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The main objective of this project was to develop, prepare, and deliver 25 g of vancomycin microcapsules, 25 g of placebo microspheres, and 5 g of vancomycin, USP to USAIDR.

In vitro release studies, in addition to antibiotic microbial assay, demonstrated that the BIOTEK vancomycin microcapsules met all the criteria stipulated in the contract. The microcapsules maintained a sustained release of therapeutic levels of vancomycin and inhibited bacterial growth of Staphylococcus aureus for 19 days. The preparation of these vancomycin microcapsules required no methylene chloride and the release of vancomycin from the microcapsules was not adversely affected by sterilization with gamma irradiation.

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# I. SUMMARY

The main objective of this project was to develop, prepare, and deliver 25 g of vancomycin microcapsules, 25 g of placebo microspheres, and 5 g of vancomycin, USP to USAIDR.

In vitro release studies, in addition to antibiotic microbial assay, demonstrated that the BIOTEK vancomycin microcapsules met all the criteria stipulated in the contract (see **Objectives** below). The microcapsules maintained a sustained release of therapeutic levels of vancomycin and inhibited bacterial growth of Staphylococcus aureus for 19 days. The preparation of these vancomycin microcapsules required no methylene chloride and the release of vancomycin from the microcapsules was not adversely affected by sterilization with gamma irradiation.

# II. INTRODUCTION

## A. Objectives

The overall objective of the project is to develop a microcapsule with at least a 10% loading level of vancomycin (w/w) which, after an initial burst of drug, will release the drug steadily for 14-21 days.

The specific objectives are:

1. To prepare several batches of vancomycin microcapsules from 50:50 Poly-DL-lactide-co-glycolide from Birmingham Polymers, Inc. using BIOTEK's proprietary composite microencapsulation technology which is based on the use of calcium sulfate, triglyceride, and PLA-PGA polymer.

2. To determine the properties of the microcapsules by measurement of vancomycin loading, size distribution, vancomycin release in vitro, surface morphology by SEM, and residual solvent.

3. To determine if the microcapsules prepared in 1 above meet the following criteria:

| | | |
|---|---|---|
| a. | Vancomycin Loading: | As high as possible, preferably above 10% |
| b. | Microsphere Size: | 50-200 microns |
| c. | In Vitro Release: | Initial burst of 10-20% on day one.<br>Sustained release of balance over a period of 14-21 days. |
| d. | Residual Solvent: | As low as possible, with Certificate of Analysis of Solvent Content |

4. To sterilize the vancomycin microcapsules by exposure to gamma radiation.

5. To supply USAIDR with the following: 5 grams of vancomycin, 25 grams of placebo polymer microspheres, and 25 grams of vancomycin microcapsules.

6. To submit at the termination of the contract a written final report describing the technical approach and methods used to formulate the vancomycin and placebo microspheres. In addition, the report shall describe all analytical methods used to characterize the vancomycin microspheres including:

   a. vancomycin core-loading;

   b. size range of the vancomycin and placebo microspheres;

   c. methods and conditions used to sterilize the vancomycin and placebo microspheres;

   d. technique used to determine the in vitro release kinetic profile of the vancomycin microspheres;

   e. results of scanning electron microscopy to assess the surface morphology of the vancomycin and placebo microspheres; and

   f. methods and conditions used to reduce the levels of residual solvents in the vancomycin and placebo microspheres.

## III. METHODS

### A. Materials

#### 1. Vancomycin

Two hundred grams of vancomycin hydrochloride USP Special (Lot No. 1232OID00) were purchased from Abbott Laboratories (No. Chicago, IL).

#### 2. Witepsol E-85

Witepsol E-85, a triglyceride, was procured from Hüls A.G. (Germany).

#### 3. Polymer

Two PLA:PGA polymers were purchased from Birmingham Polymers, Inc. (Birmingham, AL). The first was a 50:50 PDLLA-PGA (poly-D,L-lactide-co-glycolide) with an intrinsic viscosity of 0.59

dl/g Lot No. 95041. The second was a 65:35 PLLA-PGA (poly-L-lactide-co-glycolide) with an intrinsic viscosity of 1.04 dl/g, Lot No. D95078. The 50:50 polymer could not be used for coating of the microcapsules because it was too tacky, causing the microcapsules to agglomerate. Microcapsules coated with the 65:35 polymer flowed well without agglomeration.

## B. Microparticles

### 1. Preparation of Vancomycin Microsphere Core

Two methods were used to prepare vancomycin microsphere cores: hydroforming with calcium sulfate and polymer casting.

* A proprietary method for hydroforming the drug with calcium sulfate, previously developed by
* BIOTEK, was used in preparing vancomycin calcium sulfate microsphere core. The method involves
* preparing oil in water microspheres from an aqueous solution of calcium sulfate and vancomycin.
* Calcium sulfate has a unique property of rapidly hardening upon contact with water to form plaster of
* Paris. The method consists of dissolving the drug in distilled water and adding the solution to a
* carefully weighed quantity of calcium sulfate to form a "slurry". This slurry is then poured into
* vegetable oil and stirred at high speed until the calcium sulfate microspheres harden entrapping the drug.
* The microspheres are next washed to remove excess vegetable oil, and then sieved and air dried.

The polymer cast core was prepared by mixing vancomycin with 65:35 PLLA/PGA polymer in a ceramic mortar with enough acetone to form a paste. After hardening, the mixture was ground to a powder with dry ice and sieved. The 53-212 μm size fraction was collected and all oversize and undersize particles were recast, reground, and sieved.

### 2. Polymer Placebo Microspheres

Polymer placebo microspheres were prepared by solvent evaporation in which the 50:50 PDLLA-PGA polymer is dissolved in methylene chloride and this solution is carefully emulsified in a non-solvent for the polymer in the presence of an emulsifying agent. The stirred microspheres are heated to facilitate the removal of methylene chloride by continuous stirring in the non-solvent for several hours. After hardening, the microspheres are then collected, washed with distilled water and dried.

### 3. Microcapsule Preparation

* Two processes were used to prepare microcapsules from the microsphere cores. The first was by
* pan coating. This process was used to coat microspheres with triglyceride on laboratory scale
* equipment. In this method, the core vancomycin microspheres are placed in a rotating pan and either
* sprayed or mixed with a solution of the triglyceride in an organic solvent. The process is continued until
* the desired amount of coating is applied to the microcapsules.

The second process for preparing microcapsules was by air suspension coating. This process was used to coat microspheres with polymer. Vancomycin microsphere core particles are suspended in a

* Proprietary

moving air stream, which cycles them through coating and drying zones. A solution of the PLA-PGA polymer is sprayed into the centrally located coating zone with an atomizer. Spray droplets collide with the particles being coated, wetting and spreading over the surface. As the particles leave the coating zone, the coating dries. Dried particles fall into the annular chamber surrounding the coating zone and are ultimately cycled through the coating zone again. Each time the particles pass through the coating chamber they receive a coating increment. The cycling is continued until the desired coating thickness is obtained **(Nuwayser, 1984, 1987, 1988)**.

### 4. Assays of Vancomycin Microspheres and Microcapsules

Assay of vancomycin microspheres was performed spectrophotometrically at a wavelength of 282 nm. A standard reference solution of vancomycin was prepared and serially diluted. Each solution was read and the absorbance plotted versus concentration. The slope, y-intercept, correlation coefficient and standard absorbance were calculated from the calibration curve.

Vancomycin microspheres prepared with calcium sulfate were assayed by weighing out a sample into a clean 50 ml volumetric flask and filled to the mark with distilled water. After complete dissolution, the solution was read spectrophotometrically at 282 nm against a distilled water blank.

Vancomycin microcapsules were assayed by first weighing out a sample into a clean 50 ml volumetric flask. A 20 ml aliquot of methylene chloride was then introduced into the flask. The flask was then stoppered and secured, placed into a sonicating bath for 10 minutes. A 30 ml aliquot of distilled water was then introduced into the flask and the flask was sonicated for an additional 10 minutes. After sonicating, the flask was allowed to separate out into two separate phases: a top layer-aqueous phase and a lower layer-organic phase. The top layer was then eluted and read spectrophotometrically at 282 nm against a distilled water blank saturated with methylene chloride.

### 5. Microcapsule Size Distribution

Vancomycin microcapsules size distribution for each run was determined by sieve analysis through a series of Gilson sieves with decreasing mesh size ranging from 212 μm to 53 μm as these are standard mesh sizes closest to the 200 μm to 50 μm sizes requested by USAIDR.

### 6. Microcapsule Residual Solvent

Residual solvent level of the microcapsules was determined by Alpha Analytical Labs (Westborough, MA) using a gas chromatography method. No methylene chloride is expected to be detected since none was used in the production of the vancomycin microcapsules. The microcapsules were assayed for ethyl acetate and acetone. The polymer placebo microspheres were assayed for methylene chloride.

### 7. Microcapsule Sterilization and Sterility

25 g of vancomycin microcapsules Batch W8 (see **Section IV, Results**) (5 bottles of 5 g each), as well as 5 g of vancomycin hydrochloride powder USP Special and 25 g of 50:50 PDLLA-PGA

polymer placebos were sealed in Wheaton vials under a nitrogen atmosphere. These vials were sent to Isomedix (Morton Grove, IL) for sterilization by gamma irradiation ($Co^{60}$ source). Upon receipt of the irradiated samples, they were shipped to Microbiology Research Associates (Acton, MA), an FDA registered laboratory for Microbial Limits Testing and Sterility Tests as per USP 23.

### 8. Vancomycin In Vitro Release Rate From the Microcapsules

An accurately weighed quantity of microcapsules (10 mg) was placed in a pouch of fine mesh (30 μm) inert fabric, and suspended in 0.04 M pH 7.4 phosphate buffer at 37°C, shaking at a constant speed in a Dubnoff shaker bath. At given time intervals, the medium was replaced with fresh solution and the drug release rate determined. Daily measurement of release rate was routinely performed and the concentration of the drug in the elution medium does not exceed 20% of saturation. The buffer was replaced daily to insure sink conditions. The use of fine mesh pouch also insures that no microcapsules will be lost during UV measurement and elution medium replacement. Determination of this daily release rate is repeated at several times during the projected life-span of the microcapsules. For these tests, drug is quantitated by UV absorption at 282 nm, and the amount of microcapsules used is such as to assure that results will be over the detection limit. At the end of the release study, the remaining material is inspected under the microscope to assess capsule morphology and the presence of depleted capsule shells.

### 9. Antibiotic Microbial Assay of Vancomycin Microcapsules

A modified version of USP 23 Biological Tests and Assay <81> p 1690-96 was employed using Staphylococcus aureus ATCC No. 33593 (American Type Culture Collection, Rockville, MD). This organism was evenly streaked on trypticase-soy with 5% sheep blood agar (BBL/VWR Scientific, Philadelphia, PA). Vancomycin microcapsules were carefully weighed into a fine mesh polyester pouch and heat sealed. The pouch was wetted with isotonic saline and placed onto the surface of the streaked agar. The agar plates were then allowed to incubate at 37°C for 18 hours. The circular zone of inhibition was measured along three different diameters and the diameter of the pouch subtracted from each measurement. The mean was then calculated and divided by two to give the mean width of the inhibition annulus (in millimeters). The pouch was transferred to a freshly streaked plate daily until no inhibition was observed.

### 10. Scanning Electron Micrographs of the Microcapsules

Scanning electron micrographs of the microcapsules were taken at Analytical Answers, Inc., in Woburn, MA using an ISI "Topcon" Model DS-130.

## IV. RESULTS

### A. Vancomycin

A Certificate of Analysis for vancomycin hydrochloride, USP from Abbott Laboratories, is presented in **Figure 1**.

## B. Characterization of Polymers

The properties of the polymers used in preparing the polymer placebo microspheres and vancomycin microcapsules are listed in **Figure 2**. The 50:50 PDLLA:PGA could not be used for spray coating because of tackiness. The 65:35 PLLA:PGA polymer had a viscosity of 1.04 dl/g and a weight average molecular weight of 158,000 daltons.

## C. Preparation of Microspheres and Microcapsules

### 1. Microsphere Core

#### a. Preparation of Vancomycin Calcium Sulfate Microsphere Core

Vancomycin microspheres were prepared from anhydrous calcium sulfate and vancomycin hydrochloride. Microsphere batches MS2, 3, and 4 were blended to form MSBlend I (**Figure 3**). Batches 5, 6, 7 and 8 were blended to form MSBlend II. Microsphere runs MS9 through MS20 were blended together to form MSBlend III and the remaining runs MS21 through 40 made up MSBlend IV.

Average drug loading of the calcium sulfate-vancomycin microsphere batches was 14.49% ± 0.73. Each blend was assayed in triplicate and the drug loading ranged from 12.4% to 15.1% (**Figure 3**).

#### b. Preparation of Vancomycin Cast Core

Three batches of vancomycin cast core were prepared and identified as CC1A, CC1B, and CC1C. The ratio of drug to polymer was 50:50 and the assayed vancomycin loadings of these three cast cores are presented in **Figure 3** and range between 49.8% and 50.2%.

### 2. Microcapsule Preparation

#### a. Preparation of Microcapsules by Triglyceride Coating of Calcium Sulfate Cores

Five batches of vancomycin microcapsules (MC1 to MC5) were prepared. Four batches (MC1 to MC4) utilized calcium sulfate microsphere blends as starting core and one batch, MC5, used polymer cast core as the starting core.

Microsphere blend or drug-polymer cast core was introduced into a marumerizer along with a solution of triglyceride dissolved in ethyl acetate. A stream of nitrogen gas was directed into the marumerizer to facilitate the removal of ethyl acetate. As the particles were rotating in the marumerizer and the ethyl acetate evaporated, a coating of triglyceride was applied to each microsphere. This process continued until all the ethyl acetate evaporated and a free flowing powder was formed. This powder was collected and sieved, and all the microcapsules from 53 μm to 212 μm were recovered and the oversize particles discarded.

### b. Preparation of Microcapsules by Polymer Coating

Eleven batches of polymer coated microcapsules were prepared and evaluated (**Figure 3**). They were numbered W1 through W8 with two sub-batches each for runs W2, W3, and W4. Batches W1 through W8 were prepared by overcoating the following cores with polymer: calcium sulfate microspheres, polymer cast core, and triglyceride microcapsules.

Microcapsule batch W1 was unsuccessful because of the high level of triglyceride in the wall (36%) of batch MC2. During the polymer coating process, the acetone solvent softened the triglyceride sufficiently to cause severe agglomeration. Batches W2a and W2b were also unsuccessful because the starting core was microsphere blend MSBlend II, a fragile vancomycin calcium composite which fragmented during coating. The fragments also agglomerated.

The first successful polymer overcoated microcapsules were Runs W3a and W3b, which utilized a lightly triglyceride coated microcapsule Batch MC3. A heavy coating of a crystalline polymer (65:35 PLLA-PGA) was applied, 63% and 67.4%, respectively to runs W3a and W3b in order to compensate for the low triglyceride coating. The high polymer coating levels effectively reduced the overall drug loading of vancomycin to approximately 9% and reduced the day 1 burst to 33%, with a duration of 15-18 days. Microcapsule runs W4a, W4b, W5 and W6 used 50% loaded cast cores at different coating levels. Although Run W6 met the criteria for drug loading and duration, the burst of 50% on day one was excessive. Run W7 used triglyceride microcapsule core (MC5), and a 17.7% coating of 65:35 PLLA-PGA in an attempt to get a high drug loading, attenuated burst on day 1 and a duration of 14-21 days. The drug loading of 38.5% produced a drug burst (76%) on day one with an overall duration of four days.

Microcapsule run W8 was prepared by coating batch MC4 (triglyceride coated vancomycin calcium sulfate microsphere core) with 29.7% PLA:PGA. The formulation met all the criteria of initial burst, vancomycin content, and duration of release. The burst on day one was 26%, the drug loading was 10.1%, and 80% of the drug was released in 21 days. This formulation was selected for preparing a large batch which was sterilized by gamma irradiation, and sent to USAIDR.

### c. Sieve Analysis

A size distribution analysis was performed on each successful batch of vancomycin/calcium sulfate microspheres (MS1 to MS40) (**Figure 4**). Due to the large number of batches prepared, we will present only a select few. **Figure 5** is an illustration of the sieve analysis of four microsphere batches. Batch MS4, used in the preparation of MSBlend I, shows a majority of the particles (55.7%) in the desired size range of 53 μm-212 μm. Batch MS8, MS18, and MS32 used in the preparation of MSBlends II, III and IV had 89.52%, 86.22%, and 90.58% yields in the desired size range, respectively. Over 95% of all triglyceride microcapsules and polymer coated microcapsules were in the desired 53 μm-212 μm size range.

* Proprietary

### d. In Vitro Release

#### 1) Microspheres

**Figure 6** is an illustration of the release of vancomycin from microsphere batch MS1, a composite of vancomycin and calcium sulfate. The release profile is typical of vancomycin release from this type of microsphere. The day 1 burst was approximately 44% with 80% released by day 4.

#### 2) Microcapsules

**Figure 7** is a graph of the release of vancomycin from calcium sulfate MSBlend IV before and after triglyceride coating (Batch MC4). The triglyceride coating reduced the burst on day 1 from 85% to 26%.

**Figure 8** is a graph of Batch W8, the formulation which represents the ideal release of vancomycin from a microparticle. The burst on day 1 was 26% with a slow steady release over the next 20 days.

### e. Selection of Final Formulation

Batch W8 microcapsules met all the criteria set in the contract for vancomycin loading (> 10%), vancomycin release on day 1 (26%), and duration of release (21 days). This formulation was selected for preparing the 25 grams of vancomycin microcapsules required in the contract.

A total of 20 batches of vancomycin calcium sulfate microsphere core were blended to form MSBlend IV. The vancomycin content of the blend was 14.7%. This blend was next overcoated with triglyceride in a laboratory scale marumerizer. Finally, the triglyceride microcapsules were coated with 65:35 PLA:PGA in a microfluidized bed chamber. Eight coating runs were required to prepare 25 grams of microcapsules.

### f. Residual Solvent Analysis

The results of the residual solvent analysis performed by Alpha Analytical Laboratories on Batch W8 vancomycin microcapsules indicate less than 400,000 ppb or 400 ppm (0.04%) of acetone or ethyl acetate to be present in the vancomycin microcapsules **(Figure 9)**. The method employed by Alpha Analytical is GC/MS using EPA method 8260. A Certificate of Analysis from Alpha is presented in **Figure 9**.

A Certificate of Analysis for the residual solvent content of the polymer placebo microspheres is shown in **Figure 10**. The methylene chloride level was below the 5 ppb of the detection limit of the assay.

### g. Sterilization and Sterility

Batch W8 vancomycin microcapsules, as well as USP vancomycin powder and polymer placebo microspheres, were sent to Isomedix for sterilization by gamma irradiation. After sterilization the samples were sent to Microbiology Research Associates for sterility testing.

The Microbial Limits Test provides tests for the estimation of the number of viable aerobic microorganisms present and for freedom from designated microbial species in pharmaceutical articles of all kinds. The test is described in detail on page 1681 of USP 23. A Preparatory Test is conducted first to ascertain that the test specimen to which the tests are applied do not, of themselves, inhibit the multiplication, under the test conditions, of microorganisms that may be present.

Because vancomycin is a potent antimicrobial it interfered with the growth of Bacillus subtilis culture used in the Bacteriostasis/Fungistasis test. **Figure 11** is a letter from Microbiology Research Associates, Inc. stating their inability to grow Bacillus subtilis despite a dilution to 200 ml of media, as required by USP 23. The other organisms used in the Bacteriostasis/Fungistasis test, Candida albicans and Bacteriotis vulgatus, were not inhibited by vancomycin. The USP Sterility Tests are described in detail on page 1686 of USP 23 and was conducted on Batch W8. The results of this test are presented in the MRA report, which is reproduced in **Figure 12** and shows the microcapsules were sterile since no growth was formed in the two media recommended by USP 23.

### h. Effect of Gamma Irradiation on In Vitro Release

**Figure 8** also contains the in vitro release profile of microcapsule batch (W8) which was sterilized by gamma irradiation. The burst on day 1 was 23% and a near identical release of drug compared to its non-irradiated counterpart.

### i. Antibiotic Microbial Assay - USP 23

Approximately 8 mg of vancomycin microcapsules (W8) were weighed into a circular polyester pouch (pore size: 30 μm) and heat sealed. The pouch was wetted with isotonic saline and transferred daily to a freshly streaked blood agar plate. **Figure 13** is a graph of the daily mean diameter of inhibition (mm). Error bars show the standard error of the mean. The microcapsules inhibited the growth of S. aureus, ATCC No. 35393 for approximately 19 days.

### j. Scanning Electron Micrographs

**Figure 14** is an SEM of a blend of microspheres prepared using calcium sulfate as a core. The particles are rough and brittle. **Figure 15** is an SEM of the vancomycin microspheres blend, MS Blend IV, that has been overcoated with triglyceride. When compared to **Figure 14**, the microcapsules are rounder and less fragmented. The triglyceride coating provided a hydrophobic coating as well as binder which strengthens the vancomycin-calcium sulfate core.

**Figure 16** is an SEM of the triglyceride coated microcapsules which were overcoated with 65:35 PLLA-PGA to form Batch W8. A smooth coating of polymer on the microcapsules effectively sealed most of the surface pores. **Figure 17** is an SEM of these same microcapsules after sterilization by gamma irradiation. There was no apparent loss of coating or any other visual adverse effect. This was also confirmed by the similarity of the vancomycin release profile before and after irradiation (**Figure 8**).

## V. CONCLUSION

BIOTEK, Inc. prepared 25 g of sterile vancomycin microcapsules for USAIDR which release 23% of the drug on day 1, and a slow, steady release over the following 19 days. These microcapsules (Batch W8) range in size from 53 to 212 μm, and required no methylene chloride in their production. They were sterilized by gamma irradiation with no detrimental effect on either in vitro release or efficacy when tested against Staph. aureus, ATCC No. 35393 on a 5% blood agar plate.

* The microcapsules were prepared by blending 20 microsphere runs (MS19 to MS40) utilizing a water-in-oil emulsion technique containing calcium sulfate and vancomycin. This microsphere blend (MSBlend IV) was then overcoated with Witepsol E-85 triglyceride in a marumerizer using ethyl acetate as solvent. The microcapsules (Batch MC4) were then overcoated with a 65:35 PLLA-PGA copolymer using a spray coating technique that employs acetone as a solvent. The final microcapsules, Batch W8, were assayed and their vancomycin loading was 10.1%. The calculated composition of the microcapsules is 10.1% vancomycin (by actual assay), 22.9% polymer, 7.5% triglyceride, and 59.5% calcium sulfate.

In addition to the 25 g of sterilized vancomycin microcapsules, BIOTEK also prepared 25 g of polymer placebo (50:50 PDLLA-PGA) which were also sterilized by gamma irradiation, and 5 g of pure vancomycin USP. These materials were shipped to the attention of Dr. Ibrahim Barsoum on December 13, 1996.

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FIGURE 1



Quality Health Care Worldwide

# CERTIFICATE OF ANALYSIS

28-Dec-1995

VANCOMYCIN HYDROCHLORIDE, USP, SPECIAL
Lot Number 12320ID00

Manufacturing Date: 04-Dec-1995
Expiration Date: 01-Jan-1997

| Tests | Results | |
|---|---|---|
| Vancomycin | | |
| Release Anhydrous Potency | 1094 | mcg/mg |
| Release As Is Potency | 1078 | mcg/mg |
| Appearance | PASS | |
| Color | PASS | |
| pH (of a 50 mg/ml Solution) | 3.1 | |
| Solution (in Water at Room Temperature) | PASS | |
| Moisture (by KF) | 1.5 | % |
| Identification (by IR) | PASS | |
| Identification (by HPLC) | PASS | |
| Chromatographic Purity (by HPLC) | | |
| Vancomycin B | 91 | % |
| Other Peaks | 2 | % |
| Bacterial Endotoxins | <0.125 | EU/mg |
| Heavy Metals | <30 | ppm |
| APHA Color | <100 | Units |
| Solvent Residue | 0.9 | % |

Order 95447 / BIOTEK, INC.

Expiration dating based upon testing requirements as specified in compendia. The undersigned certifies this to be a true copy of the results of tests and assays conducted by ABBOTT LABORATORIES.

Beth Cunningham
Quality Assurance

FIGURE 2

POLYLACTIDE–co–GLYCOLIDE POLYMER PROPERTIES

| Copolymer Type | PLLA–PGA | PDLLA–PGA |
|---|---|---|
| Copolymer Ratio | 65/35 | 50/50 |
| Lot Number | D95078 | D95041 |
| Quantity | 1,000 g | 500 g |
| Viscosity | 1.04 dl/g | 0.59 dl/g |
| NMR | 65/35 | 51/49 |
| Residual Tin | 79 ppm | 13.2 |
| Mw | 158,000 Da | 46,500 Da |
| Mn | 98,700 Da | 29,500 Da |
| Mw/Mn | 1.6 | 1.58 |

612/96–IF2

FIGURE 3

TABLE OF VANCOMYCIN MICROSPHERES, CAST CORE AND MICROCAPSULES

| Run No. | Type | Polymer Type | Core Material | Assayed Drug Content | Coating Material | Theo. Coating Percent | Duration:80% Released | Day 1 Burst |
|---|---|---|---|---|---|---|---|---|
| MS Placebo | Microspheres | 50:50 PDLLA–PGA | N/A | 0.0% | | | | |
| MS 1 | Microspheres | CaSO4 | N/A | 13.7% | | | 2 Day | 44% |
| MSBlend I | Microsphere Blend | CaSO4 | MS2,3,4 | 14.5% | | | | |
| MSBlend II | Microsphere Blend | CaSO4 | MS5,6,7,8 | 12.4% | | | | |
| MSBlend III | Microsphere Blend | CaSO4 | MS9–20 | 15.1% | | | | |
| MSBlend IV | Microsphere Blend | CaSO4 | MS21–40 | 14.7% | | | 1 Day | >80% |
| CC 1A | Cast Core | 65:35 PLLA–PGA | N/A | 50.2% | | | 1 Day | >80% |
| CC 1B | Cast Core | 65:35 PLLA–PGA | N/A | 49.8% | | | | |
| CC 1C | Cast Core | 65:35 PLLA–PGA | N/A | 50.2% | | | | |
| MC1 | Microcapsules | Microspheres | MSBlend I | 11.3% | Triglyceride | 28.4% | 5 Days | 50% |
| MC2 | Microcapsules | Microspheres | MSBlend II | 11.1% | Triglyceride | 36.0% | | |
| MC3 | Microcapsules | Microspheres | MSBlend III | 14.9% | Triglyceride | 1.3% | | |
| MC4 | Microcapsules | Microspheres | MSBlend IV | 13.1% | Triglyceride | 12.2% | 17 Days | 25% |
| MC5 | Microcapsules | Microparticles | CC 1C | 45.3% | Triglyceride | 10.8% | | |
| W 1 | Microcapsules | | MC2 | 13.0% | 50:50 PDLLA–PGA | * | 1.5 Days | >65% |
| W 2a | Microcapsules | | MSBlend II | 13.7% | 50:50 PDLLA–PGA | * | 1–2 Days | >65% |
| W 2b | Microcapsules | | MSBlend II | 13.2% | 50:50 PDLLA–PGA | * | 1–2 Days | >65% |
| W 3a | Microcapsules | | MC3 | 9.1% | 65:35 PLLA–PGA | 63.0% | 18 Days | 33% |
| W 3b | Microcapsules | | MC3 | 8.9% | 65:35 PLLA–PGA | 67.4% | 18 Days | 33% |
| W 4a | Microcapsules | | CC 1A | 47.9% | 65:35 PLLA–PGA | 4.7% | 1 Day | >80% |
| W 4b | Microcapsules | | CC 1A | 47.0% | 65:35 PLLA–PGA | 6.8% | 1 Day | |
| W 5 | Microcapsules | | CC 1B | 42.0% | 65:35 PLLA–PGA | 18.6% | 15 Days | 63% |
| W 6 | Microcapsules | | CC 1C | 38.2% | 65:35 PLLA–PGA | 31.4% | 24 Days | 50% |
| W7 | Microcapsules | | MC5 | 38.5% | 65:35 PLLA–PGA | 17.7% | 4 Days | 76% |
| W8 | Microcapsules | | MC4 | 10.1% | 65:35 PLLA–PGA | 29.7% | 17 Days | 26% |
| W8(Irrad.) | Microcapsules | | MC4 | 10.1% | 65:35 PLLA–PGA | 29.7% | 21 Days | 23% |

612/96–fF3

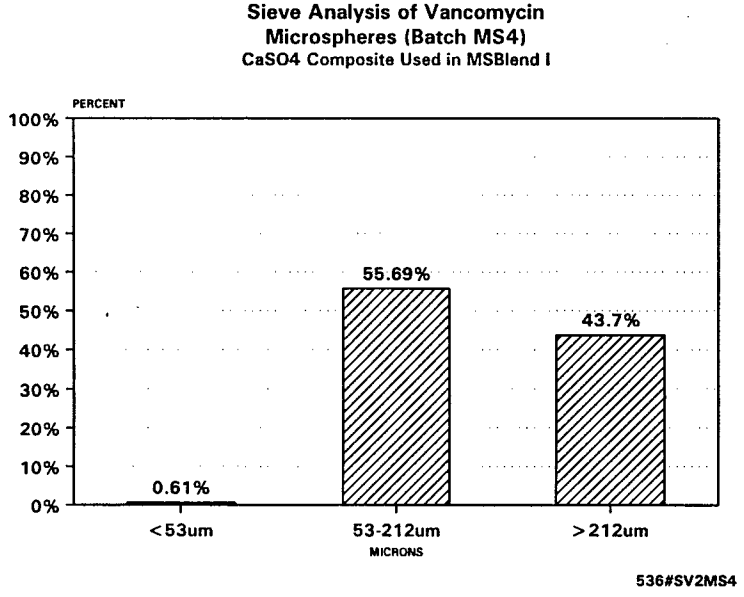
* agglomerated

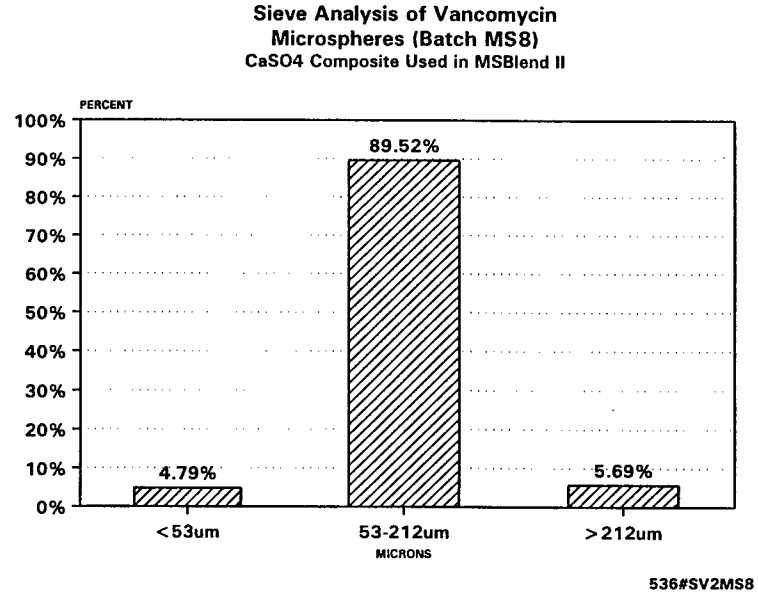
FIGURE 4

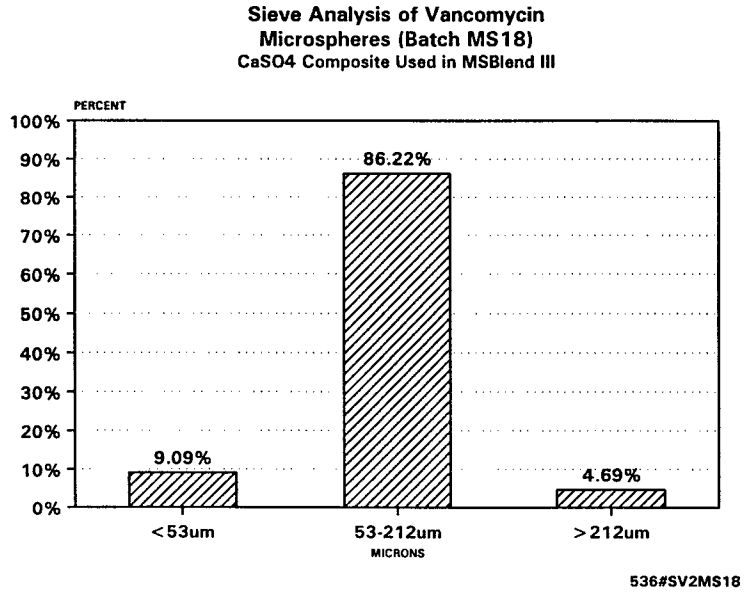
VANCOMYCIN MICROSPHERE SIZE DISTRIBUTION

| Microsphere Run No. | <53 μm Weight % | 53–212 μm Weight % | >212 μm Weight % |
|---|---|---|---|
| MS 1 | 4.35 | 22.50 | 73.13 |
| MS 2 | 0.00 | 16.94 | 83.06 |
| MS 3 | 0.00 | 29.83 | 70.17 |
| MS 4 | 0.61 | 55.69 | 43.70 |
| MS 5 | 12.14 | 77.00 | 10.85 |
| MS 6 | 9.69 | 81.63 | 8.67 |
| MS 7 | 6.21 | 88.59 | 5.20 |
| MS 8 | 4.79 | 89.52 | 5.69 |
| MS 9 | 8.59 | 85.32 | 6.09 |
| MS 10 | 4.20 | 86.49 | 9.31 |
| MS 11 | 7.49 | 85.31 | 7.20 |
| MS 12 | 4.79 | 89.52 | 5.69 |
| MS 13 | 8.31 | 88.64 | 3.05 |
| MS 14 | 8.04 | 85.53 | 6.43 |
| MS 15 | 14.08 | 79.47 | 6.45 |
| MS 16 | 14.33 | 82.09 | 3.58 |
| MS 17 | 9.01 | 85.40 | 5.59 |
| MS 18 | 9.09 | 86.22 | 4.69 |
| MS 19 | 10.76 | 82.56 | 6.69 |
| MS 20 | 12.85 | 85.47 | 1.68 |
| MS 21 | 17.67 | 36.84 | 45.49 |
| MS 22 | 14.39 | 43.86 | 41.75 |
| MS 23 | 1.35 | 40.81 | 57.85 |
| MS 24 | 13.45 | 17.34 | 69.21 |
| MS 25 | 11.30 | 64.25 | 24.45 |
| MS 26 | 17.42 | 68.86 | 13.72 |
| MS 27 | 13.29 | 81.07 | 5.64 |
| MS 28 | 10.31 | 81.54 | 8.15 |
| MS 29 | 9.98 | 46.46 | 43.56 |
| MS 30 | 19.10 | 76.29 | 4.61 |
| MS 31 | 7.15 | 86.57 | 6.28 |
| MS 32 | 3.07 | 90.58 | 6.36 |
| MS 33 | 9.55 | 87.17 | 3.29 |
| MS 34 | 0.35 | 95.54 | 4.11 |
| MS 35 | 7.56 | 85.74 | 6.70 |
| MS 36 | 0.37 | 96.76 | 2.87 |
| MS 37 | 0.59 | 96.09 | 3.32 |
| MS 38 | 0.76 | 97.82 | 1.42 |
| MS 39 | 0.46 | 98.33 | 1.21 |
| MS 40 | 3.07 | 93.84 | 3.10 |
| **Mean** | **7.76** | **74.24** | **18.00** |

FIGURE 5


Sieve Analysis of Vancomycin
Microspheres (Batch MS4)
CaSO4 Composite Used in MSBlend I
PERCENT
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.61%
55.69%
43.7%
<53um
53-212um
>212um
MICRONS
536#SV2MS4


Sieve Analysis of Vancomycin
Microspheres (Batch MS8)
CaSO4 Composite Used in MSBlend II
PERCENT
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
4.79%
89.52%
5.69%
<53um
53-212um
>212um
MICRONS
536#SV2MS8


Sieve Analysis of Vancomycin
Microspheres (Batch MS18)
CaSO4 Composite Used in MSBlend III
PERCENT
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
9.09%
86.22%
4.69%
<53um
53-212um
>212um
MICRONS
536#SV2MS18

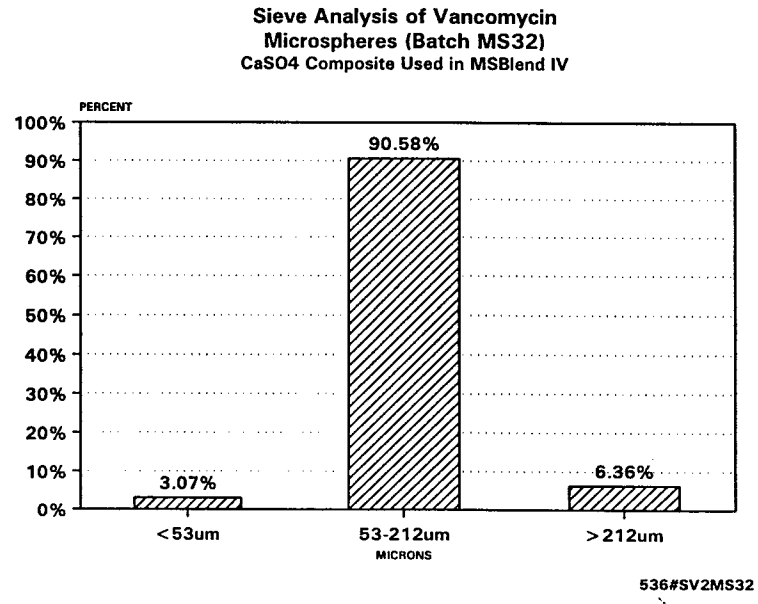

Sieve Analysis of Vancomycin
Microspheres (Batch MS32)
CaSO4 Composite Used in MSBlend IV
PERCENT
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
3.07%
90.58%
6.36%
<53um
53-212um
>212um
MICRONS
536#SV2MS32

FIGURE 6

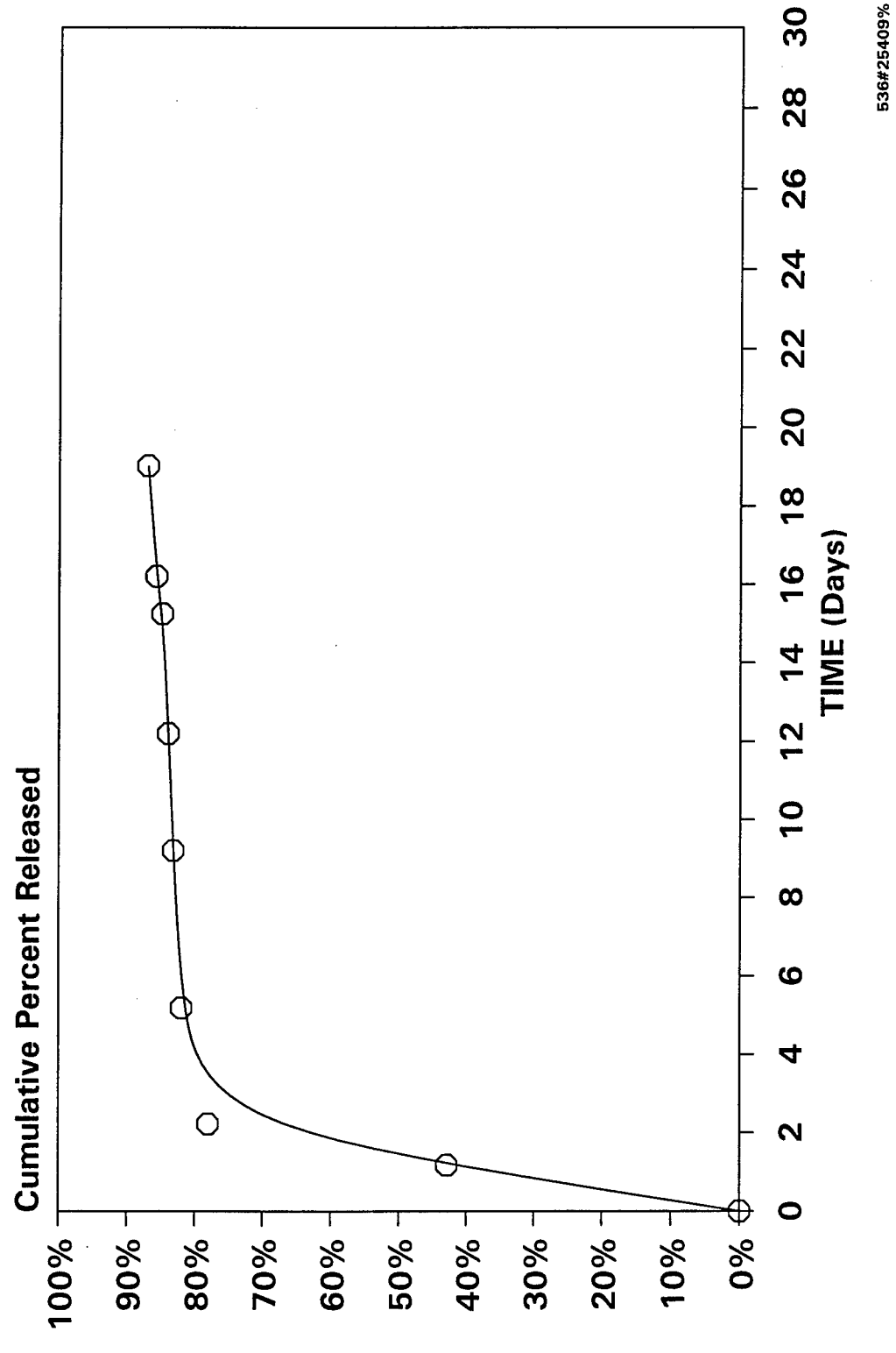

In Vitro Release of Vancomycin from
Microsphere Batch MS1 (Drug Load:13.72%)
Calcium Sulfate Composite
Cumulative Percent Released
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (Days)
536#25409%

FIGURE 7

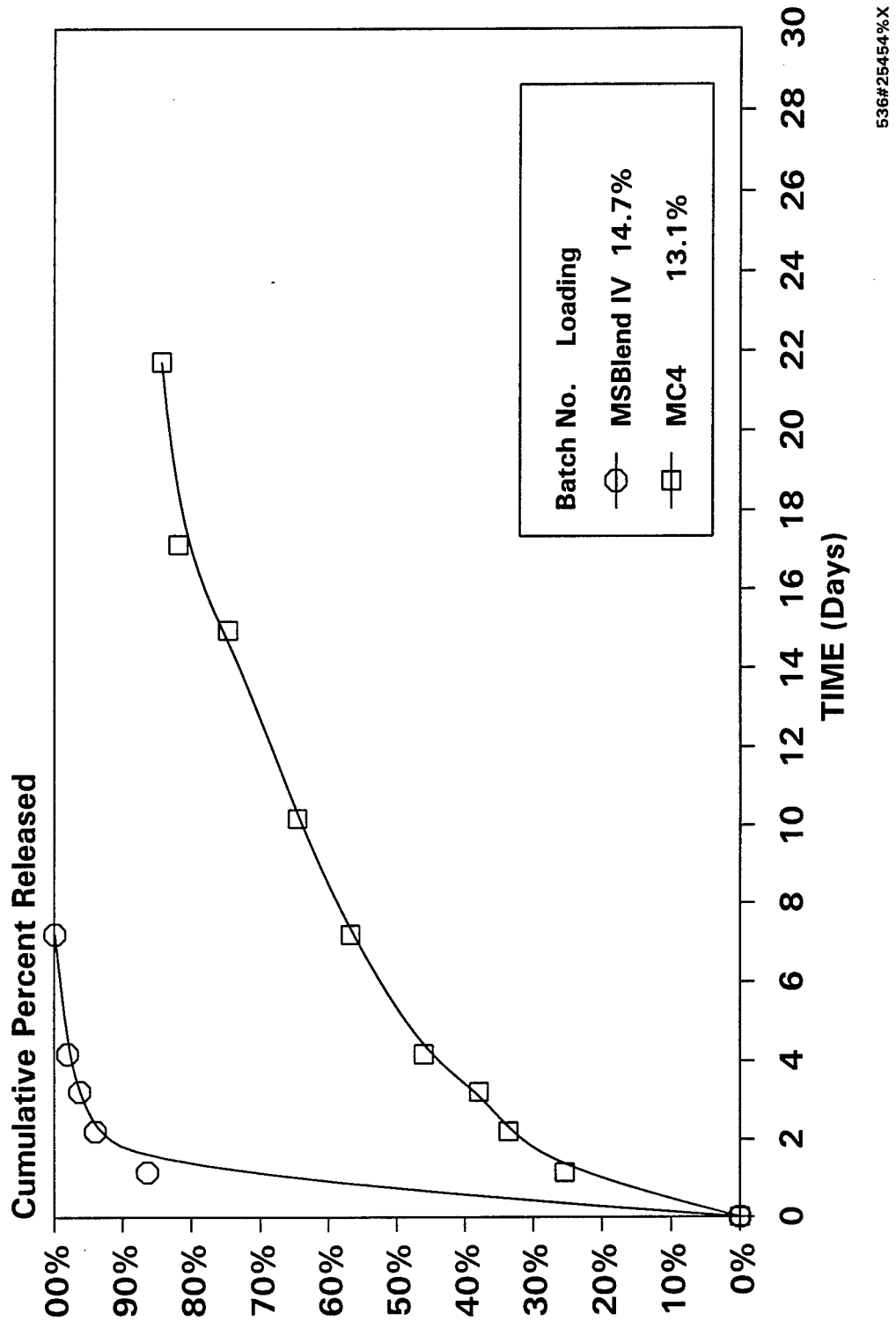
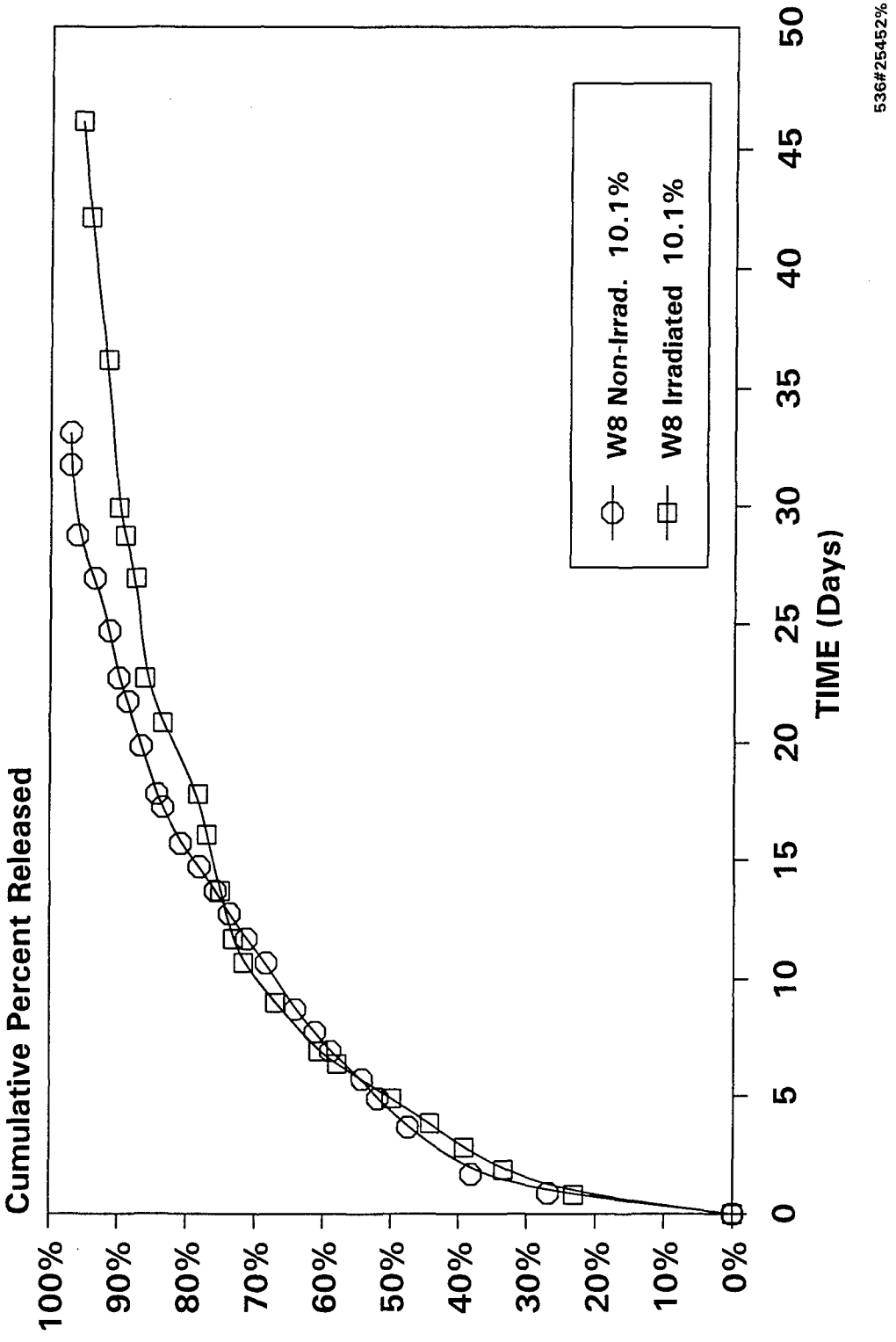

In Vitro Release of Vancomycin from
Microsphere MSBlend IV and Triglyceride
Coated Microcapsule Batch MC4-Coat:12.2%
Cumulative Percent Released
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (Days)
Batch No. Loading
MSBlend IV 14.7%
MC4 13.1%
536#25454%X

FIGURE 8




536#25452%

MA:M-MA-086 NH:200395-B/C CT:PH-0574 ME:MA086 RI:65

**Laboratory Sample Number:** L9605859-01
VANCOMYCIN
**Sample Matrix:** SOLID

**Date Collected:** 16-AUG-96
**Date Received :** 16-AUG-96
**Date Reported :** 30-AUG-96

**Condition of Sample:** Satisfactory

**Field Prep:** None

**Number & Type of Containers:** 1 Amber Glass

**Comments:**
Sample reported on as received basis.

| PARAMETER | RESULT | UNITS | RDL | REF | METHOD | DATES PREP | DATES ANALYSIS | ID |
|---|---|---|---|---|---|---|---|---|
| Volatile Organics by GC/MS | | | | 1 | 8260 | 29-Aug | 29-Aug | DB |
| Acetone | ND | ug/kg | 400000 | | | | | |
| Ethyl Acetate | ND | ug/kg | 400000 | | | | | |
| SURROGATE RECOVERY | | | | | | | | |
| Toluene-d8 | 101. | % | | | | | | |
| 4-Bromofluorobenzene | 89.0 | % | | | | | | |
| Dibromofluoromethane | 84.0 | % | | | | | | |

RDL = Reported Detection Limit = Lowest readable level in µg/kg or parts per billion (ppb).

Surrogate Recovery = The recovery of the methods internal standard.

ND = No detection = the level of the analyte in the sample is below the reported detection limit.

Comments: Complete list of References and Glossary of Terms found in Addendum I

**ALPHA ANALYTICAL LABORATORIES**
**CERTIFICATE OF ANALYSIS**

**MA:M-MA-086 NH:200395-B/C CT:PH-0574 ME:MA086 RI:65**

**Laboratory Sample Number:** L9606488-01
DML-2196
**Sample Matrix:** SOLID

**Date Collected:** 09-SEP-96
**Date Received :** 09-SEP-96
**Date Reported :** 23-SEP-96

**Condition of Sample:** Satisfactory

**Field Prep:** None

**Number & Type of Containers:** 1 Plastic

**Comments:**
Results reported on an as received basis.

| PARAMETER | RESULT | UNITS | RDL | REF | METHOD | DATES PREP | DATES ANALYSIS | ID |
|---|---|---|---|---|---|---|---|---|
| Volatile Organics by GC/MS | | | | 1 | 8260 | 23-Sep | 23-Sep | DB |
| Methylene chloride | ND | ug/kg | 5.0 | | | | | |
| SURROGATE RECOVERY | | | | | | | | |
| Toluene-d8 | 103. | % | | | | | | |
| 4-Bromofluorobenzene | 72.0 | % | | | | | | |
| Dibromofluoromethane | 76.0 | % | | | | | | |

RDL = Reported Detection Limit = Lowest readable level in µg/kg or parts per billion (ppb).

Surrogate Recovery = The recovery of the methods internal standard.

ND = No detection = the level of the analyte in the sample is below the reported detection limit.

Comments: Complete list of References and Glossary of Terms found in Addendum I

Microbiology Research Associates, Inc. 33 Nagog Park, Acton, MA 01720 • (508) 263-2624 • FAX (508) 263-2786

September 23, 1996

Mr. Phil Blaskovick
Biotek, Inc.
21-C Olympia Avenue
Woburn, MA 01801

Dear Phil,

Please find enclosed the Sterility Test results for your antibiotic products. These products all pass the USPXXIII Sterility Test. We were unable to get the *Bacillus subtilis* culture to grow in the media with these antibiotics (even after increasing the dilution to 200 ml of media). The other bacteriostatis/fungistatis microorganisms (*Candida albicans* and *Bacteroides vulgatus* did grow). This result is obviously due to the potent cidal activity of these products in mcg quantities. The USP does state that "if inspite of incorporation of suitable inactivating agents and substantial increase in the volume of diluent it is still not possible to recover the viable cultures and where the product is not suitable for membrane filtration it can be assumed that the failures to recover inoculated organisms is attributable to the bactericidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of microorganisms.

Please call me if you have any questions.

Sincerely,

James J. Barbato, M.S., M.P.H.
President

/pab
enclosure

**Microbiology Research Associates, Inc.** 33 Nagog Park, Acton, MA 01720 • (508) 263-2624 • FAX (508) 263-2786

## Sterility testing Report

Product Name: Vancomycin Microcapsules

Company: Biotek, Inc.

Control Number: NA Notebook Ref. Pg.: Bk.#:14 Pg.#:29

Sample Rec'd: 8-08-96 Test Begun: 9-04-96 Completed: 9-18-96

Project #: BIO-012 # of Units: 0.5 g

Method and Procedure:

A. Test Procedures for Direct Transfer to Test Media USPXXIII p.1686

1. _____ Liquids
2. _____ Ointments and Oils Insoluble in Isopropyl Myristate
3. X Solids
4. _____ Purified Cotton, Gauze, Surgical Dressings, Etc.
5. _____ Sterilized Devices
6. _____ Other_____

B. Test Procedures Using Membrane Filtration USPXXIII, p.1686

1. _____ Liquids Miscible with Aqueous Vehicles (less than 100ml/container)
2. _____ Liquids Miscible with Aqueous Vehicles (more than 100ml/container)
3. _____ Liquids Immiscible with Aqueous Vehicles(less than 100ml/container)
4. _____ Ointments and Oils Soluble in Isopropyl Myristate

Incubation Period _____ 7 days X 14 days _____ 21 days _____ other

Results:

1. X No growth in either medium
2. _____ Growth in Fluid Thioglycollate Medium(If growth see SOP#P007)
3. _____ Growth in Soybean Casein Digest Medium(If growth see SOP#P007)
4. _____ First retest _____ Second retest

Conclusion:

1. X In the absence of growth, the product meets the requirements for the Test for Sterility described in the USPXXIII.
2. _____ In the presence of growth the product does **not** meet the requirement for the Test for Sterility described in the USPXXIII.

9-18-96
Date

James J. Barbato, M.S., M.P.H.
President

BIOVNMIC.012

FIGURE 13

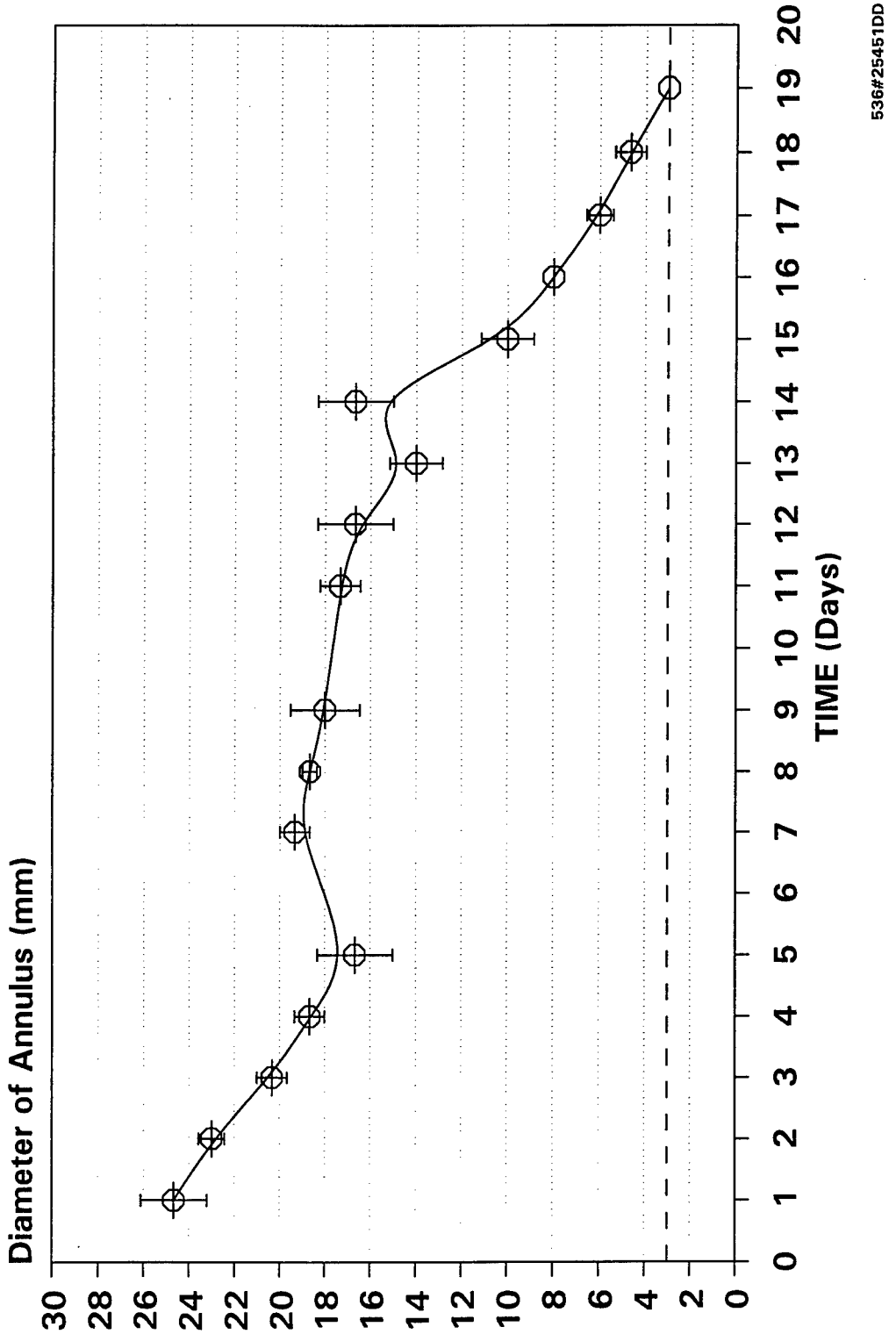

Antibiotic Microbial Assay of Vancomycin
Microcapsules against Staphylococcus
aureus ATCC# 35393 (n=3 +/- SEM)
Diameter of Annulus (mm)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
TIME (Days)
536#25451DD


FIGURE 14 Vancomycin-Calcium Sulfate Microspheres
Batch MSBlend IV Drug Loading: 14.7%

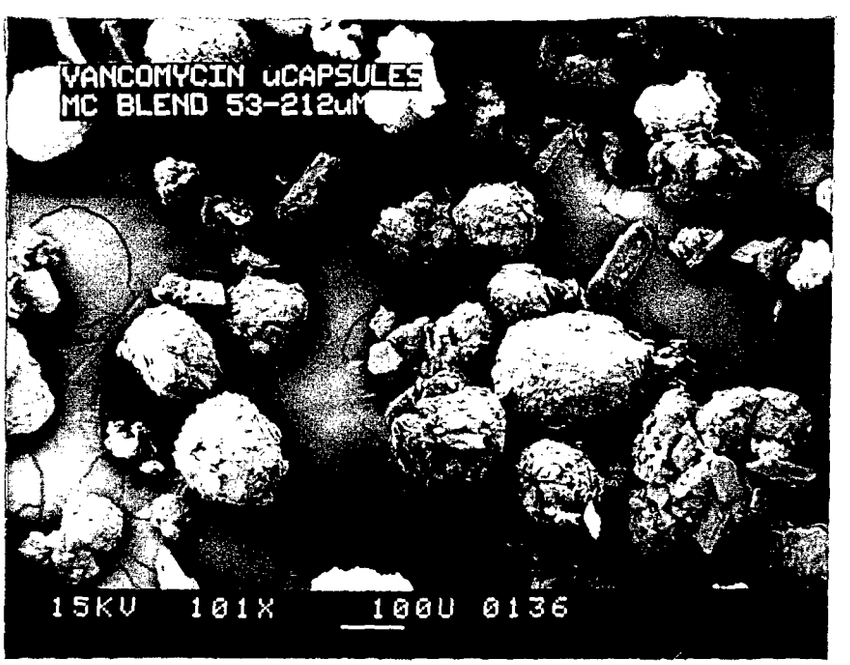



FIGURE 15 Triglyceride Coated Microcapsules
Batch MC4 Drug Loading: 13.1%

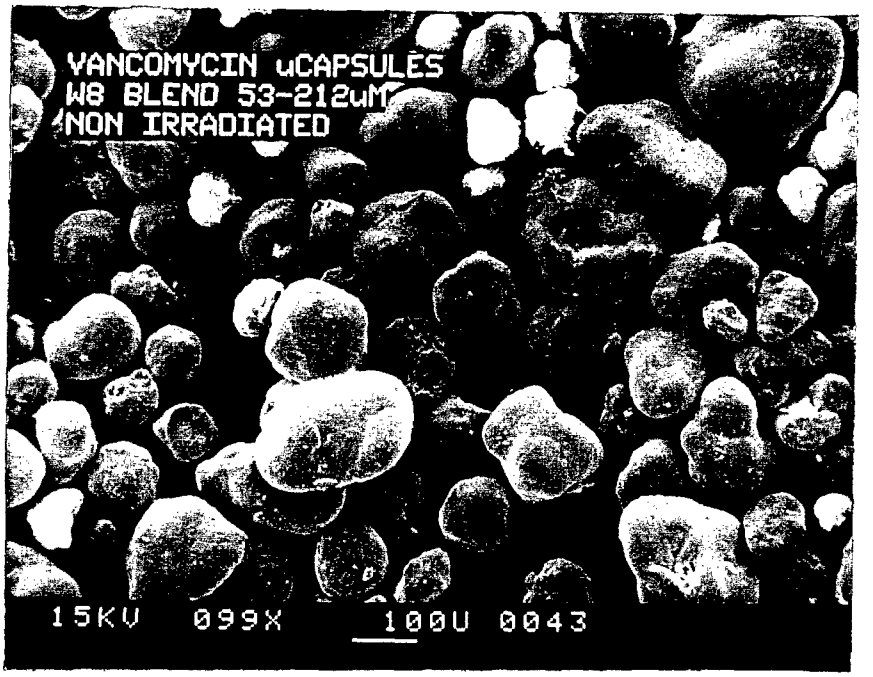



FIGURE 16 65:35 PLLA-PGA Polymer Overcoated Microcapsules Batch W8 Drug Loading: 10.1%

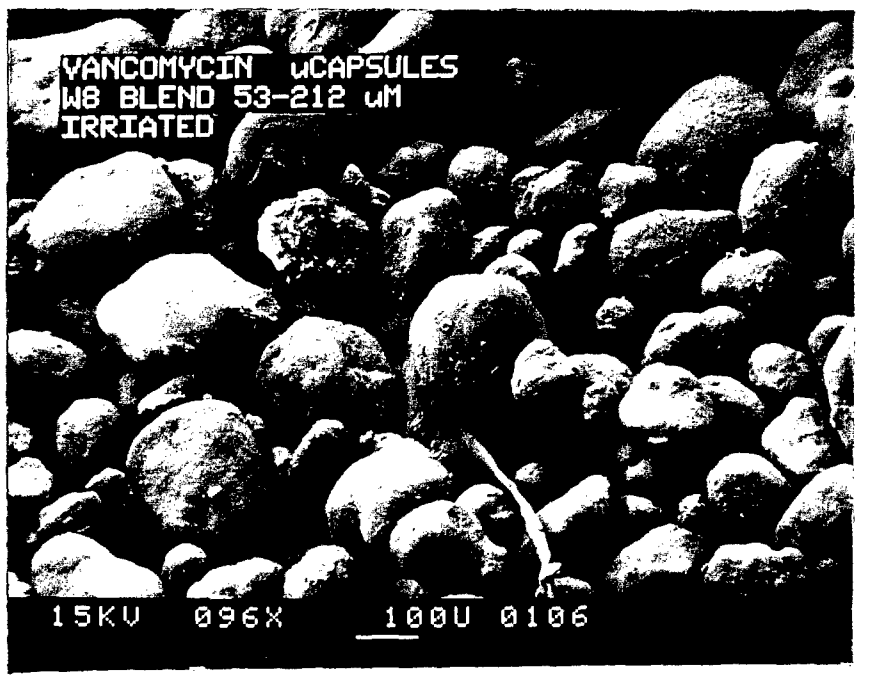



FIGURE 17 65:35 PLLA-PGA Polymer Overcoated Microcapsules (Irradiated) Batch W8 Drug Loading: 10.1%

**DEPARTMENT OF THE ARMY**
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 2 Feb 98

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Contract DAMD17-96-C-6002. Request the limited distribution statement for Accession Document Number ADB220006 be changed to "Approved for public release; distribution unlimited." This report should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Betty Nelson at DSN 343-7328 or email: betty_nelson@ftdetrck-ccmail.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Completed
2-8-2000
BN