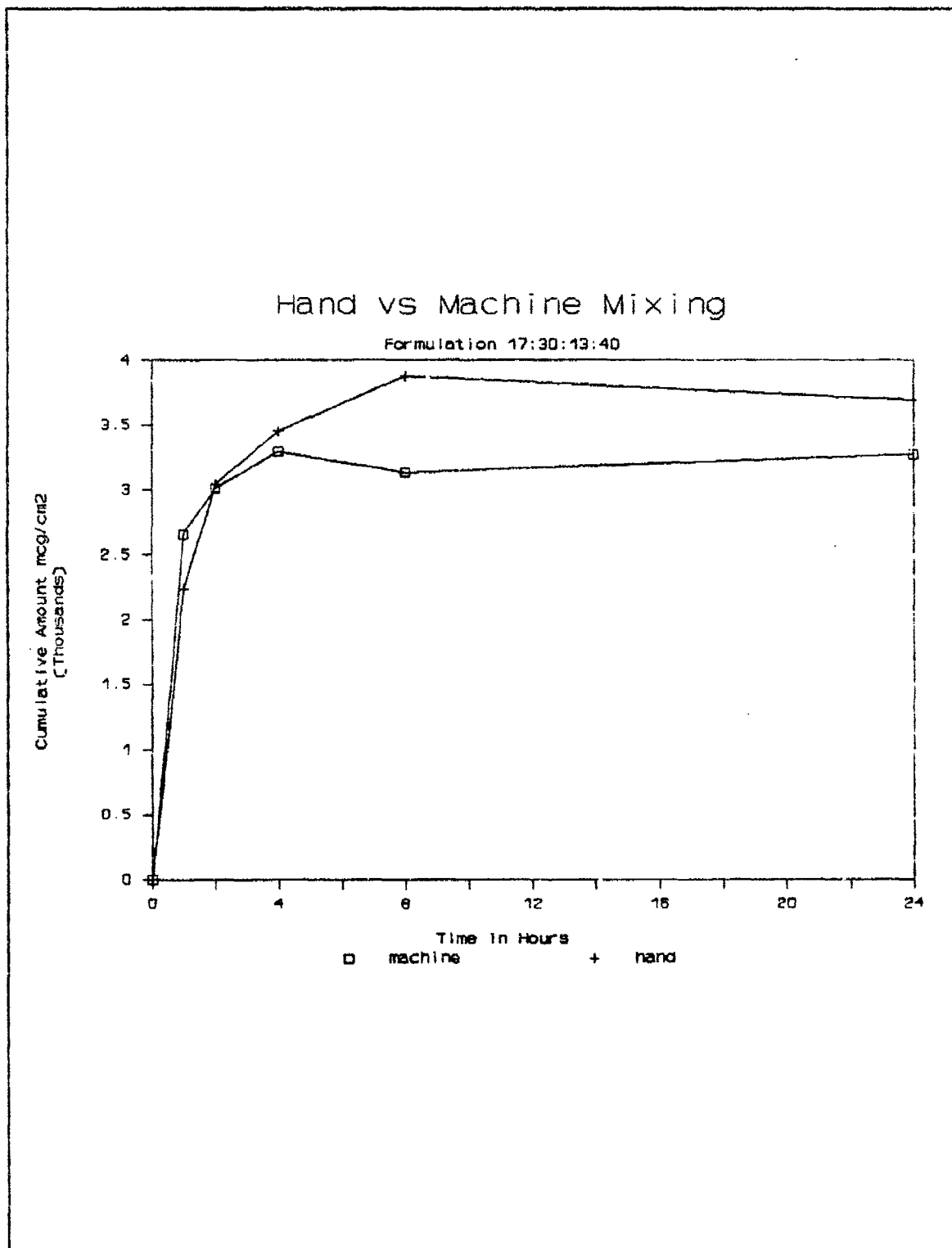


Figure 1. Effect of Mixing Methods on Release Kinetics

A

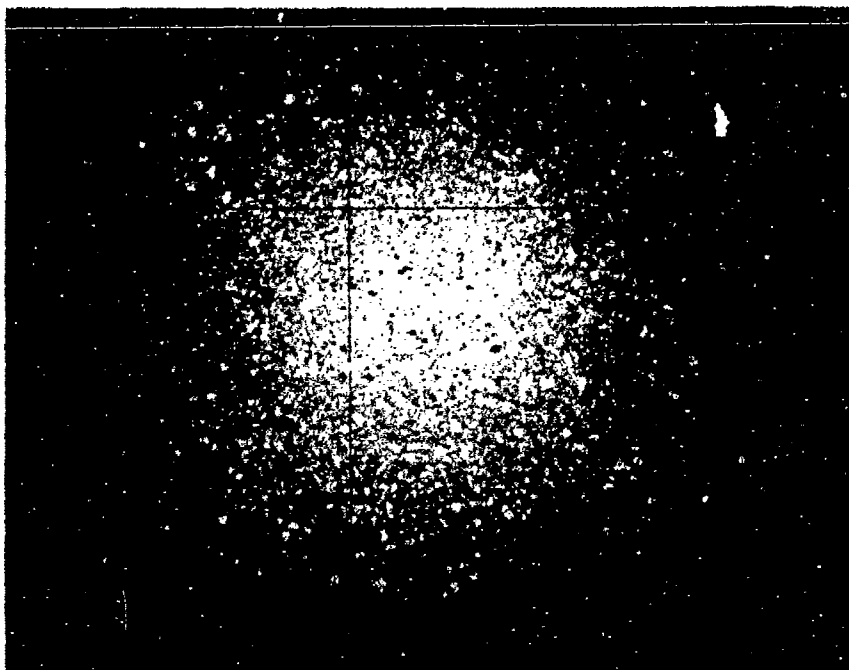


Photo 1 (20X) Clindamycin 20mg Dressing Dispersed by Homogenizer

B

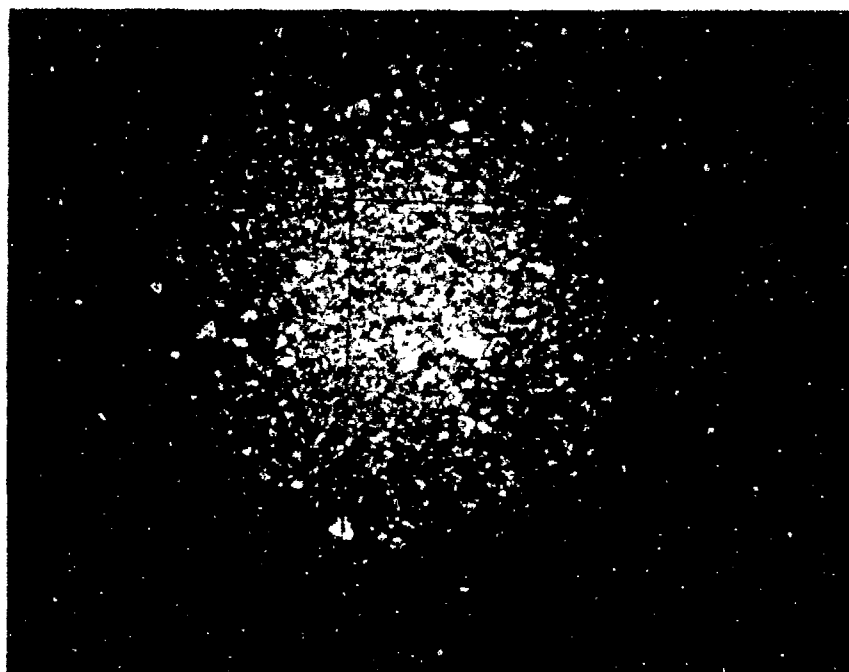


Photo 2 (20X) Clindamycin 20mg Dressing Dispersed by Mortar and Pestle

Figure 2. Comparison of Resultant Dispersion Utilizing Machine versus Hand Mixing Methods.

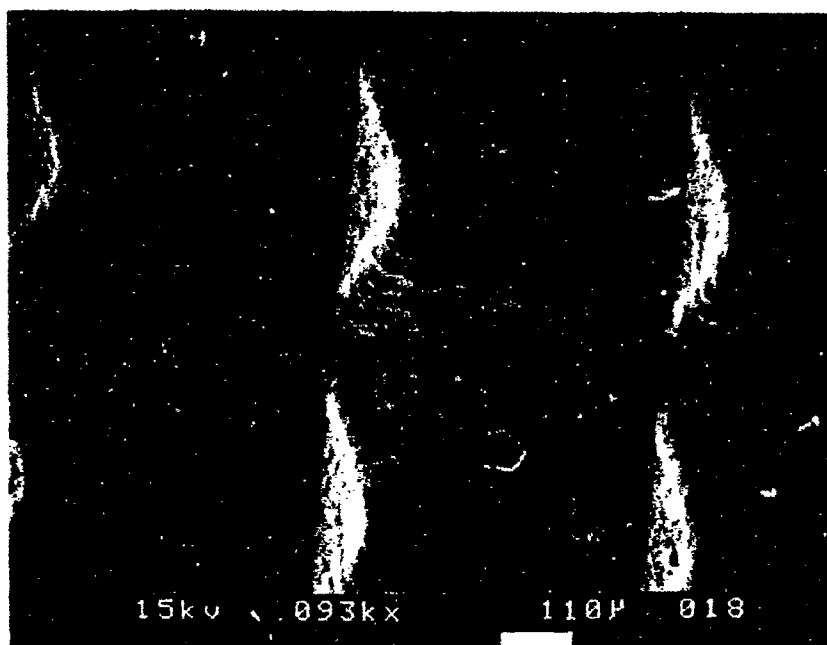
and clindamycin phosphate USP having a potency of not less than 800 mcg/mg were obtained. The certificates of analysis of the respective antibiotics used for our processing have been included in Appendix I.

C. Increase Surface Area of the Dressing

The contact surface of the wound dressing was increased by utilizing a textured surface. This technique not only increases the surface area but also increases the total amount of drug eluted or released from a dressing. The textured wound surface was obtained by casting uncured drug oligomer onto embossed polyethylene release liner prior to UV cure. The cured film bore a transposed mirror image of the polyethylene liner. Figures 3A and 3B show the surfaces of the polyethylene liner and embossed surface of the cured oligomer made by this procedure. Figure 4 illustrates the appearance of smooth versus textured surfaces utilizing standard scanning electron microscopic techniques.

The elution kinetics of the textured dressings are compared to those obtained with the smooth samples in Figure 5. The textured samples consistently showed greater drug release, and more rapid release than the smooth controls.

A



B

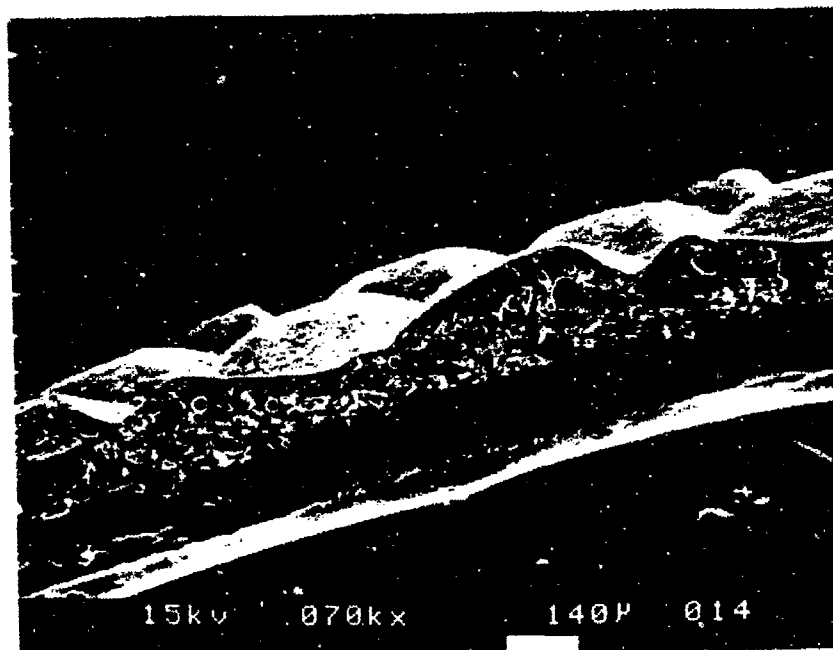
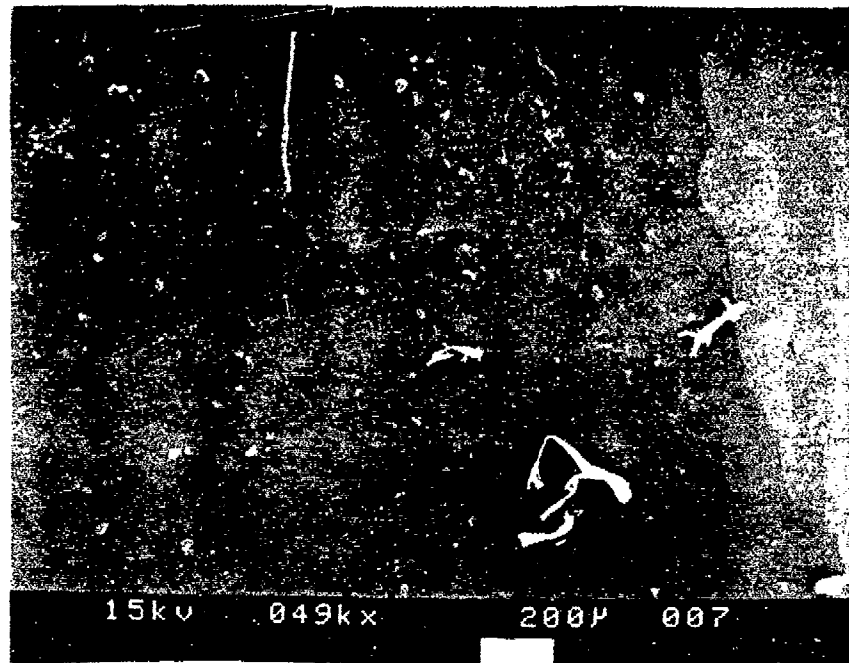


Figure 3.

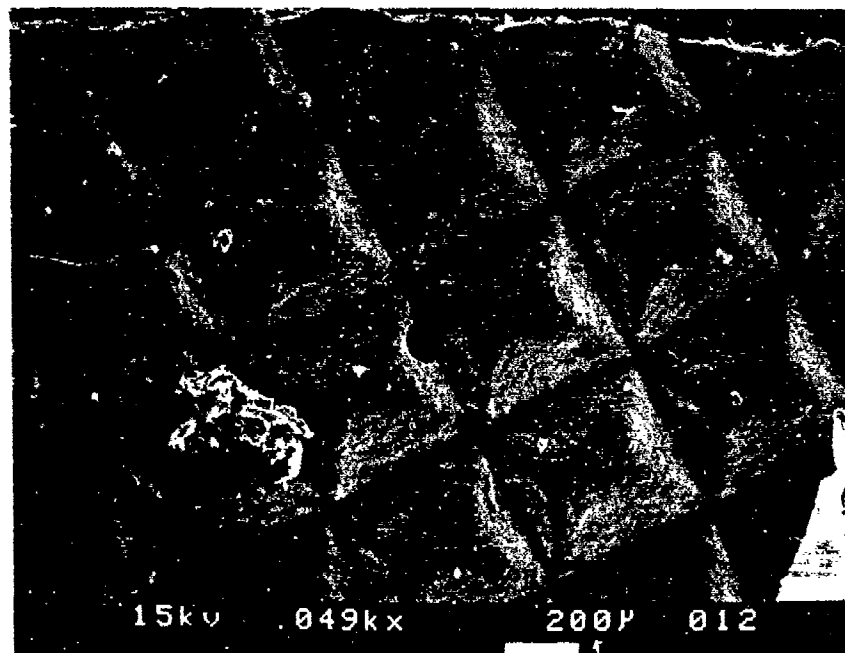
Scanning Electron Photomicrographs of the Embossed Polyethylene (A) and the Urethan is Cast Upon, and the Resultant Textured Oligomer (B).

A



Control - Wound Dressing surface

B



Experimental Wound Dressing with
Increased Surface Area

Figure 4.
Representative Appearance of Smooth
versus Textured Surfaces.

Gentamicin Sulfate Release Kinetics

(Dual Loaded Dressings-17:90:13:40 w/w)

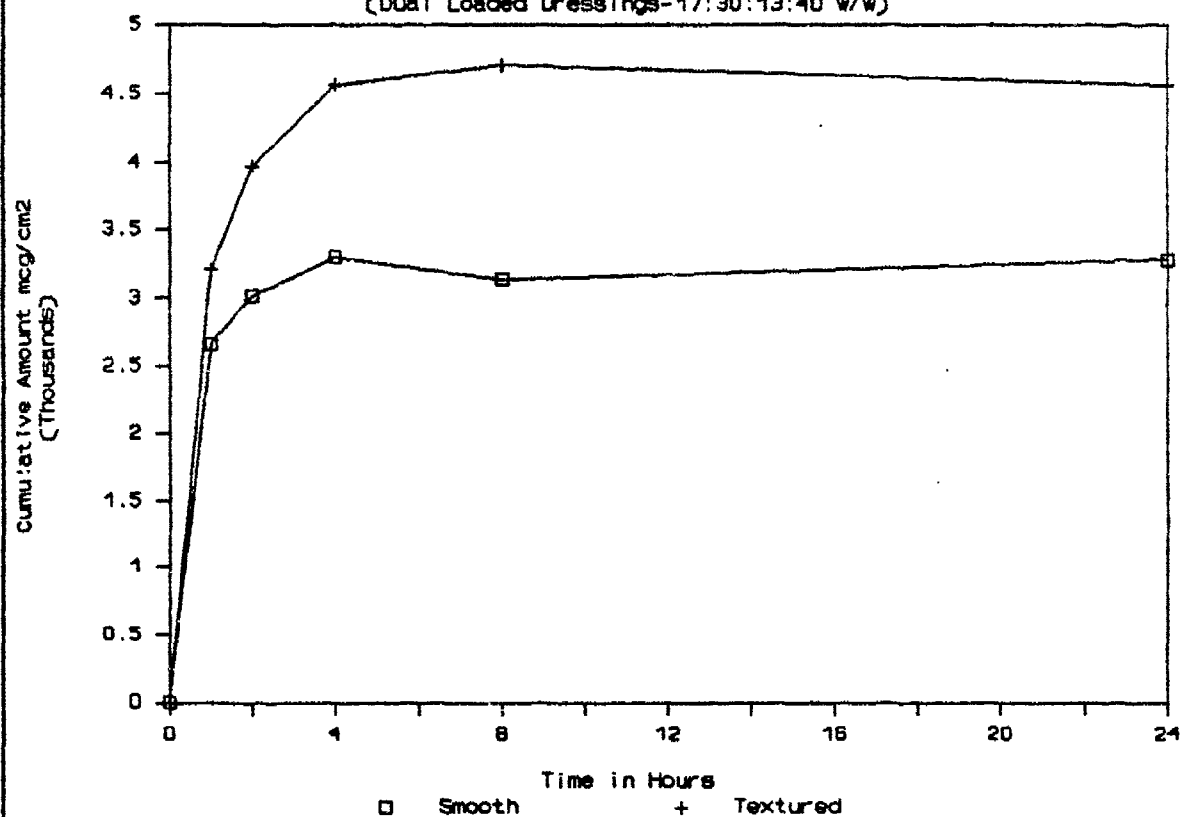


Figure 5. Effect of Increased Area on Release Kinetics

D. Increase the Hydrophilicity of the Dressing and Utilization of Barrier Technology

The release kinetics of the dressing are directly related to the hydrophilicity of the polymeric dressing⁴. The release of the water soluble drugs from the dressing indicated that the hydrophilicity of the dressing was increased due to a decrease in the hydrophobic polymer. The release kinetics of the wound dressing reported last year were obtained using samples containing only one of the drugs (gentamicin sulfate) incorporated into the dressing. However, to simulate actual release kinetics, the new dressings were loaded with both gentamicin sulfate USP and clindamycin phosphate USP. These dressings exhibited a prompt release of the drugs with minimal controlled release. The reduction of the polymeric matrix by almost 25% caused almost all of the drugs to be released from the dressing in less than 24 hours. Figures 6 and 7 are photomicrographs of the polymeric drug loaded matrices before and after elution. Based on this observation, it was decided there was a need to decrease the hydrophilicity of the dressing and thereby decrease the rate of drug release from the dressing rather than increase it.

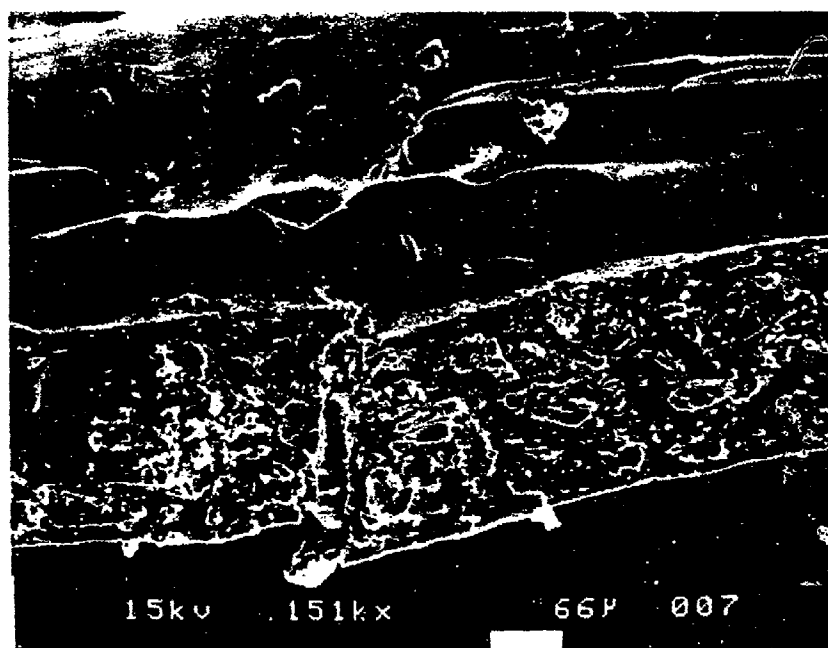
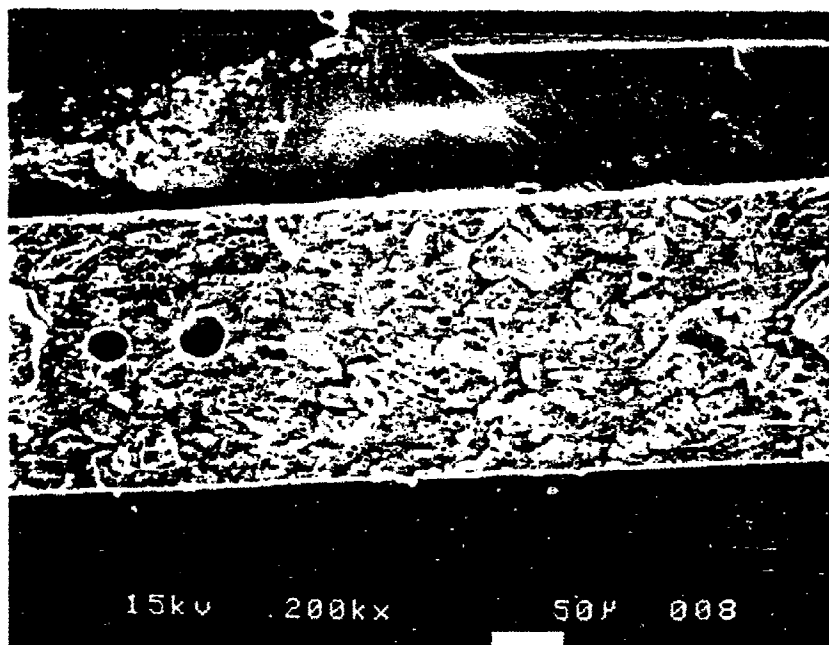


Figure 6.

Drug Impregnated Control Samples Prior to
Extraction. (Dual Loaded Dressing)

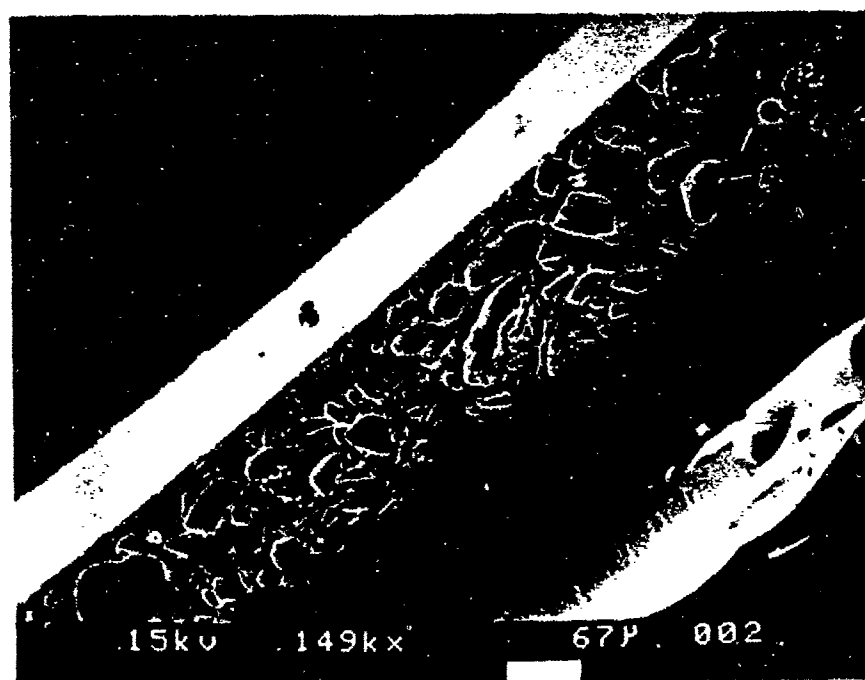
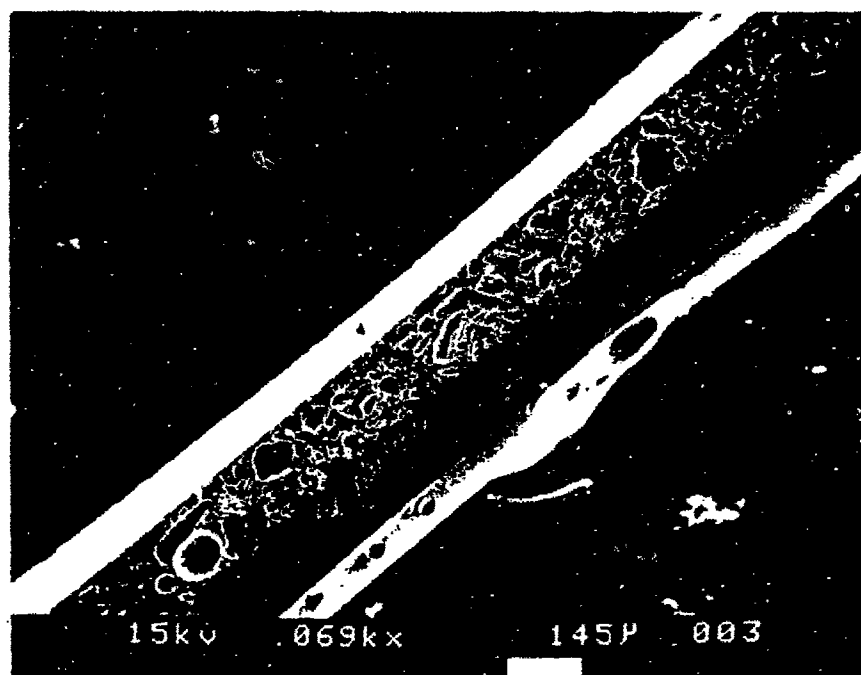


Figure 7.

Evidence of Drug Release Following Less Than 24 Hours of Extraction (Original Dual-loaded Dressing).

The subsequent series of experiments were then performed to document controlled release. The initial experiments focused on the application of a barrier layer over the island dressing. The barrier layer consisted of a one mil thick, drug free polyurethane over the island dressing. Figure 8 depicts the resultant release kinetics. Even though the elution of gentamicin was retarded, the dressing still failed to maintain sustained release of the drug for seventy two hours as required. However, the experimental results led to the conclusion that the hydrophilicity of the polymer should be reduced in order to achieve a slower release of the drugs. This was accomplished by varying the amount of polyethylene glycol (PEG), an excipient, in the formulation matrix. Figure 9 illustrates the effects of varying the concentration of PEG 300 in the matrix.

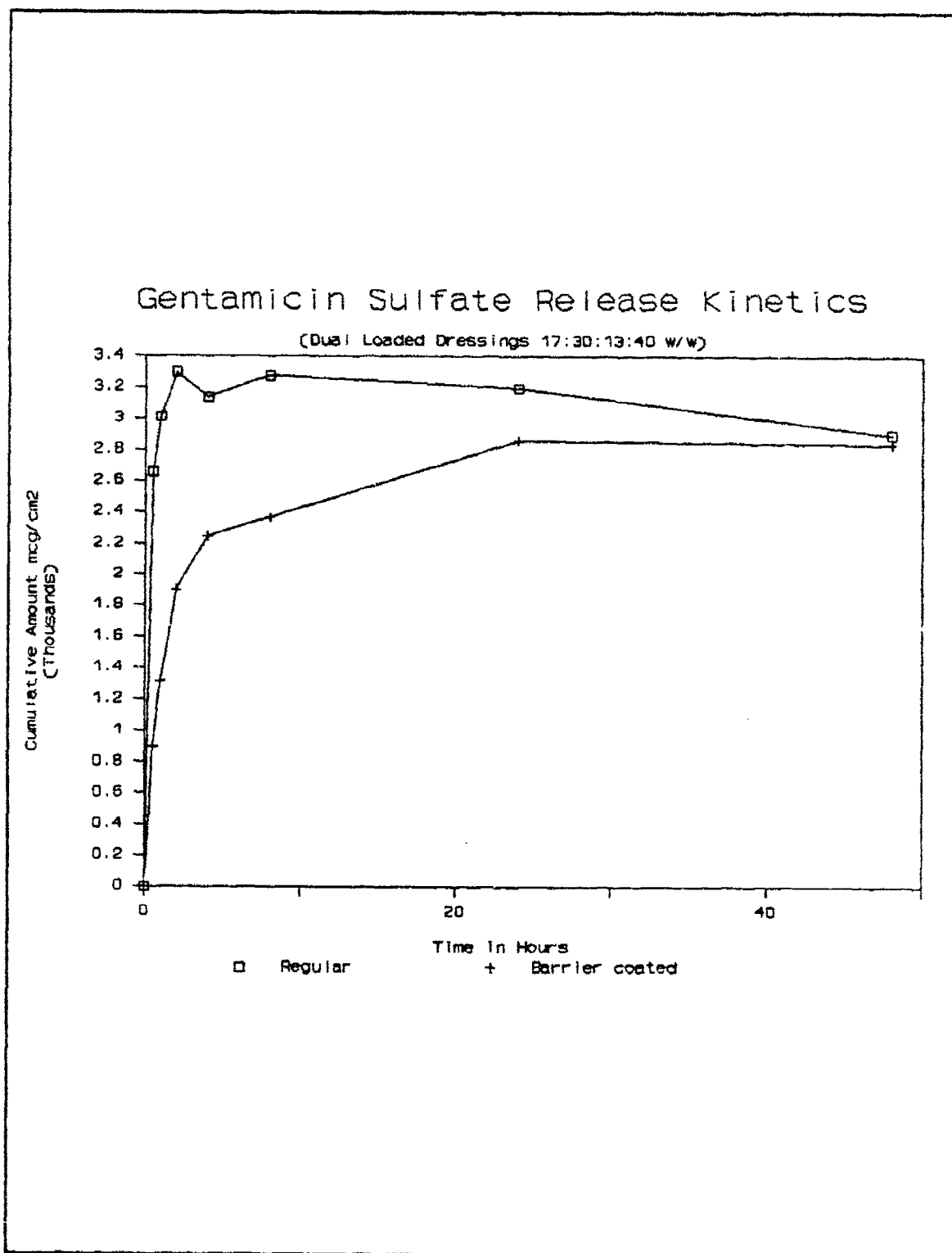


Figure 8. Effect of Barrier Coating on Release Kinetics

Gentamicin Sulfate Release Kinetics

(Effect of Varying PEG Ratio on 17:30)

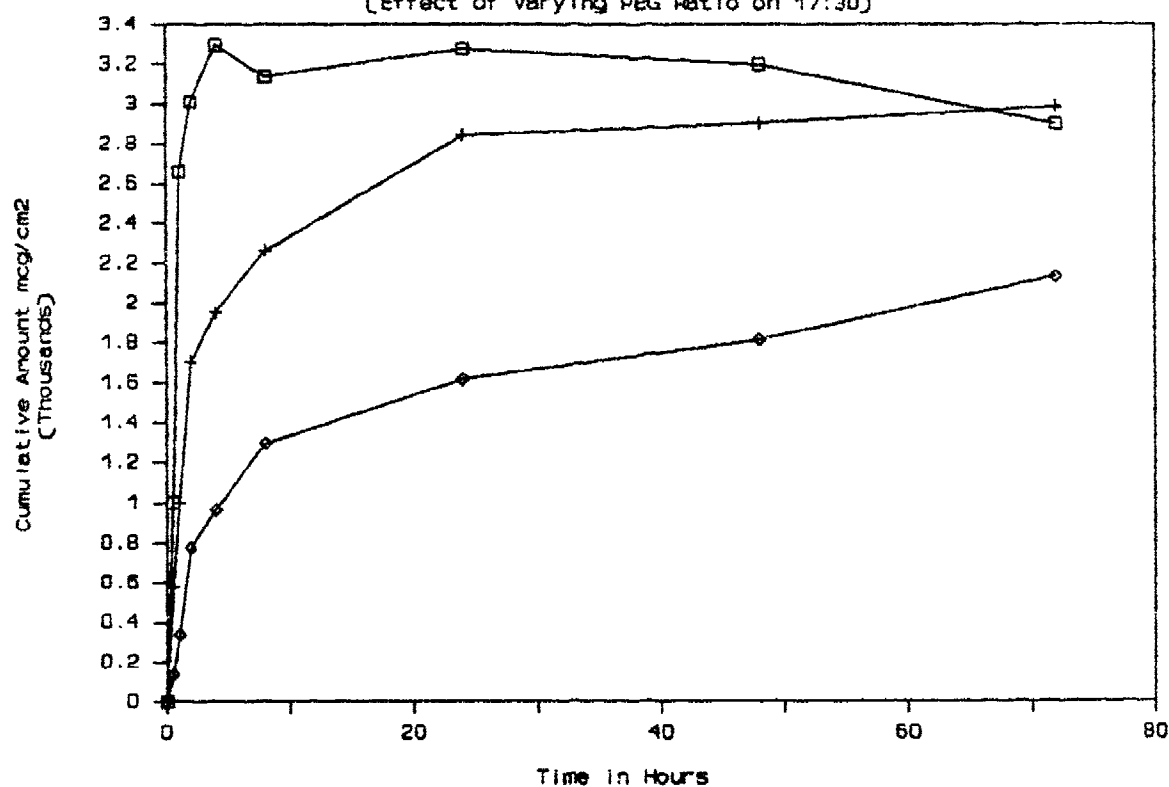


Figure 9. Effect of PEG Ratios on Release Kinetics

E. Increase Thickness of the Dressing

The amount of drug per unit area is directly proportional to the volume or the thickness of the dressing. Hence to increase the total amount of drugs being eluted, the thickness of the dressings can be increased. Table I shows the effect of drug concentration and thickness on the total amount of gentamicin sulfate released. Vapor transmission rates are inversely proportional to membrane thicknesses⁵. In the case of the ADDs, as the water soluble drug particles were extracted, the membrane became porous and more permeable. However, the effect of increased thickness on the vapor transmission was not determined.

Table I. Effect of Loading and Thickness on Release Kinetics of ADD,s containing Gentamicin Sulfate.

Loading mg %	Thickness mils	Amt. Released mcg/cm ²
16	6	1600
20	6	1900
30	6	3500
30	12	6500

F. Adhesive Testing

Table II lists the results of adhesive tests performed with Spandra^R dressings bonded to de-greased leather employing several pressure sensitive adhesives. These results showed two possible candidates as replacements for the current I 780 (Avery) pressure sensitive adhesive. Both Arcare 7400 (Adhesive Research) and I 597 (Fitchburg) adhesives showed improved bond strength under ambient conditions; the former exhibited outstanding adhesion even under wet conditions. The formulations FL 78 and L 76 (LecTec) represented an attempt to replace the solution cast pressure sensitive adhesive (PSA) with a commercially available medical grade porous hot melt adhesive; however these failed the water immersion test. Therefore, no further investigation of porous hot melt adhesives were undertaken.

Both dry and wet samples were conditioned for 24 hours before testing: ambient conditions for the dry and submersion in 37⁰C water for the wet. Peel tests were performed on an Instron Tensile Tester following the ASTM 180 degree peel method⁶.

Table II. T Peel Adhesive Test

Adhesive	Dry (g/cm)	Wet	% Change
Avery I 780 new	230.3	141.7	-38
Avery I 780 old	220.5	141.7	-36
Fitchberg I 597	259.8	224.4	-14
Adh.Res. AR 7400	289.4	313.0	+8
LecTec FL 78	177.2	84.6	-52
LecTec L 76	220.5	88.6	-60

TASK II THROUGH V

Tasks two through five required the development of an assay method for clindamycin phosphate, quantitative analysis of the release kinetics of the dual loaded dressings, manufacture of sufficient quantities of 3.5% silicone oligomer and submission of test samples for animal testing to USAIDR (see Appendix V). In addition, a follow up in vitro investigation of explanted animal dressings designed to correlate in vitro release kinetics with in vivo microbiological tests was undertaken. A summary of these activities is described in the following text.

A. In Vitro Release Kinetics of the Dermal Dressing

The release kinetics of the antibiotics, from the dual loaded dermal dressing were established, in vitro. The analytical methods developed in house (see Appendix II) helped define the release profile of both antibiotics from the dermal dressing. Prior release studies of gentamicin sulfate from dressings established a basis for formulations with various drug ratios, as well as polymer to PEG ratio. Figure 10 illustrates the release kinetics of a dual loaded dressing mixed manually. The result of the automated process is illustrated in figure 11. It should be noted that both dressings show similar release patterns; a rapid depletion of the drugs. The effect of decreasing excipient ratio yields a controlled release of drug as illustrated in figure 12. The elution kinetics of the

Hand Mixed Wound Dressings

Formulation 20:27:13:40 w/w

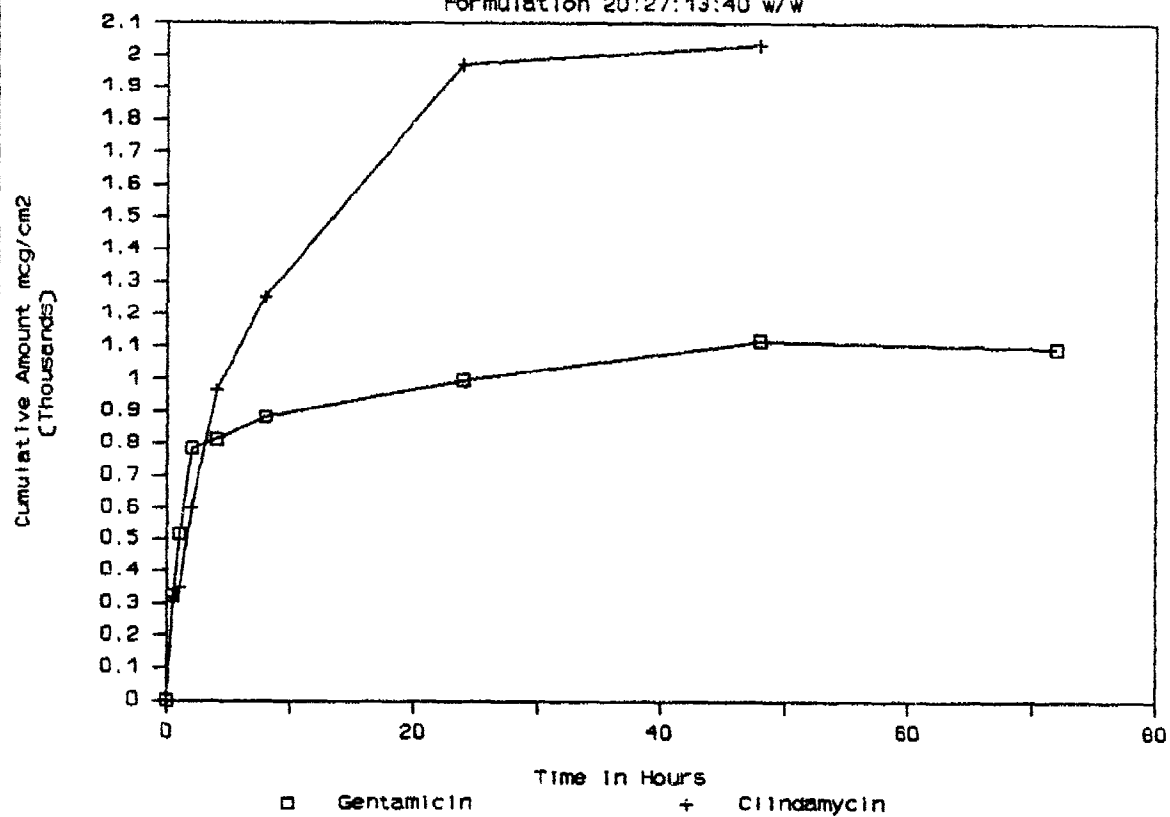


Figure 10. In Vitro Release Kinetics of Hand Mixed Dressings

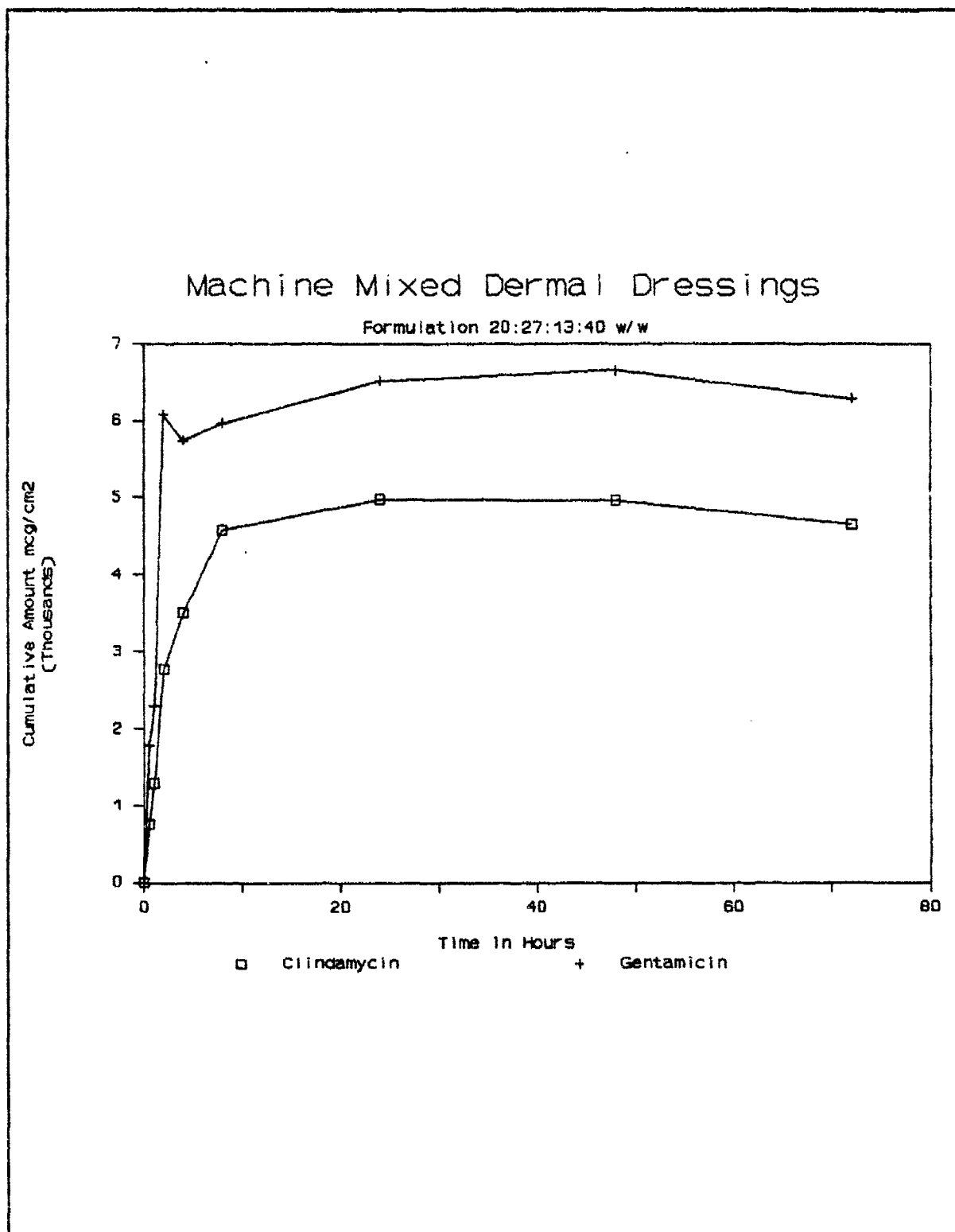


Figure 11. In Vitro Release Kinetics of Machine Mixed Thicker Dressings with 13% PEG.

Machine Mixed Wound Dressings

Formulation 17:30:1:52 w/w

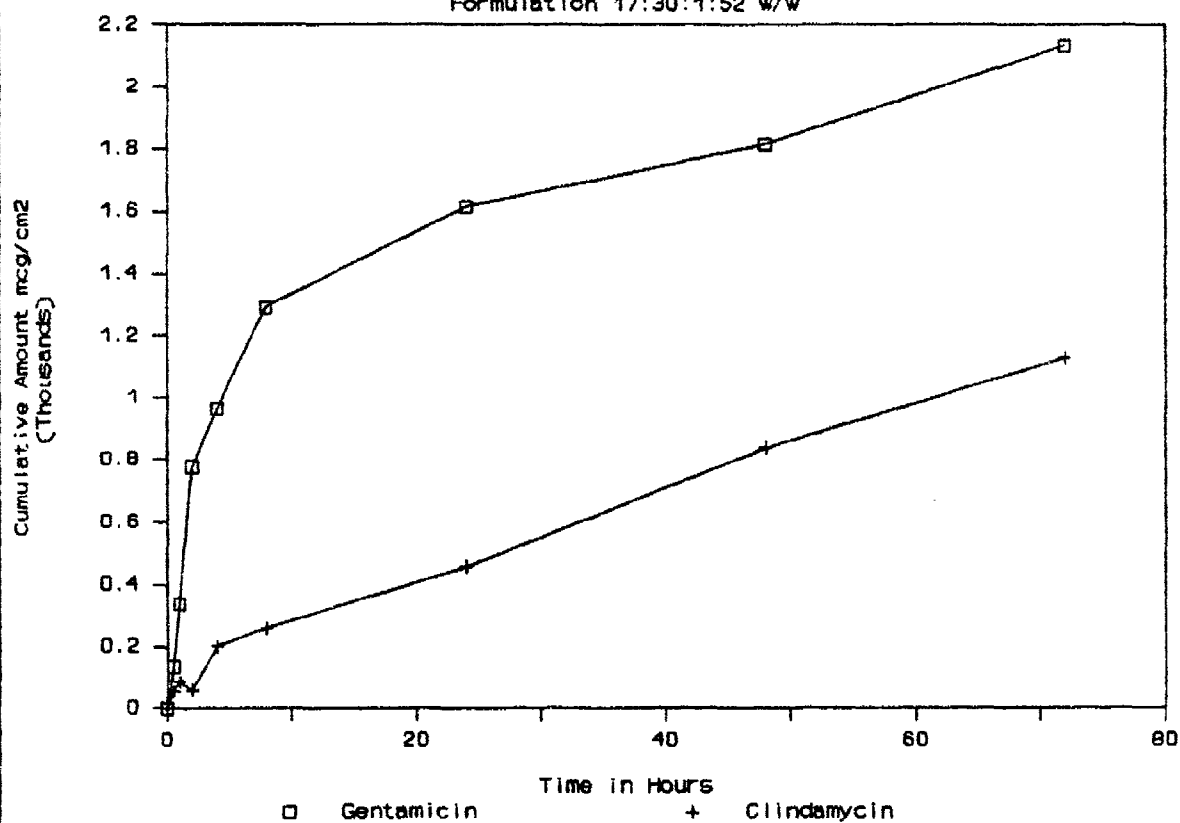


Figure 12. In Vitro Release Kinetics of Machine Mixed Dressings with 1% PEG.

dressings subjected to animal study are reported in Table III.

Table III. In Vitro Release Kinetics of ADD's

Formulation I			Formulation II		Formulation III	
Hr	C	G	C	G	C	G
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	321.3	326.1	766.6	1786.3	55.2	136.3
1	347.7	515.4	1302.5	2305.6	86.2	336.5
2	603.4	785.6	2772.3	6081.3	61.1	777.5
4	963.6	812.5	3502.0	5746.9	201.7	963.8
8	1253.0	882.7	4572.8	5965.1	260.0	1292.6
24	1973.1	998.5	4976.0	6525.3	457.9	1615.4
48	2035.4	1119.7	4968.0	6661.0	838.8	1815.3
72		1096.9	4650.9	6294.4	1129.1	2132.1

Formulation I : 20 mg Clindamycin, 27 mg Gentamicin, 13 mg PEG and 40 mg Oligomer hand mixed (6 mils).

Formulation II : 20 mg Clindamycin, 27 mg Gentamicin, 13 mg PEG and 40 mg Oligomer machine mixed (12 mils).

Formulation III: 17 mg Clindamycin, 30 mg Gentamicin, 1 mg PEG and 52 mg Oligomer machine mixed (6 mils).

B. Fabrication of Dressings for Animal Testing

Several dressings were fabricated and supplied to USAIDR for in vivo testing on guinea pigs. The dressings fabricated were with (i) extended drug release, accompanied by a burst; and (ii) a controlled drug release facilitated by lower PEG ratios, accompanied by lower peak concentrations. Additional samples were provided with a lesser amount of clindamycin and increased amounts of gentamicin. The samples submitted for animal testing are given in Table IV.

Table IV. Formulation Ratios of In Vivo Tested Dermal Dressings.

	Parts by Weight				
	#1	#2	#3	#4	#5
Clindamycin	20	20	20	17	17
Gentamicin	27	27	27	30	30
PEG 300	13	13	1	1	1
Matrix	40	40	52	52	52

#1- Hand mixed, #2-#5 Machine mixed, #5- Textured surface.

C. Follow up In Vitro Investigation of Explanted Dressings

Characterization of the ADD is dependant upon correlating the elution kinetics data generated in vitro, with the ability of the ADD to inhibit microbial growth on contaminated wounds in animals. A test protocol for comparing elution kinetics of dressings before and after animal implants was designed. USAIDR dressings were retrieved following animal tests to determine the residual amount of drug retained in each sample. The working hypothesis was that the amount of drug eluted from each dressing should be comparable to the concentration predicted by the curves of the in vitro release kinetics generated on the given lot of samples. The results assumed intimate contact of the dressing to the wound and absence of recontamination following placement of the dressing. USAIDR delivered fifteen explanted dressings for evaluation. The returns were extracted and analyzed along side respective retains which were used for controls.

Procedure:

All test samples and controls were placed in individually labeled bottles and covered with 20 milliliters of distilled water. These were sealed and placed in an ultrasonic bath for 24 hours. After extraction, they were grossly examined for loss of fluid etc. A one milliliter (1 ml) sample was removed from each bottle and filtered through a 0.22 micron membrane filter into a clean labeled

vial. These were labeled using USAIDR sample designations. The controls were similarly filtered and stored in labeled vials.

Analysis:

HPLC techniques were used to quantify the concentration of gentamicin and clindamycin in each dressing. The weight percent difference between the test sample and controls was used to calculate the amount of drug that was delivered from each dressing. Tables V, VI and VII list the raw data comparing the amount of gentamicin and clindamycin released during animal experiments with:

- a) 13% Polyethylene Glycol (PEG) Hand Mixed,
- b) 13% PEG Machine Mixed, and
- c) 1% PEG samples.

Conclusions

A statistical analysis of the data (Appendix IV) indicate there was no significant difference in the amount of gentamicin or clindamycin released from the 13% PEG machine and hand mixed samples (Tables V and VI). However, there was significantly less gentamicin released from the 1% PEG dressings and more clindamycin compared to the 13% PEG samples (Table VII). Furthermore, the 1% PEG samples were less effective than both of the 13% PEG samples based on the scrub assay results. The mean concentration of drug eluted from each sample is summarized in Table VIII.

Table V. Results of Residual Analysis - Hand Mixed (13%) ADDs

USAIDR #	Drug Eluted at Wound Site Weight %		Scrub Assay Results cfu/cm ²	
	Genta	Clinda	Test	Control
5	72.7	78.0	10 ²	10 ⁷
9	86.3	87.1	10 ³	10 ⁷
11	80.6	82.2	10 ²	10 ⁶
16	83.1	74.0	10 ¹	10 ⁷
21	84.2	---	10 ¹	10 ⁷

Formula: 20 mg clinda 27 mg genta 13 mg PEG

Table VI. Results of Residual Analysis - M/c Mixed (13%) ADDs

USAIDR #	Drug Eluted at Wound Site Weight %		Scrub Assay Results cfu/cm ²	
	Genta	Clinda	Test	
3	89.7	67.2	10 ¹	
7	---	74.4	0	
12	88.4	82.0	10 ²	
20	87.1	75.4	10 ³	
24	86.2	79.4	0	

Formula: 20 mg clinda 27 mg genta 13 mg PEG

Table VII. Results of Residual Analysis - M/c Mixed (1%) ADDs

USAIDR #	Drug Eluted at Wound Site Weight %		Scrub Assay Results cfu/cm ²	
	Genta	Clinda	Test	
2	29.6	92.3	10 ²	
6	8.1	95.5	10 ⁴	
13	17.1	90.8	10 ⁴	
17	21.8	92.3	10 ⁴	
25	48.1	---	10 ⁴	

Formula: 20 mg clinda 27 mg genta 1 mg PEG

Table VIII. Mean of Tables V - VII.

Sample	Drug Eluted at Wound Site Weight %		Scrub Assay Results cfu/cm ² Test
	Genta	Clinda	
Hand Mixed 20/27/13	81.4	80.3	10 ²
M/c Mixed 20/27/13	87.8	75.7	10 ²
M/c Mixed 20/27/1	24.9	92.7	10 ⁴

TASK VI AND VII

These tasks were deleted.

TASK VIII AND IX

These tasks focused on incorporating chlorhexidine gluconate into our antimicrobial dermal dressing, measuring the elution kinetics as well as the effectiveness of the ADDs both in vitro and in vivo. Incremental loadings were examined in combination with alternative drug excipients. Quantitative analysis conducted on the dressings employing HPLC techniques were then carried out to determine elution characteristics. Parallel microbiological assays involving zone of inhibition tests further confirmed the effectiveness of the eluted drug from the polymeric substrate. These tests showed the ADDs were active against target organisms such as Pseudomonas aeruginosa and Staphylococcus aureus.

A. Development of Chlorhexidine Gluconate ADDs

The preparation of a chlorhexidine dressing required two manufacturing steps:

- 1) formation of chlorhexidine powder and
- 2) uniform dispersion of the drug into the oligomer.

Preparation of Chlorhexidine Powder:

Fifty gram quantities of a twenty percent commercial solution of chlorhexidine gluconate were placed in drying flasks and rolled in such a manner to ensure the spreading of the sample over maximum internal surface area of the flask. Thin ice shells sublime faster than thick plugs⁷. Hence special attention at this stage was tantamount to rapid drying. The frozen sample was quickly connected to the lyophilizer by means of a 'quick seal' valve which prevented the loss of the vacuum and melting of the ice shell. The sample was left on the freeze dryer overnight whenever possible. The sample was dried until it contained less than 1% moisture⁸; initially this was noted by the absence of cold spots on the outside of the flask. The dried powder was tested by weight loss methods to determine the final purity.

B. Choice of Excipient

Several drug excipients were tested in an effort to overcome the embrittlement that was seen from failed efforts to disperse the chlorhexidine drug. These are listed in Table IX. The chlorhexidine gluconate powder was dispersed into the excipient using mechanical methods. The mixture was agitated for fifteen minutes, evacuated to remove moisture and stored in a desiccator until required. Initial dressings fabricated with propylene glycol were submitted to USAIDR for in vivo testing.

Table IX. Various Drug Excipients

Name	Viscosity cps. (25 C)	Appearance of Drug Blends 50% load
PEG 300	80	Forms hard solid
PEG 600	180	Forms hard solid
PEG 1000	---	Solid, does not form eutectic
Glycerine	1400	Dispersible paste
Propylene-	60	Dispersible fluid

C. Release Kinetics of Chlorhexidine Gluconate ADD's

The chlorhexidine gluconate dressings submitted for in vivo testing in guinea pigs showed excellent bacteriostatic activity against the test organisms. However, chlorhexidine only shows bacteriocidal activity at concentrations of 100 mcg/ml or greater⁷. Therefore it was decided that an increase in the amount of drug delivered to the wound site would be necessary if bacteriocidal conditions were to be maintained. The elution curve (generated by a modified HPLC technique - Appendix II C) for these in vivo dressings is shown in figure 13; all subsequent experiments were designed to increase the values depicted in this curve.

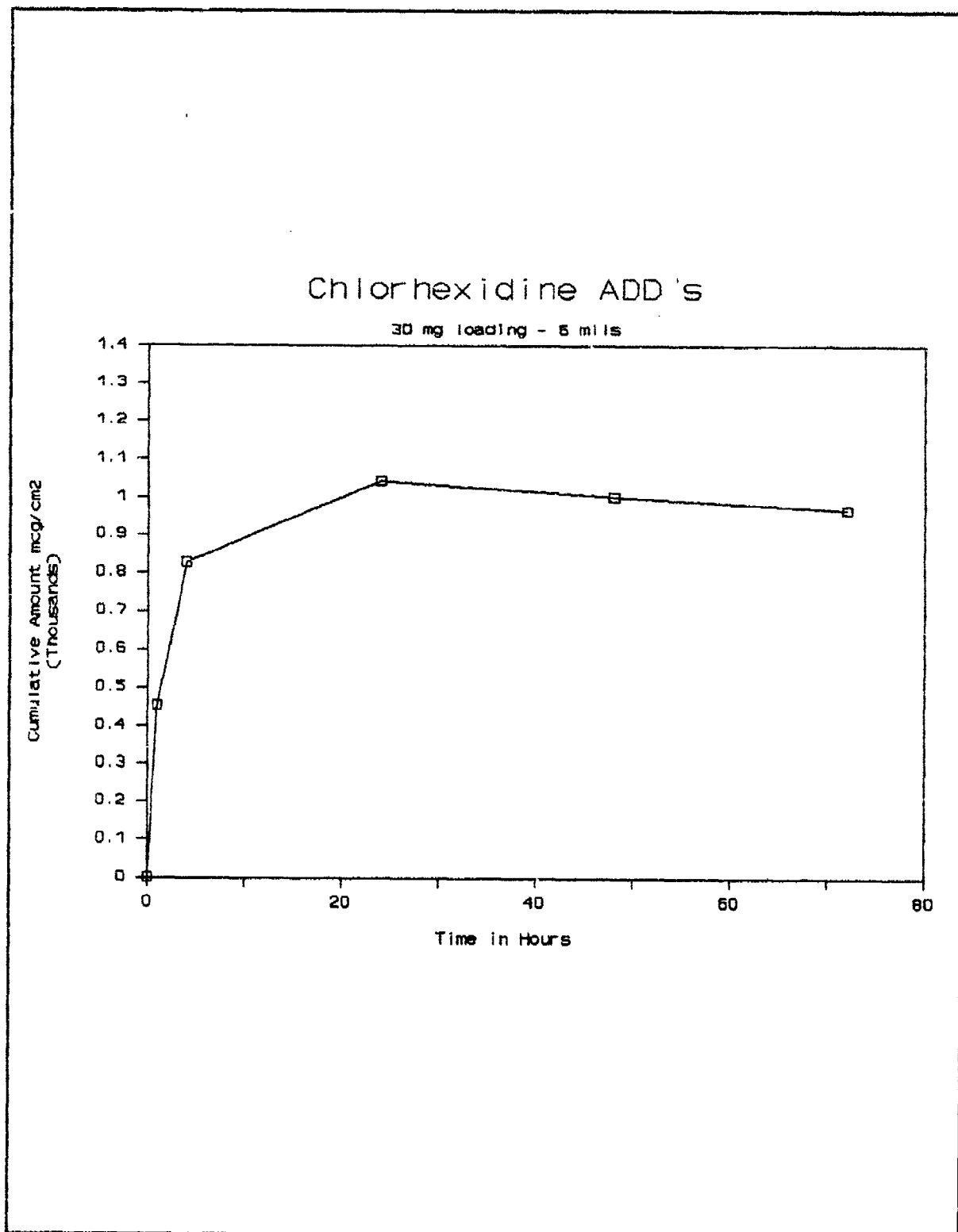


Figure 13. Release Kinetics for 30% Loaded Chlorhexidine with Propylene Glycol

An increased amount of drug to the wound site was accomplished by implementing a two step study. The first was investigating the modification of the excipient component of the dressing and second, the determination of the optimum dressing thickness for maximum drug elution.

The excipient component was modified by varying the weight ratio of propylene glycol to PEG 300. Formulations from 100% propylene glycol to 0% were tried. Dressings were made of each formulation and eluted on the Franz cell. Table X lists the results for the maximum value of drug eluted per formulation. As this table shows, the excipient with 20% propylene glycol to 80% PEG 300 elutes the maximum amount for the given concentration of chlorhexidine gluconate.

The total drug content per unit area can also be increased by an increase in the thickness of the dressing. The limiting factors determining thickness would be flexibility of the dressing and the decrease in percent elution of the total loading of drug.

The first was determined qualitatively by wearing dressings prepared at various thicknesses. These were applied to the wrist and elbow area; it was concluded that dressings in the 20 mil range were comfortable and adhered satisfactorily to the wearer. Elution studies performed upon these samples showed that the total

Table X. Maximum Drug Elution vs Excipient Ratio

Excipient Ratio	Max. Elution
PG/PEG	mcg/cm2
100	1318
50/50	1557
20/80	4041
0	1500

PG=Propylene Glycol PEG=Polyethylene Glycol 300

Formula: Drug 30/Oligomer 40/excipient 30

drug eluted increased up to a thickness of 22 mil.

Figures 14 and 15 are the elution curves for six and twenty mil dressings, respectively. Within this range, an approximate three fold increase of thickness yields a two fold increase in the total drug eluted.

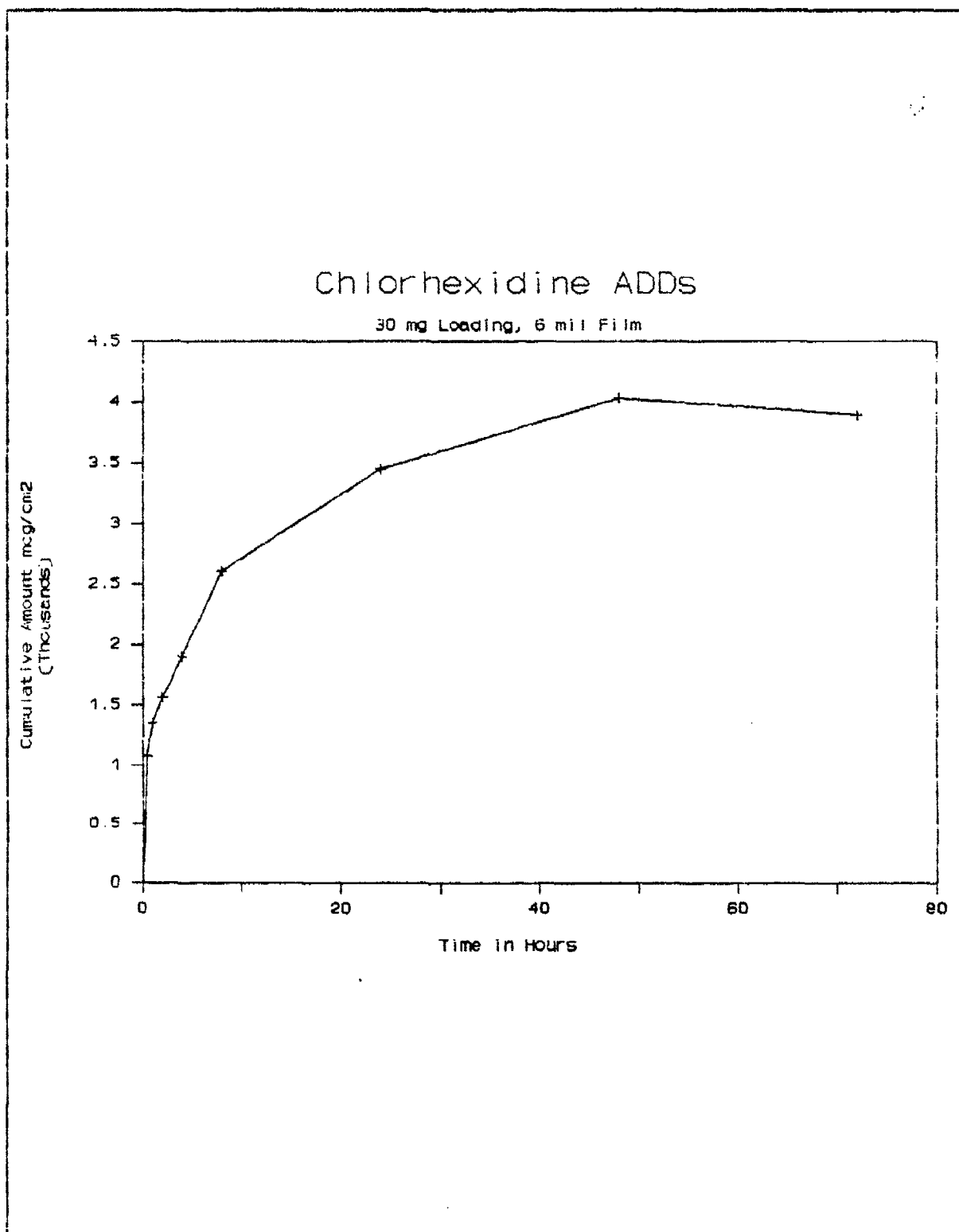


Figure 14. Release Kinetics of 30% Loaded Chlorhexidine ADD's with Excipient Blend - 6 mil Film

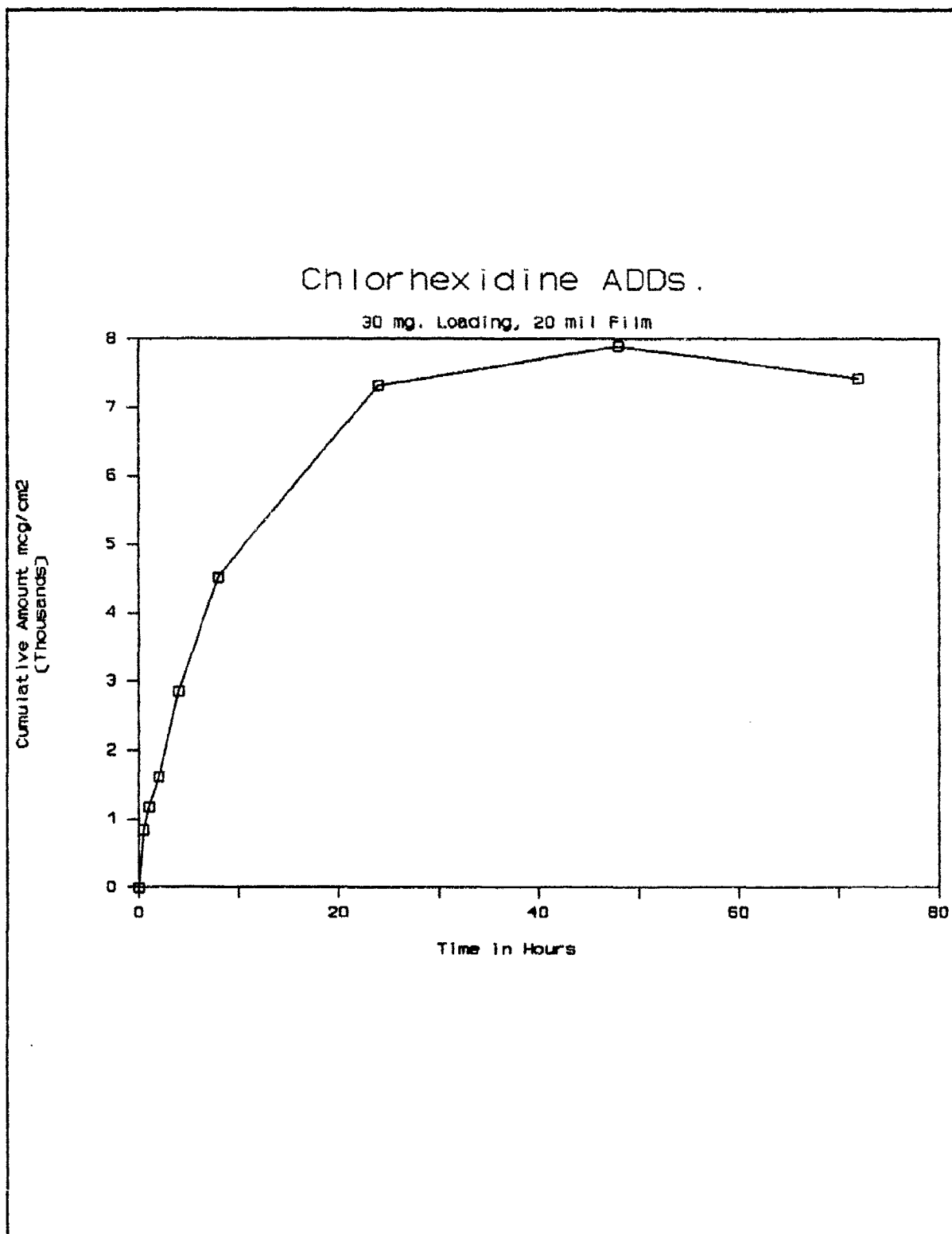


Figure 15. Release Kinetics of 30% Loaded Chlorhexidine ADD's with Excipient Blend - 20 mil Film

TASK XI

A. Selection of Antimicrobials

The medicated antimicrobial dermal dressing under development according to the terms of the USAIDR contract no. DAMD-17-88-C-8012 has to be effective against a broad spectrum of bacteria namely Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus pyogenes, and fungi such as Trichophyton species, Epidermophyton species and Candida albicans.

An ideal topical antimicrobial agent should be:

- o poorly absorbed through skin for maximum kill potential at the applied site
- o bactericidal at low local concentrations
- o as broad spectrum as possible
- o mutually compatible and complementary in spectrum with other antimicrobials.

Table XI is a summary of the available antimicrobials suited for topical medicated wound dressing¹⁰. Based on these considerations, Thermedics Inc initially developed a dual loaded

antimicrobial dressing, containing gentamicin sulfate and clindamycin phosphate. These dressings were shown to inhibit bacterial infection and aid in wound healing. However, these dressings fail to address fungal infection. Presently work is being conducted to develop dressings that will be effective against fungi as well as bacteria. A preliminary in vitro analysis was performed on several formulations composed of drugs chosen from this list.

Table XI. Summary of Antimicrobial Agents

Drug name	Bacteria				Fungi		Class
	1	2	3	4	5	6	
Clindamycin phosphate	xx	x					Rx
Gentamicin sulfate	x		xx				Rx
Silver sulfadiazine	xx	xx	xx			x	Rx
Chlorhexidine gluconate	xx	xx	xx				OTC
Neomycin							OTC
Nystatin				xx	x	xx	OTC
Miconazole				xx	x	xx	Rx
Amphotericin B	x			x	xx	xx	Rx
Tolnaftate				x	x		OTC
Ketoconazole				x	x	x	Rx
Clotrimazole						x	Rx
Carbenicillin			xx				Rx

- 1 = Staphylococcus aureus
 2 = Staphylococcus pyogenes
 3 = Pseudomonas aeruginosa
 4 = Trichophyton species
 5 = Epidermophyton species
 6 = Candida albicans

B. Microbiological Testing

The initial tests were restricted to drugs that were previously shown to be effective against Staphylococcus aureus and Pseudomonas aeruginosa when eluted from the ADDs, with the exception of silver sulfadiazine. For this initial test the Candida albicans organisms were included; however, due to cost concerns, the three remaining organisms were not tested at this time. It was decided a method of incorporating a higher concentration of silver sulfadiazine into the dressing should be resolved before testing the full matrix of organisms. The results of these in vitro tests are given in Tables XII- XIV.

Table XII. Microbiological Test Results of 30% Loaded Chlorhexidine Gluconate in Propylene Glycol Excipient

Concentrations	Avg. Zone of Inhibition (cm) for Microorganisms		
	S. aureus	Ps. aeruginosa	C. albicans
30 % Chlor. ADD	0.15	0.15	0.65
Placebos	0	0	0
+ Controls: Chlorhexidine Powder at Three Concentrations			
30% Chlor.	0.50	0.40	1.10
15%	0.35	0.25	1.05
7.5%	0.30	0.20	0.95
Formulation: Chlor. Gluconate 30/ Oligomer 40/ P.G. 30			

Table XIII. Microbiological Test Results of 30% Chlorhexidine Gluconate in Excipient Blend

Concentration	Avg. Zone of Inhibition (cm) for Microorganisms		
	S. aureus	Ps. aeruginosa	C. albicans
30 % Chlor. ADD	0.20	0.60	0.20
Placebos	0	0	0
+ Controls: Chlorhexidine Powder at Three Concentrations			
30% Chlor.	0.50	0.40	1.10
15%	0.35	0.25	1.05
7.5%	0.30	0.20	0.95

Formulation: Chlor. Gluconate 30/Oligomer 40/PG 6/PEG 24

Table XIV. Microbiological Test Results of 2% Silver Sulfadiazine with 13% PEG

Concentration	Avg. Zone of Inhibition (cm) for Microorganisms		
	S. aureus	Ps. aeruginosa	C. albicans
2% S.sulfa. ADD	0.35	0.55	0.45
Placebos	0	0	0
+ Controls: Silver Sulfa. Powder at Three Concentrations			
2% S. sulfa.	0.55	0.60	0.70
1%	0.40	0.40	0.50
0.5%	0.20	0.20	0.15

Formulation: Silver sulfadiazine 2/Oligomer 85/PEG 13

C. Incorporation of Selected Antimicrobials into ADDs

Dressings were prepared using silver sulfadiazine drug at a two percent level. These dressings were submitted for in vitro testing. The tests indicated that an increase in silver sulfadiazine concentration was warranted.

Initial trials using higher levels of silver sulfadiazine loading resulted in a dressing that failed to cure into a satisfactory film. Silver sulfadiazine as well as Nystatin are opaque powders and inhibited the polymerization of the oligomer by blocking the UV energy needed to dissociate the photoinitiator into free radicals. The use of long wave length photoinitiators is under investigation as a method to overcome this curing problem.

CONCLUSIONS

Thermedics, Inc. is developing a second generation, sustained release antimicrobial dermal dressing. This compliant adhesive dressing incorporates antimicrobial agents to facilitate wound healing. The dressing is a trilaminate composite, consisting of an outer medical grade polyurethane impregnated fabric; an antimicrobial impregnated middle laminate which serves as the sustained release layer and the acrylic-based pressure-sensitive adhesive as the third layer.

A Gentamicin/Clindamycin dual antibiotic dressing was fabricated and shown to inhibit wound infection and enhance healing. Methods were developed to improve release rates and efficacy of these ADD's by improving homogeneity through automation, increasing contact area by texturing surfaces, increasing drug loading using thicker films, and speeding drug release by using a hydrophilic matrix and using more potent drugs. However, the release of the antibiotics was too rapid over a 72 hour period. Therefore, a method to control the release rate of the antibiotics was developed by modifying the matrix composition. The resultant release rates of the antibiotics from the dressings was then characterized.

The modified dressings were subjected to a series of in vivo tests, using inoculated guinea pigs. The results of these tests

showed that the extended release dressings were less effective than those which exhibited a rapid rate of release.

The fabrication method for ADD's incorporating chlorhexidine gluconate was successfully completed. Also, the test methods to characterize the release kinetics of the chlorhexidine ADD's were developed and validated. Initial in vivo tests of these ADD's using guinea pigs exhibited excellent bacteriostatic activity. Further work is being conducted to develop ADD's with both bacteriocidal and fungicidal activity.

Previous tests employing navy seals showed the susceptibility of the adhesive to a wet environment over a prolonged time period. Therefore, work has been conducted to improve adhesion of the ADD's to moist skin.

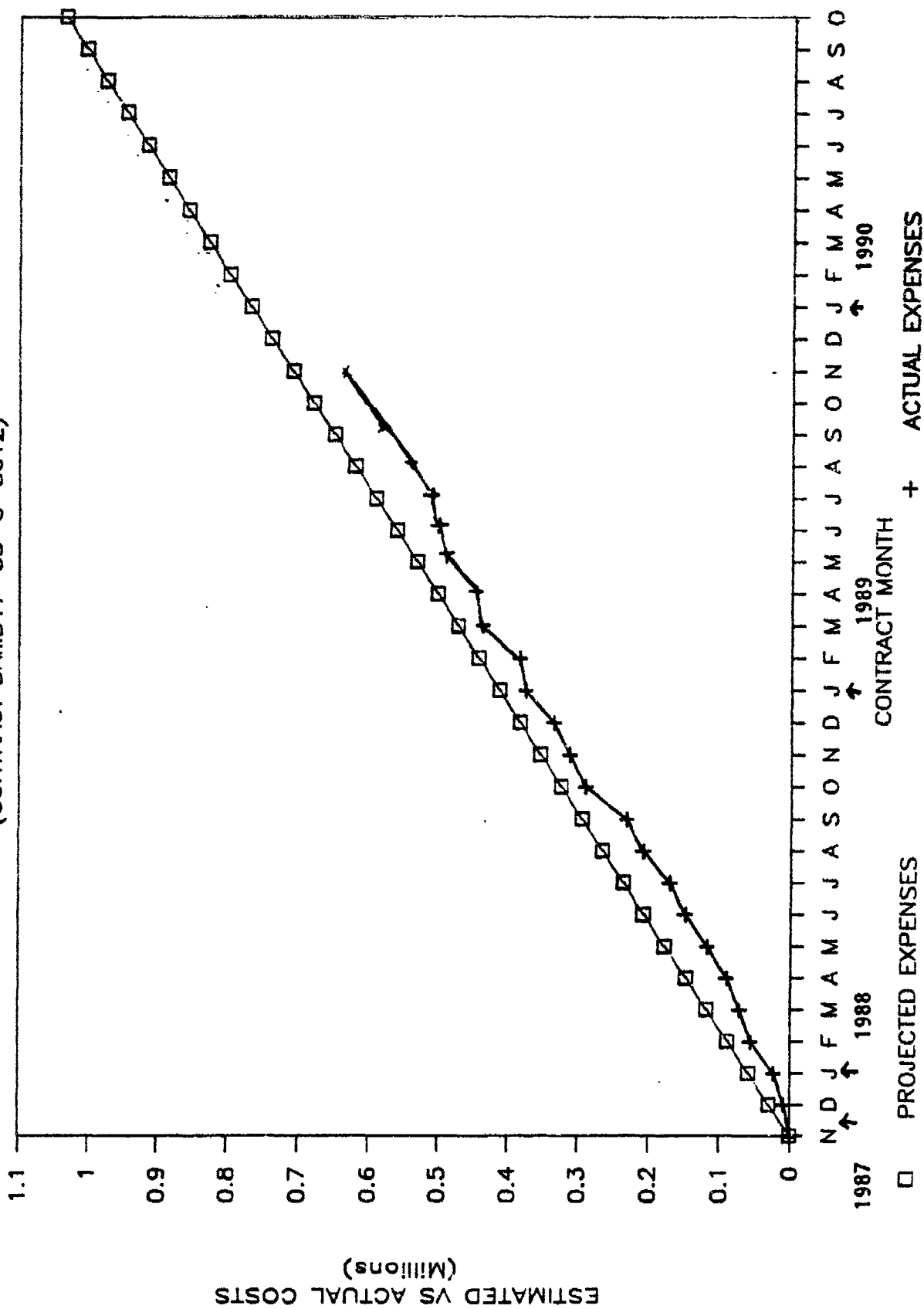
In conclusion, all tasks have been completed according to the schedule to date. The resulting dressings have been shown to meet the design requirements of being easy to apply and effective against the desired target organisms. Year 3 will focus on the development of a dressing effective against a broader spectrum of microorganisms.

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FUNDS EXPENDITURE

(CONTRACT DAMD17-88-C-8012)



APPENDIX I

CERTIFICATE OF ANALYSIS



Capitale Sociale vers. 1 n. 32 147 659 000

Pierrel S.p.A.

Indirizzo e Ufficio: 20152 Milano - Via Broletto, 96 - Tel. (02) 414011 - Telex 310015-321590 - Telefax (02) 4140400

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NDC CODE 17377-0047

NDC CODE 17377-0047

Date Capua, July 5, 1988

No. of analysis 15576

CERTIFICATE OF ANALYSIS

Product GENTAMYCIN SULFATE, non sterile - EUR.PHARM. 2nd Ed.
- USP.XXI 4 th. Suppl.

Batch No. GENTA/ 397

Test for:	EUR.PHARM. - SPECIFICATIONS	Analysis results
Description	White to cream-coloured powder.	Corresponding
Solubility	Soluble in water, insoluble in ethanol, ether, chloroform.	Corresponding
Identification	a) Infrared spectrum b) T.L.C. c) Characteristic reaction of sulphates	Corresponding Corresponding Corresponding
Assay (as Gentamicin on dry basis)	Not less than 590mcg/mg-units/mg	695 mcg/mg
pH	3.5 to 5.5	4.2
Specific optical rotation (on dry basis)	+ 107.0° to + 121.0°	+ 116.6°
Sulphate (% SO ₄) (on dry basis)	32.0 % to 35.0 %	32.6 %
Sulphated ash (residue on ignition)	Not more than 1.0 %	0.2 %
Water (K.Fischer)	Not more than 15.0 %	9.9 %
Methanol	Not more than 1.0 w/v	0.4 %
Appearance of solution	Not more than degree 6	Corresponding
Abnormal toxicity	Non toxic	Non toxic
Pyrogens	Pyrogen-free USP.XXI - SPECIFICATIONS Other than those prescribed by EUR.PHARM.	Pyrogen-free
Loss on drying	Not more than 18.0 %	10.2 %
Content of Gentamicin (HPLC)		
C ₁	25.0 % to 50.0 %	37.1 %
C _{1a}	10.0 % to 35.0 %	20.1 %
C ₂ + C _{2a}	25.0 % to 55.0 %	42.8 %
Depressor Substances	ADDITIONAL PIERREL SPECIFICATIONS Passes test	Passes test
Bacteria	Max 1 x 10 ²	Passes test
Pathogens	Absent	Absent

Approval JUNE 1988

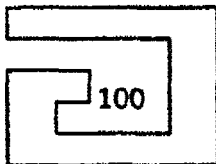
Expiration date JUNE 1992

Comments

Signature and official stamp

/ai

APPROVED



Abbott Laboratories
North Chicago, Illinois 60064
Chemical and Agricultural Products Division

06-Jan-1989

CERTIFICATE OF ANALYSIS

Clindamycin Phosphate, USP
Lot Number 23-460-CA

Tests

Results

Assay	849 mcg/mg
Appearance	Passes
Color	Passes
pH	4.0
Moisture	0.2%
ID	Passes
Crystallinity	Passes
Pyrogen	Passes
Depressor Substances	Passes

The undersigned certifies this to be a true copy of the results of tests and assays conducted by ABBOTT LABORATORIES.

ABBOTT LABORATORIES

Rolene Slininger
Quality Assurance

APPENDIX II

ASSAY METHODOLOGY
FOR
IN VITRO RELEASE KINETICS

A. Direct HPLC Method for Total Gentamicin Sulfate In Vitro Using Size Exclusion Chromatography and Electrochemical Detection¹.

Abstract

A simple and rapid HPLC method was developed to quantitate release kinetics of gentamicin sulfate, in vitro, from an antibiotic wound dressing. Wound dressings containing gentamicin sulfate were placed in Franz diffusion cells and eluted with water. Total gentamicin sulfate concentration in the eluate and in calibration drug standards were assayed by HPLC using a size exclusion column, 60⁰ A μ Porasil^R, (3x30 cm) with water as the mobile phase (1 ml/min). The antibiotic is detected by electrochemical (EC) detection. All three isomers of the drug are measured as total gentamicin. Standard concentrations from 50 to 2000 mcg/ml gave good linearity with $r^2 > 0.99$. No buffer is needed in the mobile phase at these drug concentrations. If needed, lower drug concentrations may be detected by EC. This method is direct and precise. No derivatization of gentamicin is required for detection. The method is suitable for routine quality control of gentamicin dosage forms, in vitro.

Introduction

Gentamicin is a water soluble aminoglycoside antibiotic used in the treatment of serious Gram negative bacterial infections. Like other aminoglycosidic chemotherapeutic agents, gentamicin has

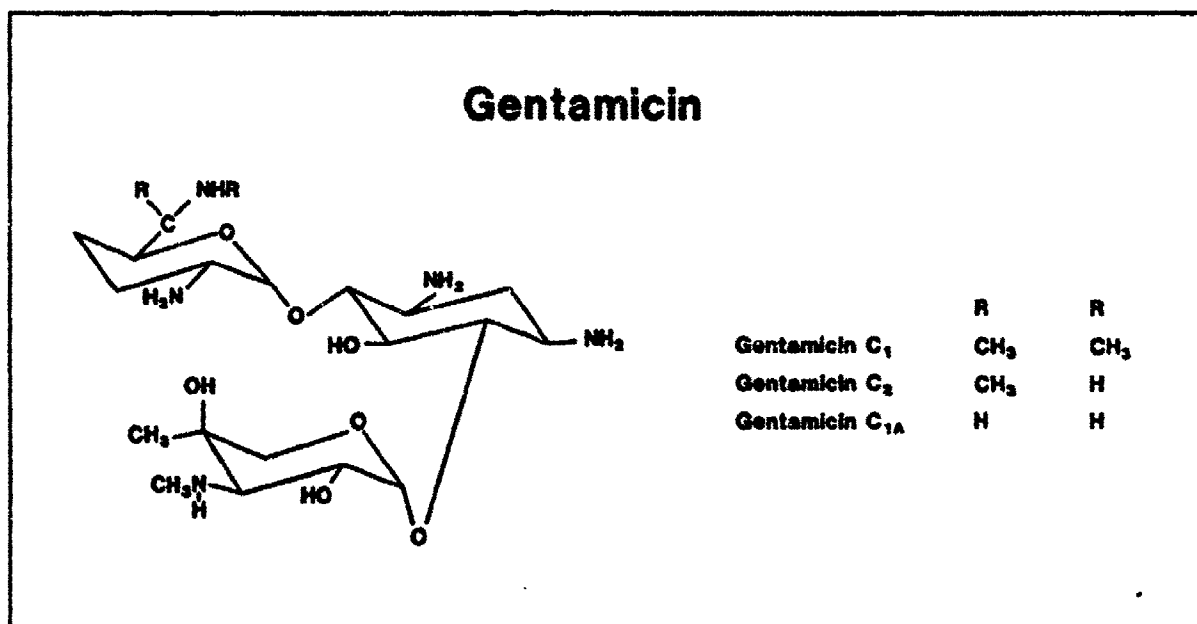


Figure A1. Structure of Gentamicin Sulfate

a narrow therapeutic range. A dermal wound dressing containing gentamicin sulfate was developed to provide a controlled release of the antibiotic after traumatic injury. Consequently, a reliable and fast method of analysis was critical. Microbiological², enzymatic³, hemagglutination inhibition⁴ and radioimmunoassays⁵ have been developed. Also several methods for the analysis of this drug in serum⁶ and plasma⁷ have been reported. However, these methods are tedious, time consuming or require the derivatization of the drug with chromophoric moieties for ultraviolet or fluorescence detection. The method reported here uses more simpler chromatographic conditions and requires no derivatization. Size exclusion chromatography or Gel Permeation Chromatography (GPC) was chosen since the resolution of gentamicin sulfate into its isomers was not necessary for drug release studies. Moreover, the high solubility of gentamicin sulfate in water allowed for the use of

an aqueous mobile phase and hydrophilic GPC column. Electrochemical detection was chosen due to the nature of the electroactivity of the drug molecule (Figure A1). The electrochemical detector relies upon the electroactive amino and amide groups present in the drug molecule. The oxidation or reduction of the aminoglycoside results in a current which is proportional to the amount of drug present.

Materials

USP grade distilled water was filtered through a 0.22 μ m membrane filter and used as the mobile phase. Chromatography was performed on a Waters Associates μ Porasil^R 60⁰ A 3 x 30 cm column, (column pressure 1800 psi) at a flow rate of 1 ml/ min., using a Waters Solvent delivery module # 570 and a Waters U6K injector. The detector used was an 'ESA' Coulochem Model 5100A fitted with a Model 5010 Standard Analytical Cell (baseline pumps 0.7 - 0.9), and the data recorded using a Waters Data Module model M730 integrator. The data for standard calibration curves were prepared (Table A1) by plotting the known drug concentrations versus the peak areas.

Method

Various standard concentrations, ranging from 2000 mcg/ml to 50 mcg/ml of gentamicin sulfate was prepared in filtered distilled water and used to prepare a calibration curve; three of which are shown in Figure A2, are the actual chromatograms and corresponding areas for the 200 μ g/ml, 400 μ g/ml and 800 μ g/ml standard

solutions. The wound dressings containing gentamicin sulfate were eluted in water from Franz diffusion cells (Figure A3). Aliquots were withdrawn (0.5 ml) at predetermined time intervals for up to 72 hours. One microliter of the sample was injected and the response recorded on a Waters Data Module model M730 integrator. Control samples were also prepared without the drug and the extracted samples were also analyzed similarly.

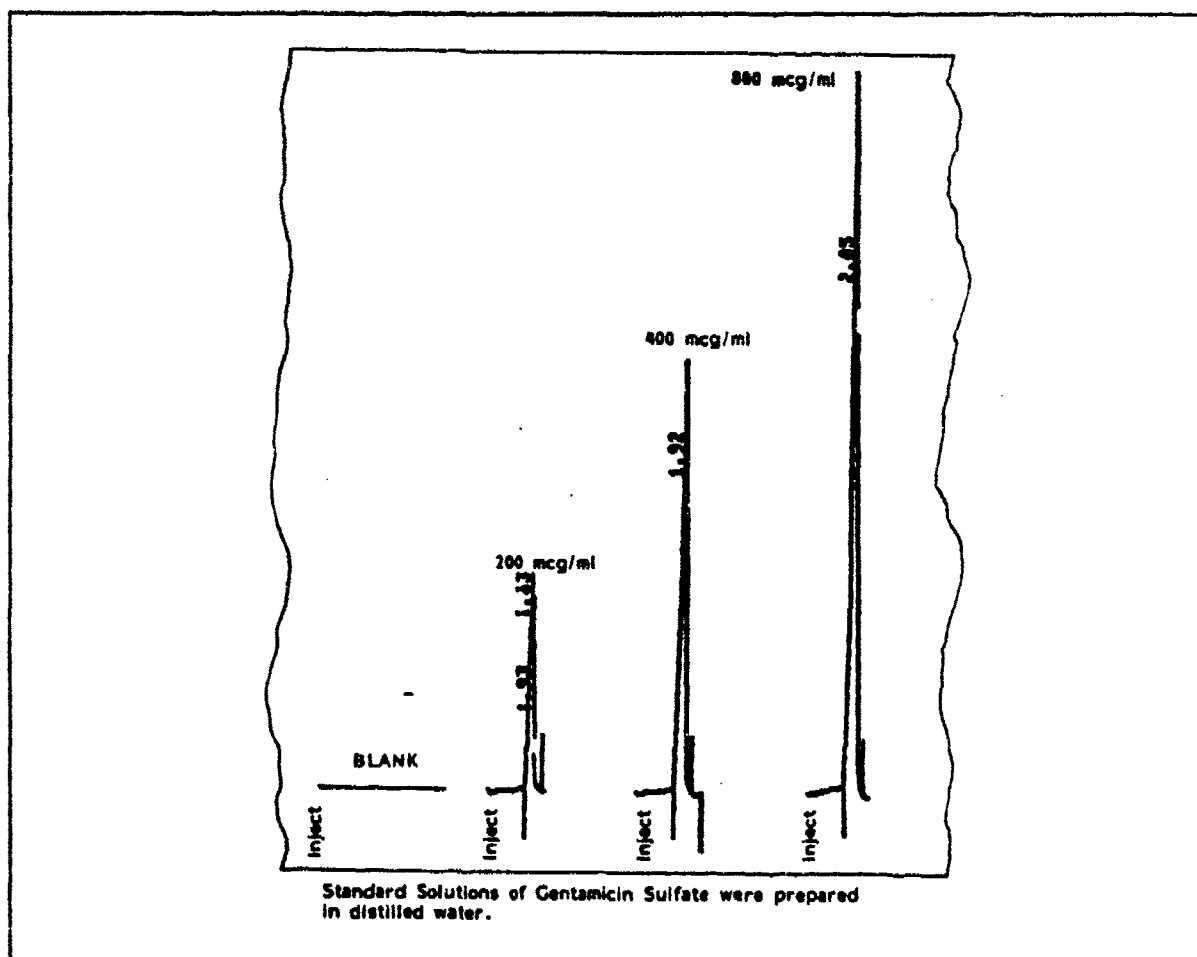


Figure A2. HPLC Chromatograms of Gentamicin Sulfate Standard Solutions

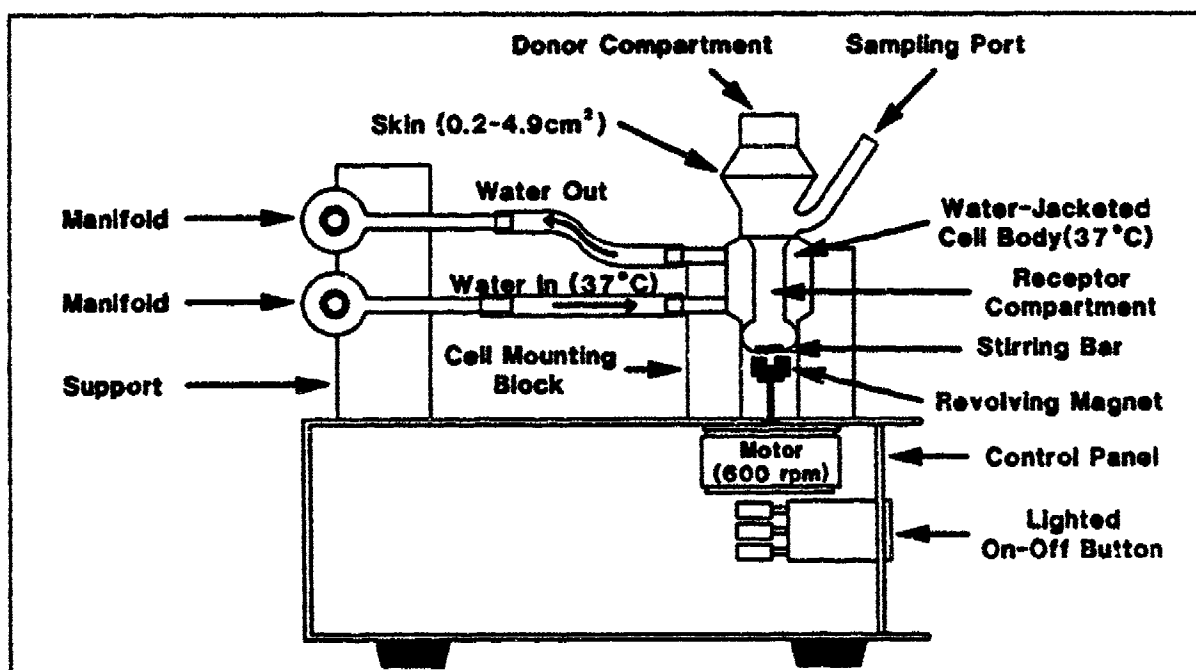


Figure A3. Finite Dose Franz Diffusion Cell

Table A1. Validation of Assay Method

Date	2/28/89	2/13/89	2/16/89	2/21/89	2/26/89	3/5/89	Mean	Std
Constant	9234	-11181	37115	32389	-27842	11923	8606.33	24939
Std Err of Y Est	18636	21887.7	27670	40627	21867.7	16429	24103	9041.66
Reg. Coef.	0.998	0.997	0.997	0.997	0.998	0.996	0.997	0.001
Corr. Coef.	0.997	0.993	0.994	0.995	0.997	0.996	0.995	0.001
No. of Observation	11	10	11	11	9	9	10	9
Degree of Freedom	9	8	9	9	7	7	8	8
X Coefficient	415.24	617.73	642	622.23	714.6	411.1	570.48	165.68
Std Err of Coef.	8.09	15.14	13.49	13.83	15.80	8.86	13.54	4.45

Standard gentamicin sulfate calibration curves from 0 to 2000 mcg/ml were run daily as indicated

B. HPLC Method for the Analysis of Clindamycin Phosphate In Vitro using Ultraviolet Detection.

This method was developed in house for the rapid in vitro these analysis of clindamycin phosphate from antimicrobial dermal dressings. The method was found to be linear and precise and could be used for determining sample concentrations as low as fifty micrograms per liter. The chromatographic conditions used for the analysis have been outlined below⁸.

Materials

The mobile phase consisted of a 77:23 v/v proportion of water:acetonitrile. Chromatography was performed on an AlTech RSil^R 250 mm x 4.6 mm 10 μ C8 column. The flow rate was adjusted to 1 ml/min using a Waters Solvent Delivery Module (model 510). Ten microliter (10 μ l) injections of the sample were introduced through a Waters U6K injector and the sample quantified by means of the Waters 484 Tunable Absorbance UV Detector, connected to a Waters M730 Data Module. Clindamycin phosphate, a thioether (Figure B1) exhibits UV absorption at 194 nm which was the wavelength chosen for quantitative analysis.

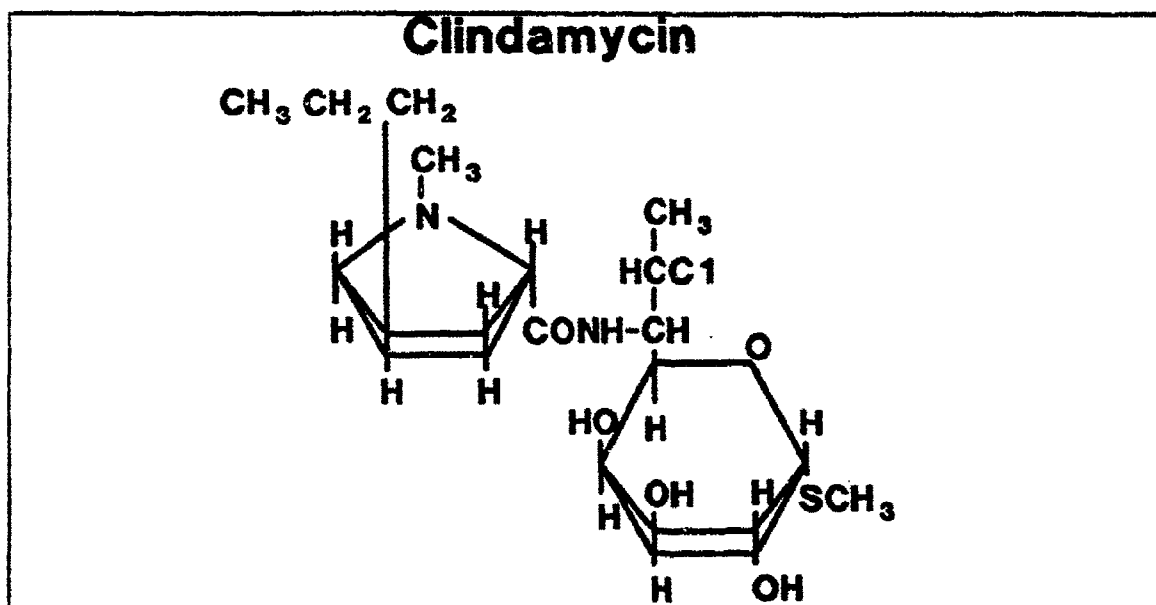


Figure B1. Structure of Clindamycin Phosphate

Method

The quantification of clindamycin phosphate released from the antimicrobial dermal dressing was made simpler by using procedures developed in-house. The method reported earlier used a Refractive Index detector which was highly sensitive to temperature fluctuations as low as $\pm 1^{\circ} \text{C}^9$. The method described here utilizes an Ultra Violet detector and is comparatively easier to handle. The method is linear and can quantitate drug solutions with concentrations as low as 50 mcg/ml. Example chromatograms for 1000 and 800 microgram per milliliter standard solutions of clindamycin phosphate, generated by this method, are shown in Figure B2. Various concentrations of clindamycin phosphate, ranging from 50 mcg/ml to 2000 mcg/ml were prepared and used for the standard calibration curve. A calibration curve was generated for each in -

vitro kinetic study by plotting the known drug concentration as the independent variable and peak areas as the dependant variable; Figure B3 depicts a typical clindamycin calibration curve.

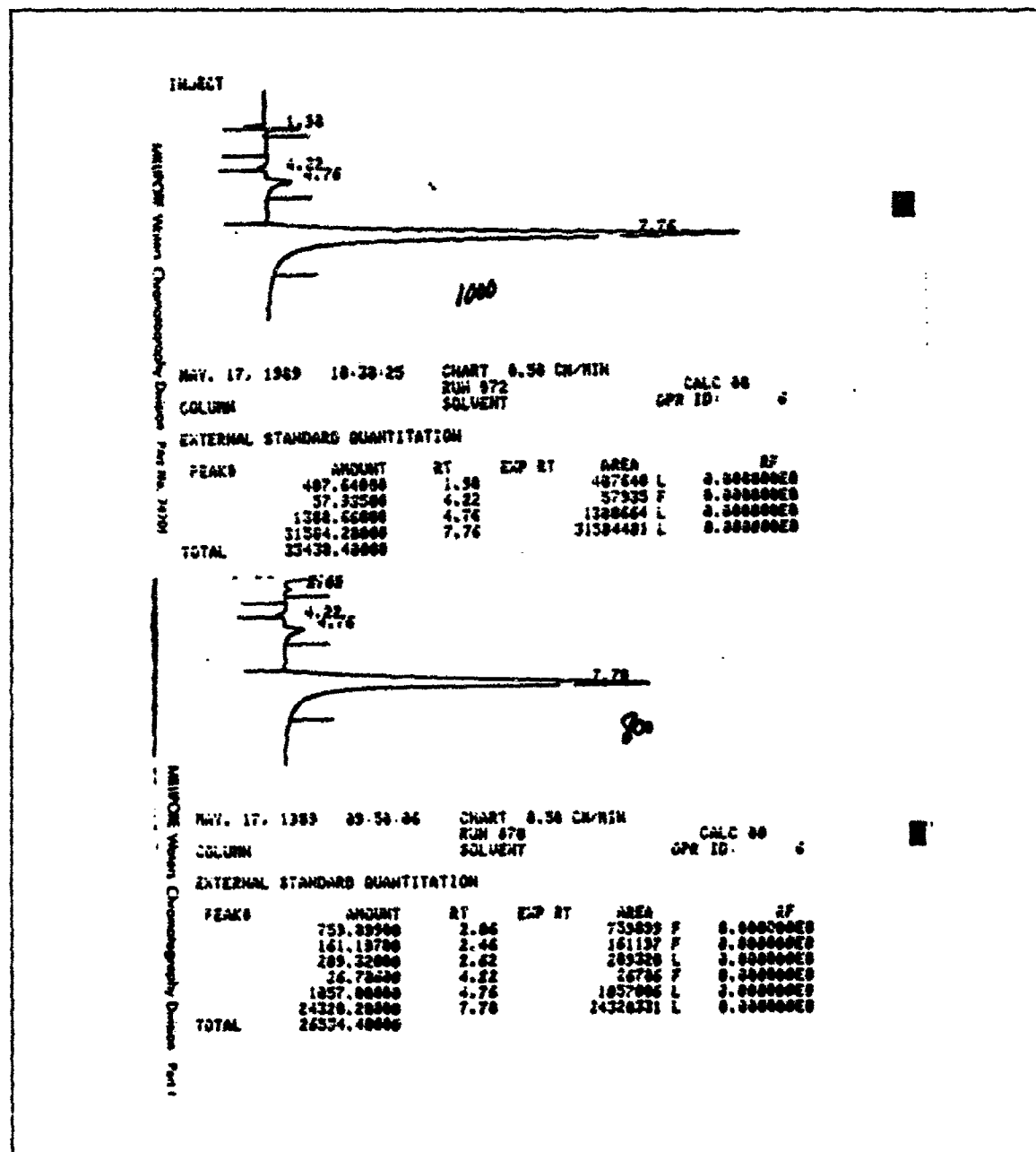


Figure B2. Chromatograms of Clindamycin Standard Solutions.

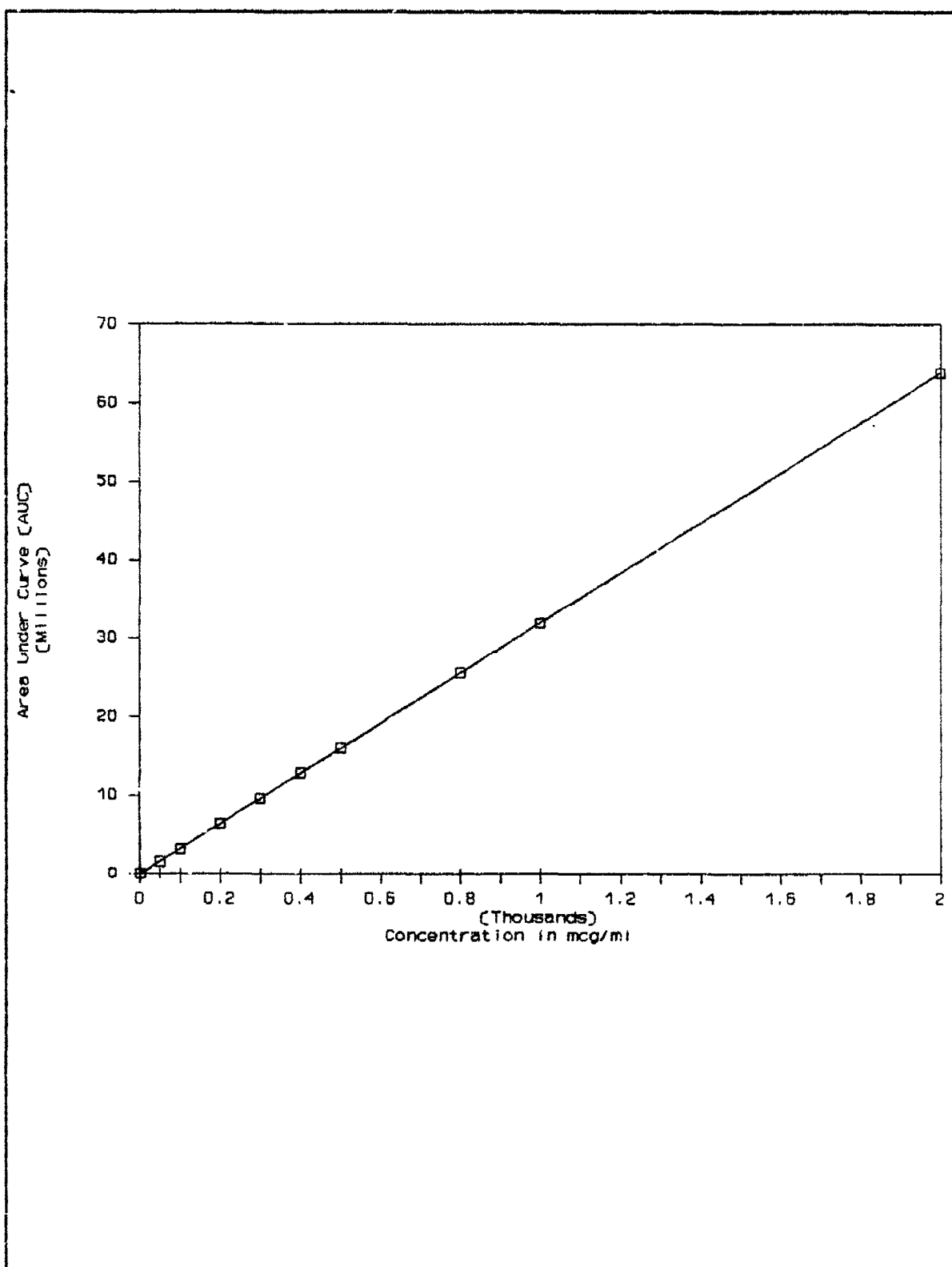


Figure B3. Calibration Curve for Clindamycin Phosphate.

C. HPLC Method for the Analysis of Chlorhexidine Gluconate In Vitro using Ultraviolet Detection.

This method is a modification of the work reported by Huston et al¹⁰. We chose to change the solvent system to a more polar one by reducing the percent methanol in the mobile phase. This was done to reduce the chance of precipitating water soluble components. The method was found to be linear and precise and can be used for determining sample concentrations as low as fifty micrograms per liter. The chromatographic conditions used for the analysis are outlined below.

Materials

The mobile phase consisted of a 70/30 v/v proportion of methanol: water, an apparent pH = 4 (adjusted with glacial acetic acid), 0.005 M heptane sulphonic acid sodium salt. Chromatography was performed on an Altech RS11 250 mm x 4.6 mm 10 μ C8 column. The flow rate was adjusted to 1.5 ml/min using a Waters Solvent Delivery Module (Model 510). One microliter (1 μ l) injections of the sample were introduced through a Waters U6K injector and the sample quantified by means of a Waters 484 Tunable Absorbance UV Detector, connected to a Waters M730 Data Module. The determination of chlorhexidine gluconate (Figure C1) was performed at 238 nm.

Chlorhexidine Gluconate

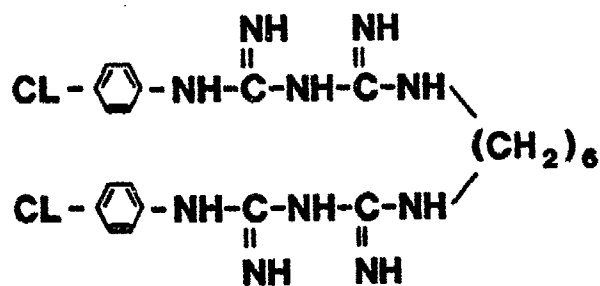
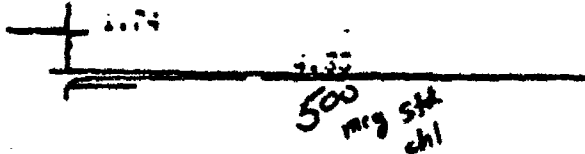


Figure C1. Structure of Chlorhexidine Gluconate

Method

The HPLC method used for quantitation of chlorhexidine gluconate is a modification of the methods used by Huston et al. This method uses a C8 column and is useful in determining drug solutions with concentrations of 50 mcg/ml and above. Example chromatograms for 500 and 1000 mcg/ml of chlorhexidine gluconate are shown in figure C2. Chlorhexidine gluconate standard solutions were prepared and used to generate a standard calibration curve, plotting concentration vs area shown in figure C3.

INJECT



AUG. 30, 1965 12:56:04

CHART 8.25 CM/MIN

COLUMN

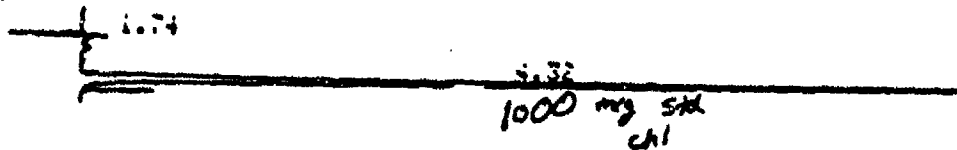
RUN 857
SOLVENT

CALC 80
CPR 10

INTERNAL STANDARD QUANTITATION

PEAKS	AMOUNT	RT	EXP RT	AREA	RF
TOTAL	2137.34000	4.35		2137955 L	0.00000000

INJECT



AUG. 30, 1965 13:25:12

CHART 8.25 CM/MIN

COLUMN

RUN 860
SOLVENT

CALC 80
CPR 10

INTERNAL STANDARD QUANTITATION

PEAKS	AMOUNT	RT	EXP RT	AREA	RF
TOTAL	4006.62000	4.32		4006633 L	0.00000000

Figure C2. Typical Chromatograms for Chlorhexidine Gluconate

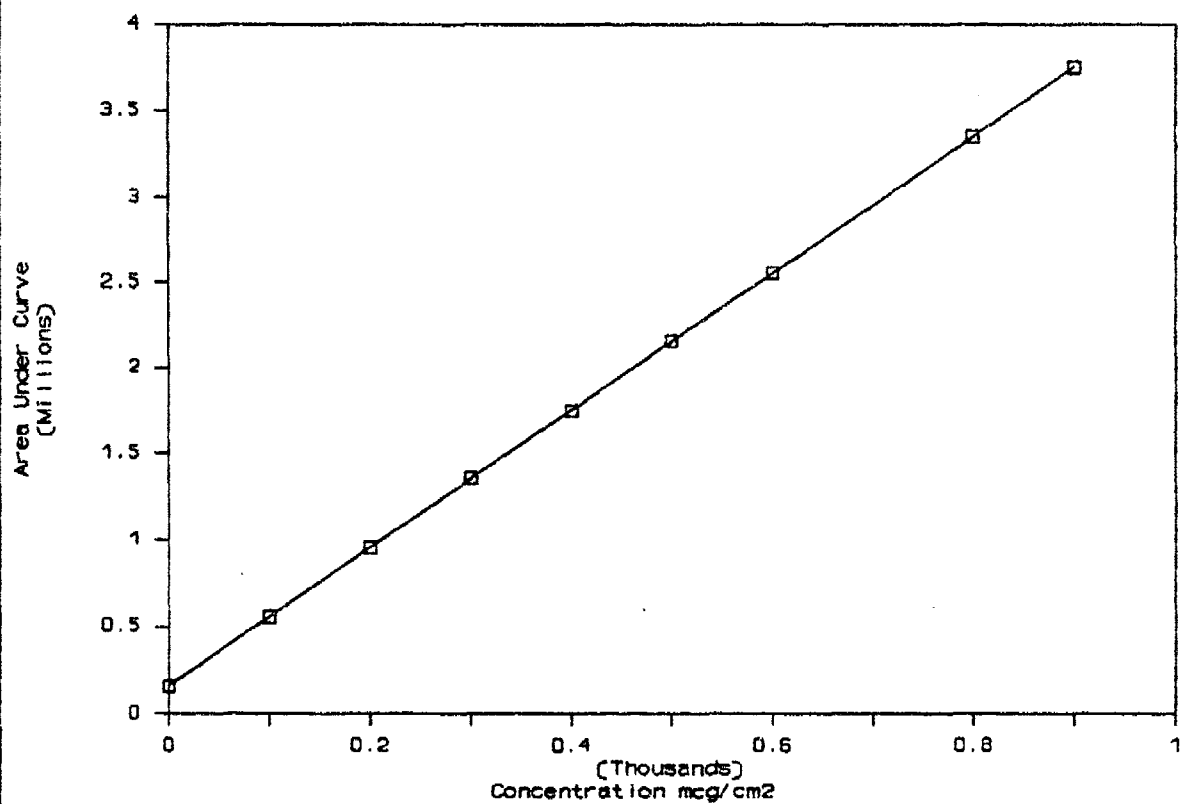


Figure C3. Calibration Curve for Chlorhexidine Gluconate

Appendix References

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APPENDIX III

DATA SHEETS

ELUTION RATE WORKSHEET FOR GENTAMICIN SULFATE "A"

TITLE : Formulation 1 - Matrix 40% Drug (17:30 C:G) 47% PEG 13% - Hand Mixed

STANDARD CALIBRATION CURVE

mcg/ml	AUC	AUC	AVG AUC	Hr.	Data of Average Values			
					dil adj			
					mcg/ml	mcg/ml	mcg/ca2	dif u/ca2
0	0	0	0	0.0	0.0	0.0	0.0	0.0
50	24896		24896	1	791.4	791.4	2239.7	2239.7
100	48002		48002	2	1057.8	1077.6	3049.7	810.0
200	85077		85077	4	1195.9	1222.4	3459.3	409.7
300	144632		144632	8	1340.8	1370.7	3879.0	419.6
400	161509		161509	24	1271.8	1305.3	3694.0	-185.0
500	211635		211635					
800	366011		366011					
1000	456087		456087					
1500	626136		626136					
2000	821954		821954					
3000	1272870		1272870					

Regression Output:

Constant 7300.993
 Std Err of Y Est 16101.25
 R Squared 0.998427
 No. of Observations 12
 Degrees of Freedom 10

X Coefficient(s) 419.4108
 Std Err of Coef. 5.263621

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
1	302441	312866	357215	392447	332549	337832	339225	29582.06
2	376003	380543	504953	533608	469245	441484	450972.6	58803.59
4	418421	403788	578588	609386	480254	562902	508889.8	79504.34
8	500997	486956	636404	690656	565203	537575	569631.8	72685.00
24	460075	450523	577186	628397	548815	579216	540702	64812.04

Formulation Wt. %
 Clindamycin 17
 Gentamicin 30
 PEG 300 13
 Oligomer 40

Date: 01/05/90
 File: HPM04K.WK1

ELUTION RATE WORKSHEET FOR GENTAMICIN SULFATE "A"

TITLE : Formulation 2 - Matrix 40% Drug (17:30 C:6) 47% PEG 13% - Machine Mixed

STANDARD CALIBRATION CURVE

mcg/ml	AUC	AUC	AVGAUC	Hr.	Data of Average Values			
					dil adj			
					mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
0	0	0	0	0.0	0.0	0.0	0.0	0.0
50	24896		24896	1	939.7	939.7	2659.2	2659.2
100	48002		48002	2	1041.2	1064.7	3013.0	353.8
200	85077		85077	4	1139.3	1165.3	3297.8	284.8
300	144632		144632	8	1080.3	1108.8	3137.8	-160.0
400	161509		161509	24	1131.0	1158.0	3277.2	139.4
500	211635		211635					
800	366011		366011					
1000	456087		456087					
1500	626136		626136					
2000	821954		821954					
3000	1272870		1272870					

Regression Output:

Constant 7300.993
 Std Err of Y Est 16101.25
 R Squared 0.998427
 No. of Observations 12
 Degrees of Freedom 10

X Coefficient(s) 419.4108
 Std Err of Coef. 5.263621

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
1	470698	464226	422984	413081	317731	319692	401402	61952.04
2	470277	461709	480688	503817	378789	368609	443981.5	51420.56
4	537886	517631	575145	514647	388776	376636	485120.1	75131.55
8	495699	488770	496319	485495	388937	407050	460378.3	44579.49
24	524583	525354	523780	507185	407946	401125	481662.1	54919.34

Formulation Wt. %
 Clindamycin 17
 Gentamicin 30
 PEG 300 13
 Oligomer 40

Date: 01/05/90
 File MMOWS.wk1

ELUTION RATE WORKSHEET FOR GENTAMICIN SULFATE "A"

TITLE : Formulation 3 - Matrix 40% Drug (17:30 C:6) 47% PEG 13% - Machine Mixed Textured

STANDARD CALIBRATION CURVE

mcg/ml	AUC	AUC	AVGAUC	Hr.	Data of Average Values			
					dil adj			
					mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
0	0	0	0	0.0	0.0	0.0	0.0	0.0
50	24896		24896	1	1134.1	1134.1	3209.4	3209.4
100	48002		48002	2	1375.4	1403.8	3972.7	763.3
200	85077		85077	4	1578.3	1612.7	4563.9	591.2
300	144632		144632	8	1624.0	1663.4	4707.5	143.5
400	161509		161509	24	1573.7	1614.3	4568.5	-138.9
500	211635		211635					
800	366011		366011					
1000	456087		456087					
1500	626136		626136					
2000	821954		821954					
3000	1272870		1272870					

Regression Output:

Constant 7300.993
 Std Err of Y Est 16101.25
 R Squared 0.998427
 No. of Observations 12
 Degrees of Freedom 10

X Coefficient(s) 419.4108
 Std Err of Coef. 5.263621

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
1	488574	443926	599054	601783	387764	376534	482939.1	90866.80
2	488574	589478	740572	783426	423247	479731	584171.3	135483.7
4	651883	652274	854632	905317	497104	454367	669262.8	166579.7
8	662455	663619	911464	928431	497953	466506	688404.6	179891.9
24	575691	585778	930850	895962	488724	527022	667337.8	177177.0

Formulation Wt. %
 Clindamycin 17
 Gentamicin 30
 PEG 300 13
 Oligomer 40

Date: 01/05/90
 File MHTWQWS.WK1

ELUTION RATE WORKSHEET FOR GENTAMICIN SULFATE "A"

TITLE : Formulation 5 - Matrix 40% Drug (17:30 C:G) 47% PEG 13% - Barrier Coat

STANDARD CALIBRATION CURVE

mcg/ml	AUC	AUC	AVG AUC	Hr.	Data of Average Values			
					dil adj			
0	0	0	0		mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
200	93613	85077	89345	0.0	0.0	0.0	0.0	0.0
300	139516	144632	142074	0.5	317.7	317.7	899.1	899.1
400	169893	161509	165701	1	457.1	465.1	1316.2	417.1
500	205079	218190	211635	2	660.0	671.4	1900.1	583.9
800	389628	317477	353553	4	778.0	794.5	2248.3	348.2
1000	473226	438949	456088	8	817.2	836.7	2367.7	119.4
1500	632447	619825	626136	24	990.5	1010.9	2861.0	493.2
2000	820956	814522	817739	48	978.4	1003.2	2839.0	-22.0

Regression Output:

Constant 11923.48
 Std Err of Y Est 16429.89
 R Squared 0.996763
 No. of Observations 9
 Degrees of Freedom 7

X Coefficient(s) 411.1877
 Std Err of Coef. 8.855547

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	119414	114366	127472	122574	171224	200320	142561.6	31925.87
1	190542	188052	182291	177469	225002	236021	199896.1	22271.09
2	292373	266887	269500	251058	314771	305238	283304.5	22558.78
4	330400	319567	318511	298018	363867	360508	331811.8	23529.65
8	366056	331631	338808	306706	380100	364396	347949.5	24775.23
24	387071	389742	386455	375254	470863	505861	419207.6	50137.68
48	406477	410703	394554	381138	448297	444226	414232.5	24546.19

Formulation Wt.% Barrier Coat
 Clindamycin 17 0
 Gentamicin 30 0
 PEG 300 13 13
 Oligomer 40 40

Date: 01/05/90
 File TCHMDWS.WK1

ELUTION RATE WORKSHEET FOR GENTAMICIN SULFATE "A"

TITLE : Formulation 4 - Matrix 52% Drug (17:30 C:G) 47% PEG 1% - Machine Mixed

STANDARD CALIBRATION CURVE

mcg/ml	AUC	AUC	AVGAUC	Hr.	Data of Average Values			
					dil adj			
					mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
0	0	0	0					
50	55507	51033	53270	0.0	0.0	0.0	0.0	0.0
100	99927	98661	99294	0.5	48.2	48.2	136.3	136.3
200	188455	187100	187778	1	117.7	118.9	336.5	200.2
300	288826	286636	287731	2	271.8	274.7	777.5	441.0
400	364824	324044	344434	4	333.8	340.6	963.8	186.3
500	474313	438364	456339	8	448.4	456.8	1292.6	328.8
800	789422	774537	781980	24	559.6	570.8	1615.4	322.8
1000	902733	862094	882414	48	627.5	641.4	1815.3	199.9
1500	1284640	1265390	1275015	72	737.7	753.4	2132.1	316.8
2000	1648090	1592540	1620315					

Regression Output:

Constant 32388.68
 Std Err of Y Est 40527.03
 R Squared 0.994792
 No. of Observations 11
 Degrees of Freedom 9

X Coefficient(s) 822.2324
 Std Err of Coef. 19.82996

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	69778	78237	66443	73953	70167	73350	71988	3735.269
1	109555	101428	156685	151560	122735	133081	129174	20287.34
2	254797	244364	238807	238707	282702	275840	255869.5	17502.43
4	285362	263534	333738	309130	297737	351421	306820.3	29256.87
8	369017	350095	458842	388766	438755	401044	401086.5	37716.03
24	443379	468896	462866	456571	590356	532968	492506	52205.59
48	586556	527540	502015	558508	522656	592539	548302.3	33555.19
72	682918	649083	584799	600280	632450	684144	638945.6	37745.44

Formulation Wt. %
 Clindamycin 17
 Gentamicin 30
 PEG 300 1
 Oligomer 40

Date: 01/05/90
 File MH1WDMS.WK1

ELUTION RATE WORKSHEET FOR GENTAMICIN SULFATE "A"

TITLE : Formulation 6 - Matrix 47% Drug (17:30 C:G) 47% PEG 6% - Machine Mixed

STANDARD CALIBRATION CURVE

Baseline m.v. =	0.67		
mcg/ml	AUC	AUC	AVGAUC
0	0	0	0
100	9934	9244	9589
400	32798	32594	32696
800	73340	72652	72996
1000	87987	88529	88258
1500	149876	149693	149785

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	203.8	203.8	576.8	576.8
1	348.4	353.4	1000.3	423.5
2	593.4	602.1	1703.9	703.6
4	674.5	689.3	1950.7	246.8
8	782.4	799.3	2262.0	311.3
24	984.6	1004.1	2841.7	579.7
48	1001.3	1025.9	2903.4	61.8
72	1030.8	1055.9	2988.1	84.7

Regression Output:

Constant	-3014.94
Std Err of Y Est	5325.554
R Squared	0.992868
No. of Observations	6
Degrees of Freedom	4

X Coefficient(s) 97.74030

Std Err of Coef. 4.141756

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	19191		14618	16904.5	2286.5
1	32300		29766	31033	1267
2	67990		41973	54981.5	13008.5
4	74723		51091	62907	11816
8	78549		68371	73460	5089
24	103718		82714	93216	10502
48	107254		82457	94855.5	12398.5
72	110477		85004	97740.5	12736.5

Formulation	Wt. %
Clindamycin	17
Gentamicin	30
PEG 300	6
Oligomer	47

Date: 01/05/90
File: HPM6WDWS.WK1

ELUTION RATE WORKSHEET FOR GENTAMICIN SULFATE "A"

TITLE : Formulation 1A - Matrix 40% Drug (20:27 C:G) 47% PEG 13% - Hand Mixed (Control)

STANDARD CALIBRATION CURVE

Baseline m.v. = 0.96				Data of Average Values			
mcg/ml	AUC	AUC	AVGAUC	dil adj			
0	0	0	0	Hr.	mcg/ml	mcg/ml	mcg/cm2
50	38588	43035	40812	0.0	0.0	0.0	0.0
100	86936	74426	80681	0.5	115.2	115.2	326.1
200	170295	144271	157283	1	179.2	182.1	515.4
300	217049	218719	217884	2	273.1	277.6	785.6
400	289120	298956	294038	4	280.3	287.1	812.5
500	367067	351491	359279	8	304.9	311.9	882.7
800	667458	650916	659187	24	345.2	352.8	998.5
				48	387.0	395.7	1119.7
				72	377.9	387.6	1096.9
							-22.8

Regression Output:

Constant -7885.31
 Std Err of Y Est 20677.25
 R Squared 0.992016
 No. of Observations 8
 Degrees of Freedom 6

X Coefficient(s) 796.7004
 Std Err of Coef. 29.17830

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	78442	78707	89554	82890	79918
1	131952	132557	151293	132882	135131
2	194669	157759	257361	245649	217273
4	193935	206452	240910	243093	207129
8	228913	234225	284366	248748	214662
24	270946	236076	337358	295622	236627
48	302877	282992	374233	356752	245228
72	300247	280009	350572	354323	238002

Formulation	Wt. %	Date:	01/08/90
Clindamycin	20	File	CONTRILUS.WK1
Gentamicin	27		
PEG 300	13		
Oligomer	40		

ELUTION RATE WORKSHEET FOR CLINDAMYCIN PHOSPHATE "A"

TITLE : Formulation 1A - Matrix 40% Drug (20:27 C:G) 47% PEG 13% - Hand Mixed (Control)

STANDARD CALIBRATION CURVE

= 194 nm				Data of Average Values			
mcg/ml	AUC	AUC	AVGAUC		dil adj		
0	0	0	0	Hr.	mcg/ml	mcg/ml	mcg/cm2 dif u/cm2
50	1148997	1006489	1077743	0.0	0.0	0.0	0.0
100	2544774	2603734	2574254	0.5	113.5	113.5	321.3 321.3
200	5544018	5308287	5426153	1	120.0	122.9	347.7 26.3
300	8630856	9289376	8960116	2	210.2	213.2	603.4 255.7
400	11744341	11240270	11492706	4	335.2	340.5	963.6 360.2
500	14353331	14589522	14471427	8	434.4	442.7	1253.0 289.4
800	24082994	24204230	24143612	24	686.3	697.2	1973.1 720.1
1000	29085733	29995557	29540645	48	702.1	719.2	2035.4 62.3

Regression Output:

Constant -330386.
 Std Err of Y Est 287201.4
 R Squared 0.999324
 No. of Observations 9
 Degrees of Freedom 7

X Coefficient(s) 30047.68
 Std Err of Coef. 295.3541

HR.	A cell		B cell		C cell		AVG.	STD.
0	0	0	0	0	0	0	0	0
0.5	2902175	2922789	2400592	2241820	3961771	4058836	3081330	701914.9
1	3246804	3298448	3005012	3097991	3532118	3473870	3275707	187628.3
2	6055210	6040659	6761836	5866296	5736043	5457017	5986176	401316.4
4	7713557	8253399	9048754	9733692	12138739	11566626	9742461	1627103.
8	11824111	12631820	15442572	13847193	10959334	11622334	12721227	1516474.
24	20554768	20030271					20292519	262248.5
48					21363961	20165401	20764681	599280

Formulation	Wt. %	Date:	01/08/90
Clindamycin	20	File	CONTRLWS.WK1
Gentamicin	27		
PEG 300	13		
Oligomer	40		

ELUTION RATE WORKSHEET FOR CLINDAMYCIN PHOSPHATE "A"

TITLE : Formulation 4 - Matrix 52% Drug (17:30 C:G) 47% PEG 1% - Machine Mixed

STANDARD CALIBRATION CURVE

= 194 nm				Data of Average Values				
mcg/ml	AUC	AUC	AVGAUC	Hr.	dil adj			
0	0	0	0		mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
50	1070975	1008280	1039628	0.0	0.0	0.0	0.0	0.0
100	2553967	2483211	2518589	0.5	19.5	19.5	55.2	55.2
200	6390241	6317663	6353952	1	30.0	30.5	86.2	31.0
300	9020223	9399093	9209658	2	20.9	21.6	61.1	-25.1
400	12653510	12887853	12770682	4	70.8	71.3	201.7	140.6
500	15673513	15551133	15612323	8	90.1	91.9	260.0	58.3
800	26065418	25514544	25789981	24	159.6	161.8	457.9	198.0
				48	292.4	296.4	838.8	380.9
				72	391.7	399.0	1129.1	290.3

Regression Output:

Constant -404035.
 Std Err of Y Est 290459.7
 R Sq. ad 0.999050
 No. of Observations 8
 Degrees of Freedom 6

X Coefficient(s) 32564.72
 Std Err of Coef. 409.8764

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	265634	273809	241985	183322	231676.1
1	555689	555689	851233	299428	572546.5
2	203006	203006		347270	275138
4		2704669	2911848	1075513	1900182.
8	2397293	2295985	3135161	1962048	2529910
24	3632907	3578147	5617802	5190922	4792033.
48	6838044	7138016	10956186	9760751	9118410.
72	9240678	8986557	14489059	14738679	12350528

Formulation	Wt. %	Date:	01/08/90
Clindamycin	17	File	MM1MDMS.WK1
Gentamicin	30		
PEG 300	1		
Oligomer	52		

ELUTION RATE WORKSHEET FOR CHLORHEXIDINE GLUCONATE "A"

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

STANDARD CALIBRATION CURVE

= 238 nm

Data of Average Values

dil adj

mcg/ml	AUC	AUC	AVGAUC	Hr.	mcg/ml	mcg/ml	mcg/cm2	dif 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 160604.9
 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. 157.4463

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
1	818991	798989	781430	802816	645912
4	1231903		1210578	1194239	1212240
8	1105076		1220024	1069403	1131501
24	1620899		1307438	1509503	1479280
48	1731935		1191389	1324270	1415864
72	1569226		1199845	1354928	1374666

Formulation Wt.%
 Chlorhexidine 30
 Propylene glycol 30
 Oligomer 40

Date: 01/04/90
 File CHG3030.WK1

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

Data of Average Values

dil adj

mcg/ml	AUC	AUC	AVGAUC	Hr.	mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
0	0	0	0	0.0	0.0	0.0	0.0	0.0
100	651553	635565	643559	0.5	382.4	382.4	1082.1	1082.1
500	2514236	2497240	2505738	1	469.1	478.7	1354.7	272.6
1000	5739827	5778169	5758998	2	542.2	553.9	1567.6	212.9
2000	11708183	11925306	11816745	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	-146.3

Regression Output:

Constant -115536.
 Std Err of Y Est 235621.5
 R Squared 0.998220
 No. of Observations 5
 Degrees of Freedom 3

X Coefficient(s) 5917.422
 Std Err of Coef. 144.2520

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	1973020	2799643	1668525	2147062.	477894.7
1	2620418	2870018	2490857	2660431	157356.3
2	3299509	3454345	2524517	3092790.	406771.4
4	4385335	3985082	2980627	3783681.	590887.9
8	5521057	6586390	3640809	5249418.	1217771.
24	6885551	8071294	5941804	6966216.	871229.8
48	7539842	10411254	6519321	8156805.	1647678.
72	7648551	8775144	7026920	7816871.	723565.5

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

Date: 01/04/90
 File CH630624.WK1

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

				Data of Average Values				
				dil adj				
mcg/ml	AUC	AUC	AVGAUC	Hr.	mcg/ml	mcg/ml	mcg/cm2	dif u/cm2
0	0	0	0	0.0	0.0	0.0	0.0	0.0
100	651553	635565	643559	0.5	298.2	298.2	843.8	843.8
500	2514236	2497240	2505738	1	409.0	416.4	1178.5	334.7
1000	5739827	5778169	5758998	2	561.7	571.9	1618.6	440.0
2000	11708183	11925306	11816745	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 0.998220
 No. of Observations 5
 Degrees of Freedom 3

X Coefficient(s) 5917.422
 Std Err of Coef. 144.2520

HR.	A cell	B cell	C cell	AVG.	STD.
0	0	0	0	0	0
0.5	1577230	1770000	1599073	1648767.	86186.76
1	2133259	2495282	2285272	2304604.	148426.1
2	2678531	4144504	2801945	3208326.	663891.9
4	5226736	7641817	4427834	5765462.	1366282.
8	9556287	11281685	6767740	9201904	1859769.
24	14175602	17875577	12858062	14969747	2123968.
48	16012158	17583839	14447817	16014604	1280276.
72	15717876	14444287	14892344	15018169	527497.9

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

Date: 01/04/90
 File CHT30624.WK1

APPENDIX IV

STATISTICAL ANALYSIS

Appendix IV is designed to provide supplementary statistical analyses in support of data outlined on page 29. The percent of drug eluted at the wound site was tabulated and reported on that page. Statistical analyses using these data were performed to define the differences in drug elution, if any, between each of the sets of dressings. The variables used for these mathematical analyses are defined as follows:

VAR1 % Gentamicin eluted at wound from hand mixed 20/27/13 ADD
VAR2 % Gentamicin eluted at wound from machine mixed 20/27/13 ADD
VAR3 % Gentamicin eluted at wound from machine mixed 20/27/1 ADD
VAR4 % Clindamycin eluted at wound from hand mixed 20/27/13 ADD
VAR5 % Clindamycin eluted at wound from machine mixed 20/27/13 ADD
VAR6 % Clindamycin eluted at wound from machine mixed 20/27/1 ADD

The results of these analyses indicate no statistical differences in drug elution between samples having test hypotheses that are not rejected. VAR1 compared with VAR2 as well as VAR4 compared to VAR5 do not show statistical differences in their release rates. Comparison of samples resulting in rejected test hypotheses indicate statistical differences in their release rates. The following comparisons show differences in their release rates:

VAR1 with VAR3, VAR2 with VAR3, VAR4 with VAR6, and VAR5 with VAR6.

Two-Sample Analysis Results

Sample Statistics:	ARMY.VAR1	ARMY.VAR2	Pooled
Number of Obs.	5	4	9
Average	81.38	87.85	84.2556
Variance	27.767	2.32667	16.8683
Std. Deviation	5.26944	1.52862	4.1071
Median	83.1	87.75	86.2

Conf. Interval For Diff. in Means:	95	Percent	
(Equal Vars.) Sample 1 - Sample 2	-12.9867	0.0467287	7 D.F.
(Unequal Vars.) Sample 1 - Sample 2	-12.9149	-0.025092	4.8 D.F.

Conf. Interval for Ratio of Variances:	0	Percent
Sample 1 + Sample 2		

Hypothesis Test for H_0 : Diff = 0
vs Alt: NE
at Alpha = 0.05

Computed t statistic = -2.34835
Sig. Level = 0.051212
so do not reject H_0 .

File A:ARMY 9/ 6/89

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row	VAR1	VAR2	VAR3	VAR4	VAR5	VAR6
1	72.7	89.7	29.6	78.0	67.2	92.3
2	86.3		8.1	87.1	74.4	95.5
3	80.6	88.4	17.1	82.2	82.0	90.8
4	83.1	87.1	21.8	74.0	75.4	92.3
5	84.2	86.2	48.1		79.4	

Sample Statistics:	Number of Obs.	ARMY.VAR2	ARMY.VAR3	Pooled
Average		87.83	24.94	52.9
Variance		2.33667	228.253	131.432
Std. Deviation		1.52862	15.108	11.4644
Median		87.75	21.8	48.1
Conf. Interval For Diff. in Means:		95	Percent	
(Equal Vars.) Sample 1 - Sample 2		44.7195	81.1005	7 D.F.
(Unequal Vars.) Sample 1 - Sample 2		44.2086	81.6114	4.1 D.F.
Conf. Interval for Ratio of Variances:		0	Percent	
Sample 1 + Sample 2				
Hypothesis Test for H0: Diff = 0		Computed t statistic = 8.18019		
vs Alt: NE		Sig. Level = 7.90438E-5		
at Alpha = 0.05		so reject H0.		

	ARMY.VAR1	ARMY.VAR3	Pooled
Sample Statistics: Number of Obs.	5	5	10
Average	81.38	24.94	53.16
Variance	27.767	228.253	128.01
Std. Deviation	5.26944	15.108	11.3142
Median	83.1	21.8	60.4
Conf. Interval For Diff. in Means:	95	Percent	
(Equal Vars.) Sample 1 - Sample 2	39.9343	72.9457	8 D.F.
(Unequal Vars.) Sample 1 - Sample 2	37.9939	74.8861	5.0 D.F.
Conf. Interval for Ratio of Variances:	0	Percent	
Sample 1 + Sample 2			
Hypothesis Test for H0: Diff = 0	Computed t statistic = 7.88742		
vs Alt: NE	Sig. Level = 4.83514E-5		
at Alpha = 0.05	so reject H0.		

Two-Sample Analysis Results

	ARMY.VAR4	ARMY.VAR5	Pooled
Sample Statistics: Number of Obs.	4	5	9
Average	80.325	75.68	77.7444
Variance	31.6092	31.852	31.7479
Std. Deviation	5.6222	5.64376	5.63453
Median	80.1	75.4	78

Conf. Interval For Diff. in Means:	95	Percent	
(Equal Vars.) Sample 1 - Sample 2	-4.2953	13.5853	7 D.F.
(Unequal Vars.) Sample 1 - Sample 2	-4.40806	13.6981	6.6 D.F.

Conf. Interval for Ratio of Variances:	0	Percent
Sample 1 + Sample 2		

Hypothesis Test for H0: Diff = 0 Computed t statistic = 1.22891
vs Alt: NE Sig. Level = 0.258809
at Alpha = 0.05 so do not reject H0.

Two-Sample Analysis Results

	ARMY.VAR4	ARMY.VAR6	Pooled
Sample Statistics: Number of Obs.	4	4	8
Average	80.325	92.725	86.525
Variance	31.6092	3.9225	17.7658
Std. Deviation	5.6222	1.98053	4.21495
Median	80.1	92.3	88.95

Conf. Interval For Diff. in Means:	95	Percent	
(Equal Vars.) Sample 1 - Sample 2	-19.695	-5.10496	6 D.F.
(Unequal Vars.) Sample 1 - Sample 2	-20.9135	-3.88649	3.7 D.F.

Conf. Interval for Ratio of Variances:	0	Percent
Sample 1 + Sample 2		

Hypothesis Test for H0: Diff = 0 Computed t statistic = -4.16048
vs Alt: NE Sig. Level = 5.94119E-3
at Alpha = 0.05 so reject H0.

Two-Sample Analysis Results

	ARMY.VARS	ARMY.VARS	Pooled
Sample Statistics: Number of Obs.	5	4	9
Average	75.68	92.725	83.2556
Variance	31.852	3.9225	19.8822
Std. Deviation	5.64376	1.98053	4.45895
Median	75.4	92.3	82

Conf. Interval For Diff. in Means: 95 Percent
 (Equal Vars.) Sample 1 - Sample 2 -24.12 -9.97 7 D.F.
 (Unequal Vars.) Sample 1 - Sample 2 -23.951 -10.139 5.2 D.F.

Conf. Interval for Ratio of Variances: 0 Percent
 Sample 1 + Sample 2

Hypothesis Test for H_0 : Diff = 0 Computed t statistic = -3.69847
 vs Alt: NE Sig. Level = 7.36513E-4
 at Alpha = 0.05 so reject H_0 .

APPENDIX V

TABLE OF DELIVERIES

Table of Deliveries

Year 2

No.	Formulation	Delivered	Date
1	Hnd Mxd 20/27/13 Dual Antibiotic	10	Feb. '89
2	M/c Mxd 20/27/13 Dual Antibiotic	10	
3	M/c Mxd 17/30/1 Dual Antibiotic	10	
4	Placebos	10	
5	Textured 17/30/1 Dual Antibiotic	10	Apr. '89
6	Placebos	10	
7	M/c Mxd 20/27/13 Dual Antibiotic	10	May. '89
8	Placebos (2.5" x 2.5")	10	
9	Chlorhexidine gluconate	20	Jun. '89
10	Placebos	10	
11	Adhesive dressings	125	