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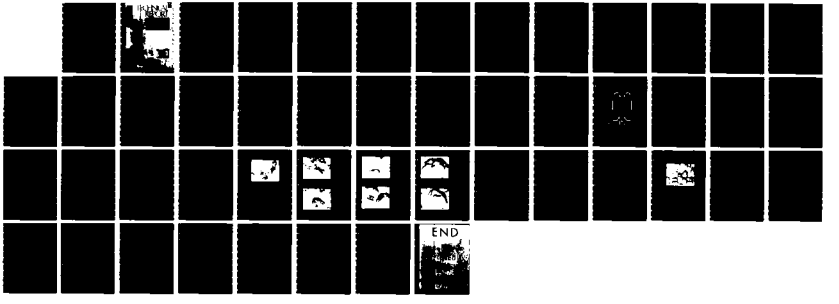

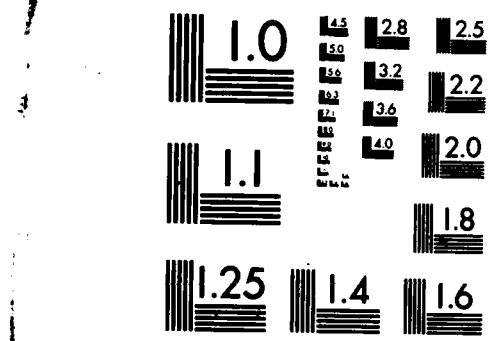

MICROCOPY RESOLUTION TEST CHART
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TECHNICAL REPORT

ABCOR, INC. 850 MAIN ST. WILMINGTON, MASS. 01887

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RESEARCH AND DEVELOPMENT IN PREVENTIVE DENTISTRY

Final Report
1/1/79 - 12/31/79
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Abcor, Inc.
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850 Main Street
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Lidocaine hydrochloride was microencapsulated in a biodegradable polymer, polyL(-)lactide, using an air suspension coating technique. Eight coating runs produced microcapsules of various sizes and polymer/drug ratios. The final run (L-8) produced 50% drug microcapsules. These microcapsules of 125-149 μm size range, released about 50% of their drug in 2.5 hours into an agitated aqueous solution at 37° C.
Anesthesia was demonstrated using the sciatic nerve block test in

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20. Abstract (continued)
the rat. Microcapsules were injected in aqueous suspensions and compared to injection of lidocaine solution. Because of problems of injecting large quantities of 125-149 μm suspensions, 4% lidocaine in a faster-releasing microcapsule suspension (74-105 μm fraction) was compared to a 4% lidocaine solution. A delayed block was evident, but an increased duration was not achieved with these microcapsules at this lidocaine concentration.

micrometers

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FOREWORD

James Ronald Lawter, Ph.D., served as Principal Investigator on this program until August 15, 1979, at which time Dr. Lawter left Abcor. David L. Williams, Ph.D. then served as the interim principal investigator. Other Abcor personnel involved with this program included Kenneth R. St. John, M.S., Barron W. Tenney, B.S., and Theresa Daley. George Camougis, Ph.D. of New England Research, Inc. (Worcester, Massachusetts), served as a consultant to the program.

We greatly appreciate the interest and direction afforded by Project Officer, Colonel William R. Posey and by Colonel Duane E. Cutright, Commander, Institute of Dental Research.

Abcor, Incorporated, and its Animal Care Committee endorse the "Principles of Laboratory Animal Care" formulated by the National Society of Medical Research, and the "Guide for the Care and Use of Laboratory Animals", published by the National Institute of Health (Publication No. NIH74-23, 1974). All investigations involving animal experimentation, as well as animal care, were conducted in conformity with these principles.

I. INTRODUCTION

A. OBJECTIVE

The objective of this project was to demonstrate the feasibility of producing a long-lasting local anesthetic for application to avulsive maxillofacial wounds. Local anesthetics in standard dosage forms are usually effective for only a few hours. In cases where longer-lasting pain relief is necessary, additional anesthetic must be administered. This could be avoided by use of a sustained-release local anesthetic.

Desired characteristics of the microencapsulated local anesthetic include a size sufficiently small for syringe injection and an effective period of twenty-four hours or more. However, shorter effective periodsor larger microcapsules could be useful in particular situations. Thus, larger microcapsules could be applied directly to avulsive wounds.

B. RESEARCH PLAN

Lidocaine hydrochloride, a widely used local anesthetic, was microencapsulated with a biodegradable polymer, poly-L(-)-lactide, using a fluidized bed coating technique. A series of microcapsule batches with different coating thicknesses and sizes were prepared. In vitro drug release rates were measured for several of these microcapsule batches, and the most promising ones were evaluated in vivo by the sciatic nerve block test in the rat.

C. TECHNICAL BACKGROUND

1. Local Anesthetics

Since Freud and Koller introduced cocaine as a local anesthetic in 1884, several types of local anesthetics have played an important role in clinical medicine.

Local anesthetics offer the advantage of allowing a surgical plane of anesthesia to be attained while the patient remains fully conscious. In general surgery, local anesthetics are administered in several ways including topically, by infiltration, and as a nerve block. The same agents are also employed in spinal anesthesia (Ritchie and Cohen, 1975). In dental surgery, the two primary techniques for achieving localized anesthesia are by local infiltration of the nerve endings and blockage of nerves or nerve branches (Bennett, 1974).

Chemical structures of some common local anesthetics are shown in Table I. The presence of an amine group linked via an amide, ester, or ether intermediate chain to an aromatic ring is characteristic of these anesthetics. The structures of the base forms of the drugs are shown. For injection, hydrochloride, or other salts, are commonly used. Partial conversion to the base form occurs in vivo due to the neutral environment and the weakly basic properties of the aliphatic amine group.

Local anesthetics block the conduction of nerve action potentials in those nerve fibers and nerve endings exposed to a sufficiently high concentration of the agent.

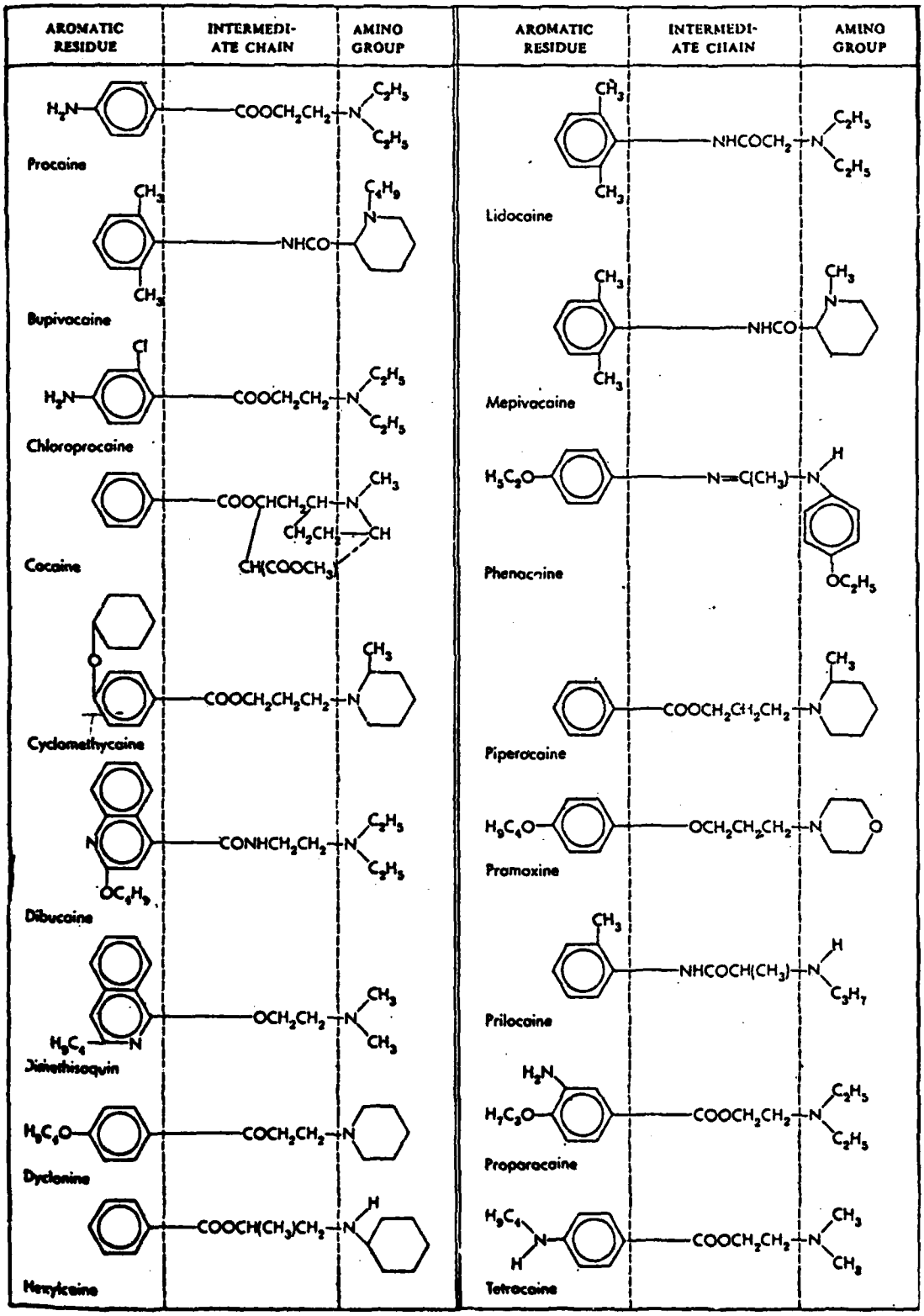
While there may be an effect on the metabolic activities of the nerve tissue, it has been found that the major effect of the anesthetic agent is at the membrane (deJong, 1970). The local anesthetics appear to interfere with the transmembrane movement of sodium and potassium ions which is necessary for the conduction of nerve action potentials.

The duration of effect of local anesthetics is dependent upon the period of time which the level of the agent remains at, or above, a minimum effective concentration. This period of time can be lengthened by decreasing absorption of the agent into the blood stream (localization), by increasing the dosage, or by repeated administrations of the drug.

Table I

CHEMICAL STRUCTURES OF LOCAL ANESTHETICS

(Source: Ritchie and Cohen, 1975)



Anesthetics have been marketed with longer durations than the lidocaine and procaine, which are commonly used, but these agents such as tetracaine and bupivacaine have also been found to be much more toxic (Bennet, 1974; Ritchie and Cohen, 1975). An appealing idea is to formulate an anesthetic agent which exhibits the lower toxicity of lidocaine, while providing much longer duration than has been seen to date for even the long-lasting agents. Microencapsulation offers this possibility.

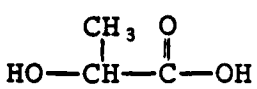
2. Microencapsulation with Polylactide

The term microencapsulation is applied to a process in which a particle, the "core", is encased by another substance, the "wall material", to form a distinct package. When the wall material is a solid, the microcapsules can be handled as a powder even though the core may be fluid. Microencapsulation refers to encapsulation of small particles; it is customary to consider particles of a few thousand microns in diameter or less as microcapsules.

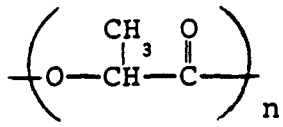
The core material may be a pure substance, a solution, or a mixture. The wall material may be any substance which can be deposited on the core material. The choice of wall material is dictated by the use of the capsule. Encapsulated material may be released from the microcapsule by removing the wall, such as by dissolution or rupture, or by permeation through the wall. Diffusion theory indicates that a steady state may be established in which the release of a drug from microcapsules will be constant with time if the wall separates two solutions with invariant concentration-time behavior.

An encapsulation technology based on fluidized bed coating of solid core particles with a biodegradable polymer, poly-L(-)-lactide was utilized in this program.

Poly-L(-)lactide is a polymer of lactic acid. Lactic acid and the polymer derived from it have the following structures.



Lactic Acid



Polylactide

Lactic acid is present in body tissues as a normal intermediate in the metabolism of carbohydrates. The notation L(-) indicates that the polymer is prepared from one of the two possible stereo-isomers of lactic acid. The stereo-regularity is not lost during the polymerization process.

The paramount requirement for a successful bioabsorbable polymer is the biological compatibility of the degradation products from the polymer. Polylactide has been found in numerous instances to be compatible with body tissues (Kulkarni, et al, 1966).

The rate of in vivo degradation of polylactide depends upon a number of factors including stereo-regularity, molecular weight, and morphology of the implant. The stereo-regular polylactides display the longest in vivo integrity, and the in vivo lifetime increases with increasing molecular weight.

As a part of an Abcor program sponsored by NICHD (Contract No. NO1-HD-3-2738), polylactide coated on nylon beads and rods and as a film were implanted in female New Zealand rabbits for varying lengths of time to determine the rate of in vivo degradation. Figure 1 shows the results of this study. It can be seen that the polymer is degraded by contact with the physiological environment. The polymer molecular weights may be obtained by referring to Figure 2.

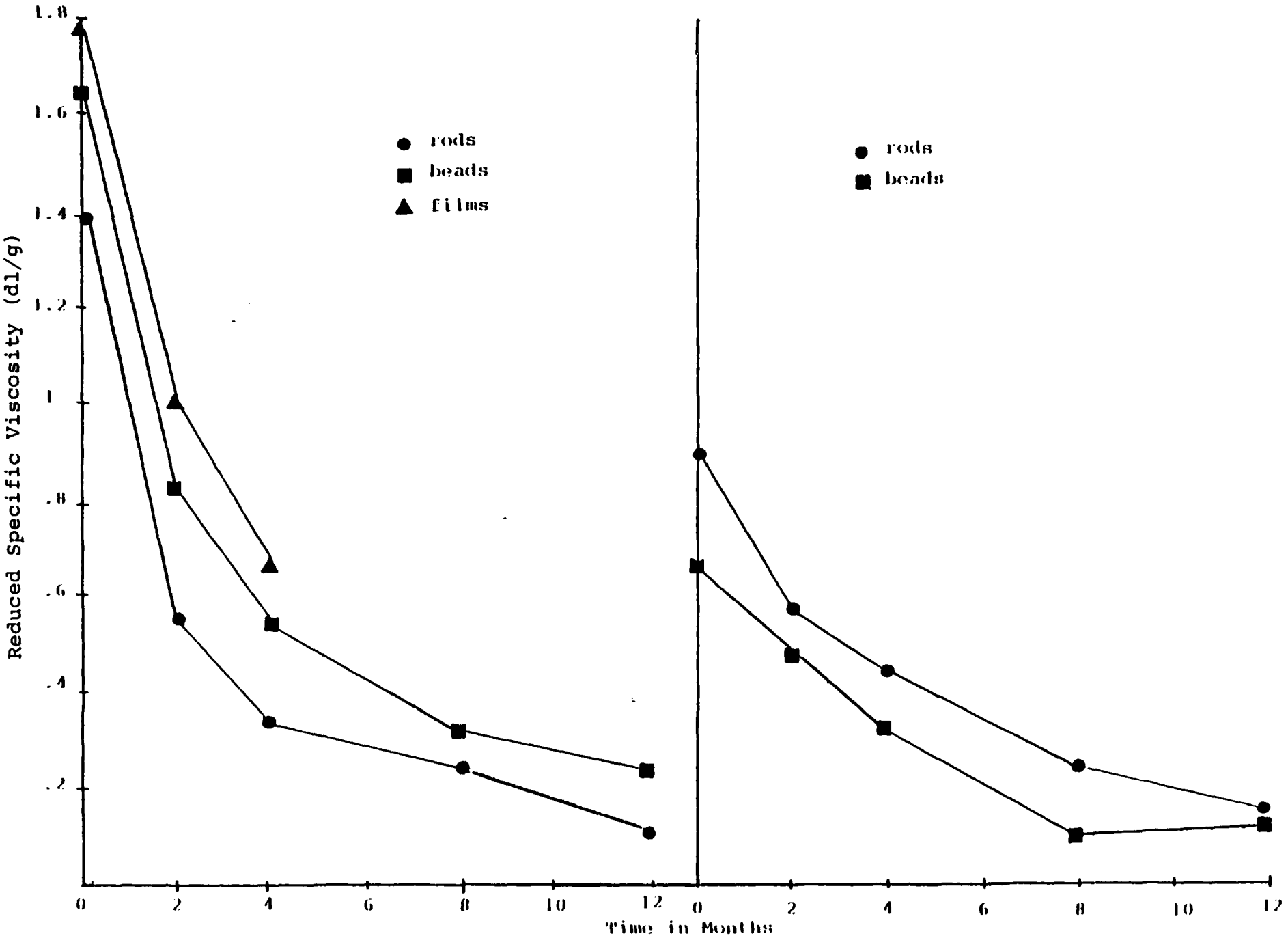
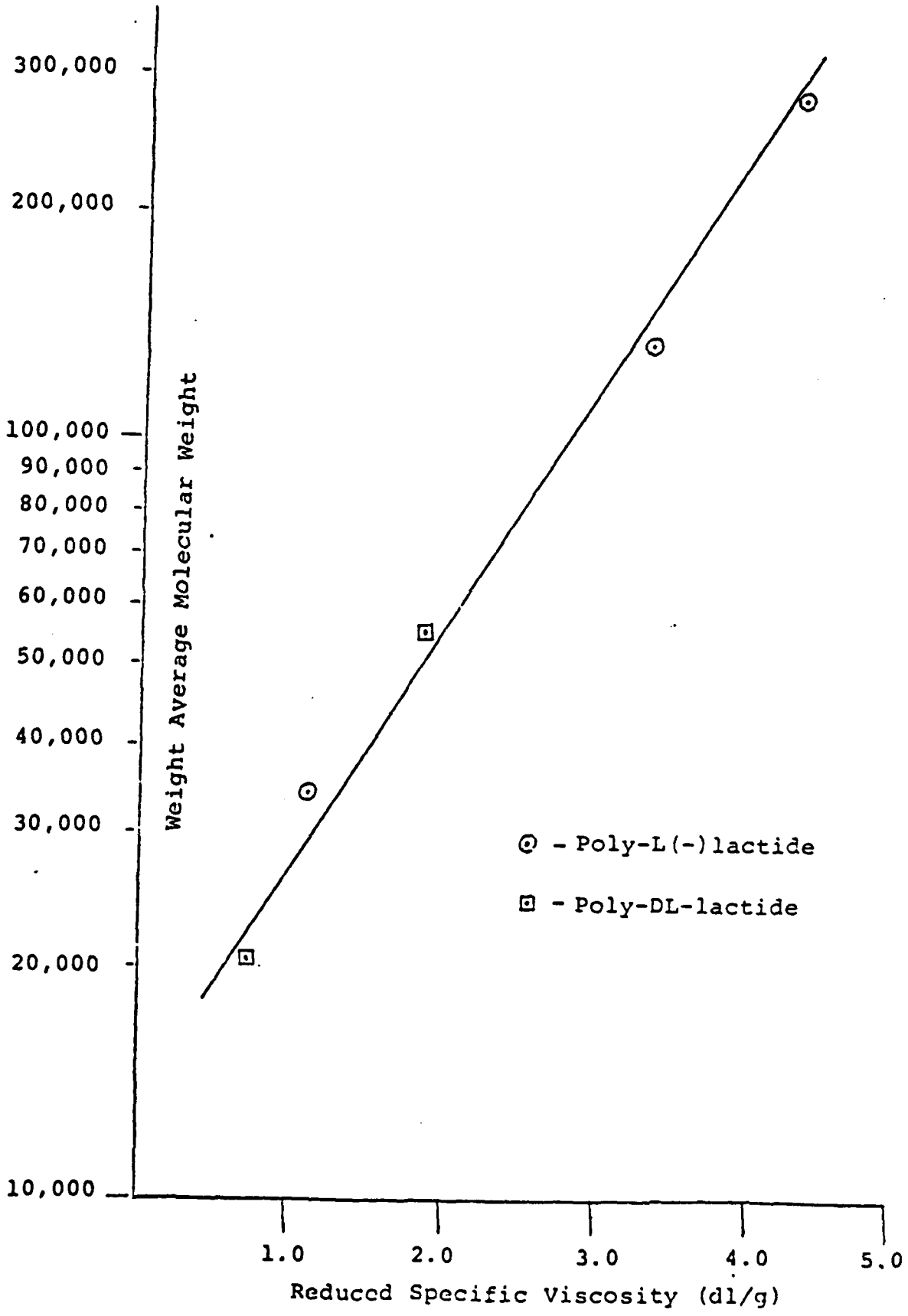


Figure 1 - In Vivo Polymer Degradation Data

Figure 2

CORRELATION BETWEEN WEIGHT AVERAGE MOLECULAR WEIGHT OF POLYLACTIDES AND REDUCED SPECIFIC VISCOSITY



3. Drug Release from Microcapsules

Fick's law of diffusion predicts that if a drug is enclosed in an inert membrane and if the drug concentration within the enclosure is constant and the concentration in the environment outside the membrane is held constant, a steady state will be established in which the diffusion rate of drug through the membrane will be constant in time (Baker and Lonsdale, 1974). A constant concentration inside a microcapsule may be obtained with a core which is a saturated solution of a drug (and for which undissolved drug is present) or with a core of a pure material. Constant concentration exterior to an implanted microcapsule would occur if the rate of drug release from a microcapsule is smaller than the rate at which the drug diffuses away from the microcapsule through surrounding tissues.

The core of the polylactide-coated lidocaine microcapsule will consist of a saturated aqueous solution of the drug (water enters the core region by diffusion through the polylactide wall).

For spherical microcapsules, the steady state drug release rate is given by:

$$\frac{dm}{dt} = \frac{4\pi R_o R_i DK\ (C_c - C_o)}{R_o - R_i}$$

Where:

$\frac{dm}{dt}$ = Mass of drug released per unit time

R_i = Radius of core

R_o = Radius of microcapsule

D = Diffusion constant of the drug in the wall material

K = Partition coefficient of the drug between the wall material and the surrounding medium (assumed to be the same inside and outside the microcapsule)

C_c = Concentration of drug in the core

C_o = Concentration of drug outside the microcapsule

The steady state release rate may be selected by choice of the parameters on the right-hand side of the above equation. If the drug and the wall material are given, the diffusion constant and the partition coefficent are determined. "C_o" will be determined by the environment of the microcapsule. Thus, in designing a drug delivery system with a predetermined drug (core) material, we actually only have at our disposal the geometric parameters, "R_o" and "R_i".

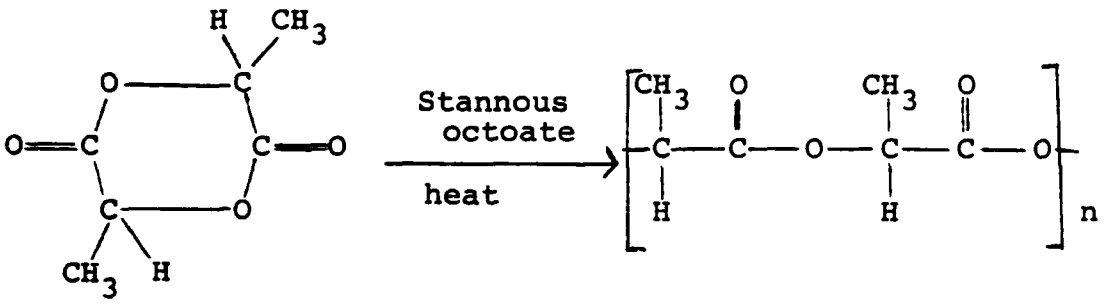
"R_o" is limited by the desire that the microcapsules be injectable. Experimentation at Abcor has shown that a 20 gauge needle is the largest size that should be used for injecting microcapsules. Larger sizes may result in bleeding with concomitant loss of microcapsules.

For highly water soluble substances such as lidocaine hydrochloride (which has an aqueous solubility of about 2 grams/ml), substantial osmotic pressure may develop within the microcapsule core and the microcapsule membrane may rupture. Such a mechanism may contribute substantially to, or may dominate, drug release.

II. EXPERIMENTAL METHODOLOGY

A. COATING POLYMER SYNTHESIS AND CHARACTERIZATION

The coating polymer, poly-L(-)lactide was prepared by a ring opening polymerization of L(-)lactide dimer using stannous octoate as a catalyst. The reaction involved is represented by the following equation:



Lactide Polylactide

Lactide dimer purchased commerically is not sufficiently pure for synthesizing polylactide of the desired molecular weight. The crude dimer obtained from Boehringer Ingelheim was purified by recrystallization from ethyl acetate to obtain starting material of sufficient purity.

Polymerization was carried out batchwise in bulk (500 gram batches). The polymerization of larger batches is difficult to control due to the exothermic nature of the reaction. The general synthesis procedure is described below.

Five hundred grams of purified L(-)lactide and a Teflon® coated magnetic stirring bar are placed in a one liter flask provided with a thermowell. The flask is heated under vacuum in a 120°C oil bath for thirty minutes in order to remove traces of volatile materials. Dry nitrogen is introduced to release the vacuum. The bath temperature is raised to 180°C. To the lactide, which is stirred vigorously, 0.2 ml of stannous octoate catalyst

(6 percent in mineral oil) is added. About fifteen minutes after the catalyst addition, the polymer mixture reaches its maximum temperature. About thirty minutes after the introduction of catalyst, the flask is removed from the oil bath and allowed to cool to room temperature.

The polymer is a tough solid which adheres tenaciously to glass. The flask containing the polymer is shattered, the polymer dissolved in methylene chloride, and the solution filtered to remove the glass. Isopropanol is added to precipitate the polymer, which is recovered and air dried.

Typically, a yield of about 80 percent of fractioned polymer with a reduced specific viscosity (RSV) of about 1.0 dl/g is obtained with dimer which has been recrystallized two or three times (melting point is 98.5-99.5°C). Polymer from several synthesis batches were blended together to give a homogeneous mixture of polymer for use in this program.

A number of properties of the 1.0 dl/g poly-L(-)lactide have been determined at Abcor. These include molecular weight determinations by gel permeation chromatography, infrared spectra, nuclear magnetic resonance spectra, differential thermal analysis, polymer sticking temperature, optical rotation, and density. Since composition and spectral analysis are similar for various polylactides, the only important variable is the molecular weight distribution. This affects the mechanical and physico-chemical properties of the polymer. For routine purposes, only the reduced specific viscosity (RSV) of each batch of polymer prepared is determined as an indicator of molecular weight.

According to the Mark-Houwink equation, the weight average molecular weight is related to the intrinsic viscosity in the following manner:

$$[\eta] = KM^a$$

In this equation, "M" is the weight average molecular weight, "η" is the intrinsic viscosity, and "K" and "a" are constants for a particular polymer solvent system. For most polymers the value of "a" has been found to be approximately 0.7.

The intrinsic viscosity is calculated from the experimental values as follows:

t_o = flow time of solvent through a viscometer

t = flow time of dilute polymer solution through the same viscometer.

η_{rel} = relative viscosity = $\frac{t}{t_o}$

η_{sp} = specific viscosity = $\eta_{rel} - 1 = \frac{t - t_o}{t_o}$

C = concentration in grams per deciliter

$\eta_{sp/C}$ = reduced specific viscosity (R.S.V.)

$[\eta]$ = intrinsic viscosity = $\lim_{c \to o} \frac{\eta_{sp}}{C}$

The reduced specific viscosity of polylactide synthesized at Abcor is determined in a 0.1 percent dioxane solution at 25±0.1°C, and is expressed in deciliters per gram (dl/g). A correlation between weight average molecular weight (Mw) and GPC and RSV is shown in Figure 2 for various Abcor polylactides.

B. CORE MATERIAL PREPARATION

Lidocaine hydrochloride was purchased from Sterling Organics in powder form. The melting point of the Sterling material was checked and found to be consistent with literature values for lidocaine hydrochloride hydrate. The term "lidocaine" will be used to refer to this material.

Sieve analysis of the drug (Table II) showed that the particle size was too large for direct coating as received. The size distribution was so broad that sieving to recover a fraction of small size would be uneconomical. Because of this, we decided to grind the material to a smaller size before coating. Two comminution methods were employed: ball milling and micronization.

Table II

Size Distribution of Lidocaine Hydrochloride

Size Range (microns)	Weight Percent	Cum. Wt. Percent
>210	43.0	100.0
177-210	12.5	57.0
149-177	10.0	44.5
105-149	18.1	34.5
74-105	2.9	16.4
44-74	11.3	13.5
<44	2.2	2.2

The <105 micron sieve fraction of the ball milled material was coated in microencapsulation Run L-1. Micronized material was coated without sieving in subsequent runs. One kilogram of lidocaine was micronized by Sturdevant Mill Company, Dorchester, Massachusetts, using a 4-inch micronizer at 2 lbs/hr. Material was less than 15 μm (99%), and most of the lidocaine was in the 1 micron range.

C. MICROENCAPSULATION

Lidocaine microcapsules were prepared by an air suspension coating process (Wurster, 1959). A schematic of the process equipment is shown in Figure 3. Particles to be coated are gently suspended in an upward moving air stream, which cycles them through coating and drying zones. A polymer solution is sprayed into the coating zone through a pneumatic nozzle. Spray droplets collide with the particles being coated and become attached. As the particles leave the coating zone, warm air dries the coating. After drying, the particles fall into an annular chamber surrounding the coating chamber 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. Particle size and size distribution are determined by several variables, each of which contribute to either collision frequency or sticking probability. When the particles are large, they may be individually coated. Below a certain size, small particles first agglomerate by sticking. A subsequent coating of the agglomerated particle ensues.

Process variables and the values of the variables employed in this contract are discussed in Section III.

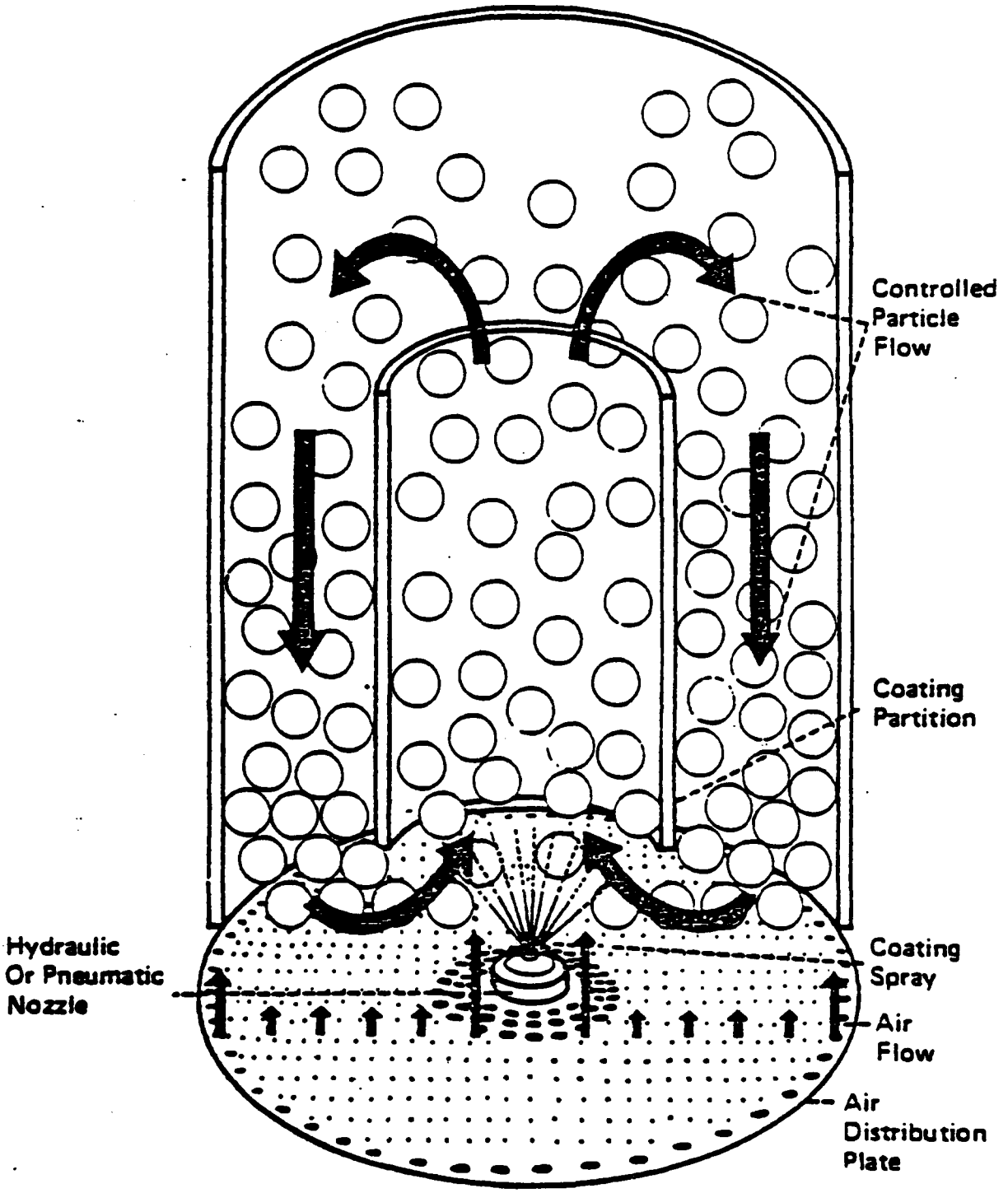


Figure 3 Fluidized Bed Coating Unit

D. CHARACTERIZATION OF MICROCAPSULES

1. Size Distribution

Microcapsule size distributions were determined by sieve analysis. A representative sample (∿30 grams) was taken from a 100-400 gram batch of microcapsules with the aid of a riffle sampler. Sample size was further reduced to about 3-4 grams by coning and quartering. The sieve analysis itself was performed with an Allen Bradley Sonic Sifter®. The sieves were preweighed and a weighed quantity of microcapsules was placed in the upper sieve. The sieves were agitated and then reweighed to determine the weight of microcapsules in each size fraction.

2. Assays

Assays for the drug content of microcapsules were performed by dissolving an accurately weighed quantity of microcapsules in dioxane and reading the absorbance of the solution at 263 nm with a spectrophotometer. The quantity of drug present was determined from a standard curve prepared with pure drug. Assays were conducted in triplicate.

3. Scanning Electron Micrographs

Scanning electron micrographs were obtained for intact microcapsules and for sections of microcapsules by Eastern Analytical Laboratories, Inc., of Burlington, MA. Sections were prepared by cutting microcapsules with a razor blade. Samples were usually examined at magnifications of 100x, 500x, and 1,000x.

4. <u>In Vitro</u> Release Rate Measurement

Drug release rates were determined by suspending a known mass of microcapsules in a known volume of an aqueous solution and periodically measuring the concentration of the drug in solution by spectrophotometry. After each measurement, the

solution was replaced with a fresh one. From the solution volume and concentration, the quantity of drug released was computed. This quantity divided by the length of time since the last measurement is the average drug release rate over the time interval.

An aqueous buffered solution (pH - 7.4) containing a 0.01% Hyamine (bacteriostat) was employed as a suspending solution for these studies. These solutions were thermostated at 37°C in a metabolic shaker bath. The vessel used for the release studies was a specially designed L-shaped test tube. The shape of this container promotes good mixing of the release solution when used in a metabolic shaker and thereby reduces local drug concentration gradients in the solution since this might affect the release rate. The microcapsules were placed in a tea-bag like structure constructed from fine polyester mesh for convenience in separating the microcapsules from the suspending solution.

Release measurements were conducted in triplicate. Release rate data was stored on magnetic tape and plotted as percent drug release versus time.

III. RESULTS

A. MICROENCAPSULATION RUNS

Eight coating runs, designated L-1 through L-8 were made during the course of this work. A general description of the coating equipment and the coating process is presented in Section II-C. Run L-1 was conducted to investigate the behavior of lidocaine in the coating equipment. Runs L-2 and L-3 were designed to provide lidocaine coated to the 10 percent level with polylactide for further coating in Run L-4. In Run L-5, the effect of process air temperature on the coating process was studied. Runs L-6 through L-8 were made to produce more highly coated microcapsules similar to those prepared in Runs L-2 through L-4 for *in vitro* and *in vivo* study.

Coating process variables included core material properties, coating polymer, coating polymer solvent and solution concentration, the type of atomizer used for spraying the polymer solution, atomizer air pressure, and the fluidization air temperature and flow rate. Values of these variables are listed in Table III.

The coating equipment used for this program has an air sweep system designed to dislodge material which has become deposited on its inner surfaces. This system consists of several air nozzles positioned on a revolving framework inside the coating unit. The air sweep system was activated for a 15 second period every 2.5 minutes in all runs.

Process air exiting the coating unit passes through a fabric filter system to remove suspended solids and return them to the coating chamber. Goretex fabric filters were used in this system for all runs.

Table III

Coating Process Variables

Variable	Value	Run
Core Material	Ball Milled lidocaine	L-1
	Micronized Lidocaine	L-2, L-3, L-6, L-7
	10% Polylactide-Coated, Micronized Lidocaine	L-4, L-8
Coating Polymer	0.89±0.03* dl/g Poly-L(-)lactide	L-1 through L-8
Solvent	Methylene Chloride (Fisher Certified Reagent Grade)	L-1 through L-8
Coating Solution Concentration	3% Weight/Volume	L-1 through L-8
Solution Addition Rate	4 to 20 ml/min	See Text
Atomizer	2850/70, Pneumatic Type Spraying System Co., Wheaton, IL	L-1 through L-8
Atomizer Pressure	25 psig	L-1 through L-8

* Mean±Standard Deviation for three determinations

1. Run L-1

Core material for Run L-1 was 400 grams of <105 micron ball milled lidocaine. A relatively high coating solution addition rate, 20 ml/min, was selected for this run in order to minimize its length. The faster the solution addition rate, the more rapidly a given coating level can be achieved; however, at some point the rate of addition will exceed the rate of solvent evaporation. When this occurs, the particles remain "sticky" and agglomerate. A high rate of addition was employed to explore the practical upper limit for this important process variable.

Samples were taken from the coating equipment at nominal coating levels (weight of polymer added/weight of polymer plus weight of core material) of 4%, 8%, 12%. Large agglomerates were found at the 12% level. Sieve analyses showed that substantial agglomeration had occurred between 4 and 8 percent (see Table IV).

On the basis of information obatained from L-1, the high addition rate was retained for the two succeeding runs L-2 and L-3. Also, these runs were stopped at the 5 percent level and the coated drug was sieved to remove large agglomeration.

2. Run L-2, L-3, and L-4

Run L-2 and L-3 were conducted to produce lidocaine coated to the 5 percent level for further coating in L-4. Core material for L-2 and L-3 was micronized lidocaine (440 grams for each run).

In Runs L-2 and L-3, polylactide was applied until the nominal coating level reached 5 percent. This material was sieved to recover the <44 micron fraction which was further coated in L-4. The weight of this fraction from L-2 and L-3 was 436 grams.

In Run L-4, a sample was taken at the 20% coating level for *in vitro* evaluation. The run was continued to the 25 percent level.

TABLE IV

Microcapsule Size Distribution (Weight Percent)

Run #	Size (μm)	0% Wt%	4% Wt%	5% Wt%	8% Wt%	20% Wt%	26% Wt%	31% Wt%	40% Wt%	50% Wt%
L-1	>177	5.0	12.8	12.8						
	149-177	2.4	4.1	4.1						
	125-149	4.9	10.8	10.8						
	105-125	5.8	15.8	15.8	---	---	---	---	---	---
	74-105	19.6	19.4	19.4						
	44-74	19.2	24.7	24.7						
	<44	43.1	12.4	12.4						
L-2	>177	0		7.5						
	149-177	0		6.0						
	125-149	0		14.4						
	105-125	0	---	8.0	---	---	---	---	---	---
	74-105	0		20.1						
	44-74	0		21.5						
	<44	100		22.5						
L-3	>177	0		15.6						
	149-177	0		7.4						
	125-149	0		10.3						
	105-125	0	---	10.5	---	---	---	---	---	---
	74-105	0		21.1						
	44-74	0		15.3						
	<44	100		19.7						
L-4	>177			0		6.9	23.1			
	149-177			0		4.0	9.0			
	125-149			0		7.2	11.7			
	105-125	---	---	0	---	7.7	13.2	---	---	---
	74-105			0		23.4	14.0			
	44-74			0		29.8	22.3			
	<44			100		21.1	6.7			
L-8	>177							1.7	6.0	5.5
	149-177							1.0	4.2	9.9
	125-149							7.2	15.6	20.5
	105-125	---	---	---	---	---	---	7.5	11.8	20.2
	74-105							13.1	12.9	28.7
	44-74							53.0	49.6	13.0
	<44							16.5	5.9	2.4

of this material were coated to the 50 percent level. Samples were taken at the 31 and 40 percent levels for *in vitro* study. A low addition rate, 4.5 ml/min was utilized in L-8 to reduce agglomeration. A higher process air temperature was also employed to decrease the rate of solvent agglomeration.

B. MICROCAPSULE CHARACTERISTICS

1. Size Distribution

Microcapsule size distribution, obtained by sieve analyses as described in Section II-D-1 are reported in Table IV. This data indicates the relative yields of microcapsules within a given size range. In several instances, the entire lot of microcapsules was sieved at some point in the coating process. This must be taken into account in computing absolute yields.

The size data indicates a general trend towards larger sizes as the coating level increases. This is due not only to increase in size of microcapsule due to addition of polymer coating but also to agglomeration of microcapsules.

2. Morphology

Figures 4 - 10 are scanning electron photomicrographs of lidocaine microcapsules from Runs L-4 and L-8. Figures 4 - 8 show the change in appearance of microcapsules during the coating process. As is shown in the cross-sectional view of Figure 4, drug particle aggregates are initially coated with a thin shell of polymer. These apparently agglomerate (Figure 5) and eventually acquire a smooth coating (Figures 6 - 8).

Figures 9 and 10 show the appearance of the microcapsules before and after drug release (in an aqueous buffer solution, pH=7.4). Pockmarks and some cracks appear on the microcapsules. It is possible that some drug is released through these surface defects.

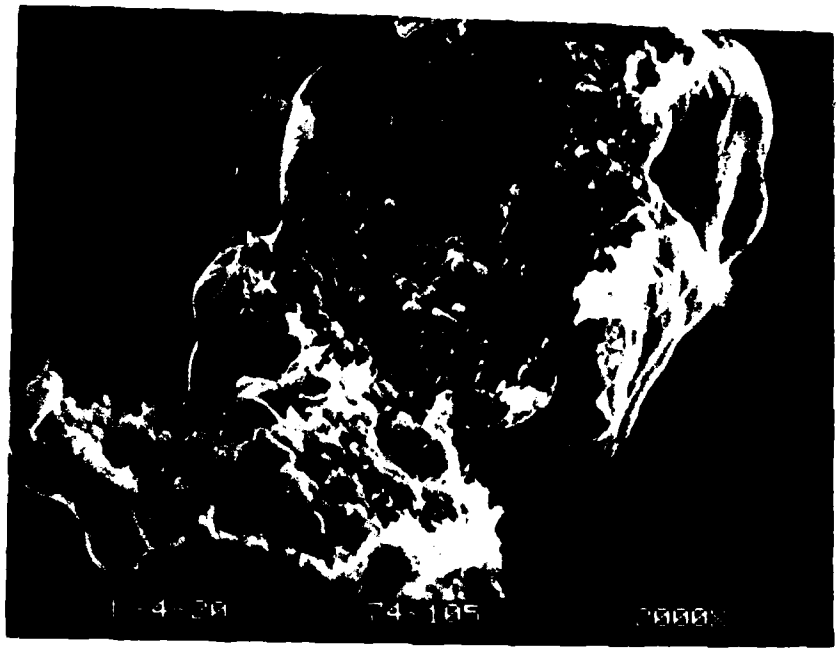


FIGURE 4

Photomicrograph of Cross-section of Lidocaine Microcapsules from Run L-4.

Coating Level: 20%
Size Fraction: 74-105 microns
Magnification: 2000X

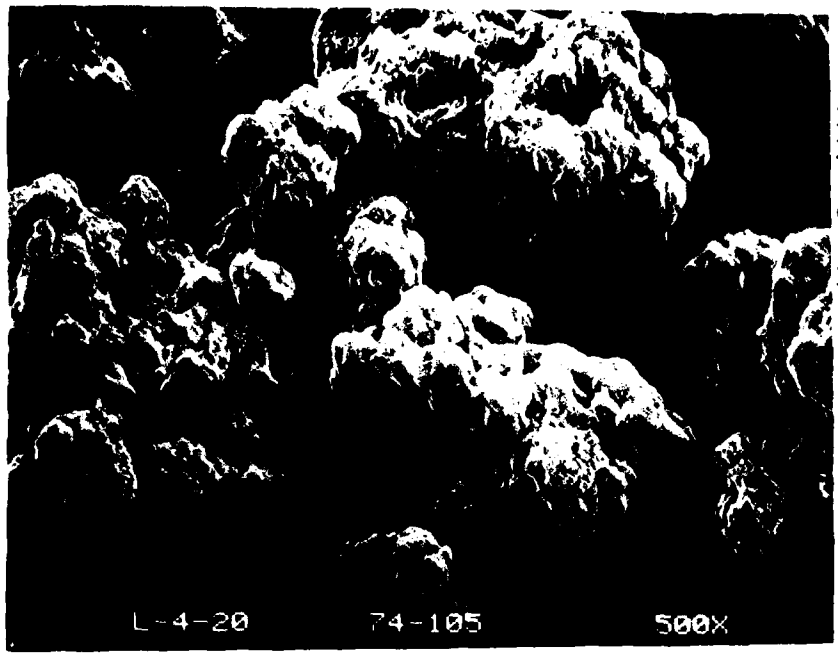


FIGURE 5

Photomicrograph of Lidocaine Microcapsules from Run L-4

Coating Level: 20%
Size Fraction: 74-105 microns
Magnification: 500X

FIGURE 6

Photomicrograph of Lidocaine Microcapsules from Run L-8

Coating Level: 30%
Size Fraction: 125-149 microns
Magnification: 500X

FIGURE 7

Photomicrograph of Lidocaine Microcapsules from Run L-8

Coating Level: 40%
Size Fraction: 125-149 microns
Magnification: 500X

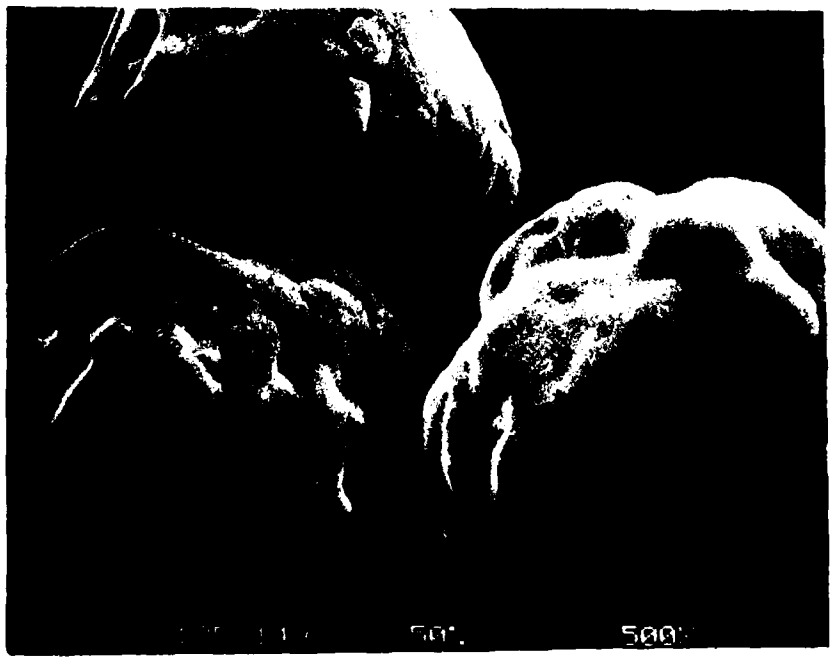


FIGURE 8

Photomicrograph of Lidocaine Microcapsules from Run L-8

Coating Level: 50%
Size Fraction: 125-149 microns
Magnification: 500X

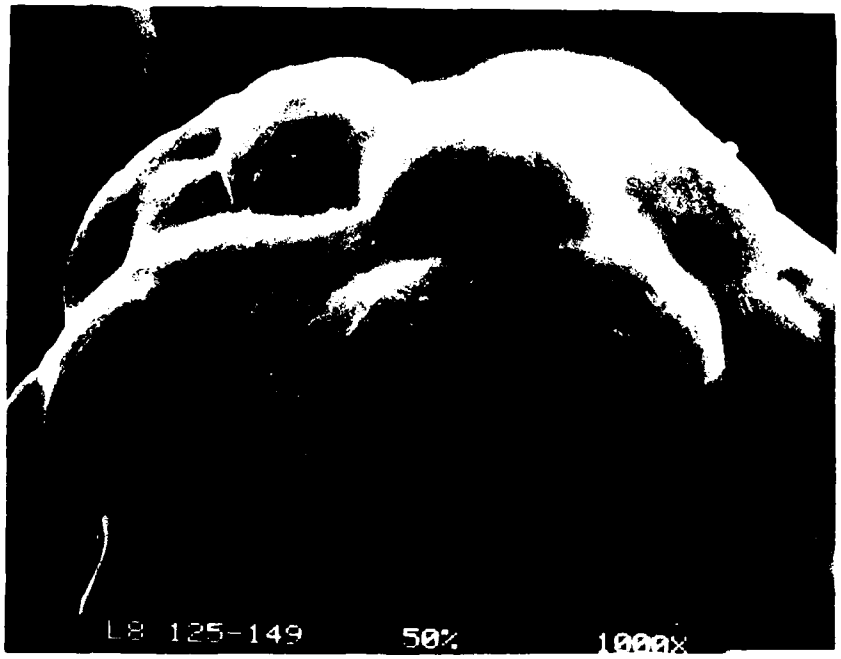


FIGURE 9

Photomicrograph of Lidocaine Microcapsules from Run L-8 Before Diffusion Study

Magnification: 1000X

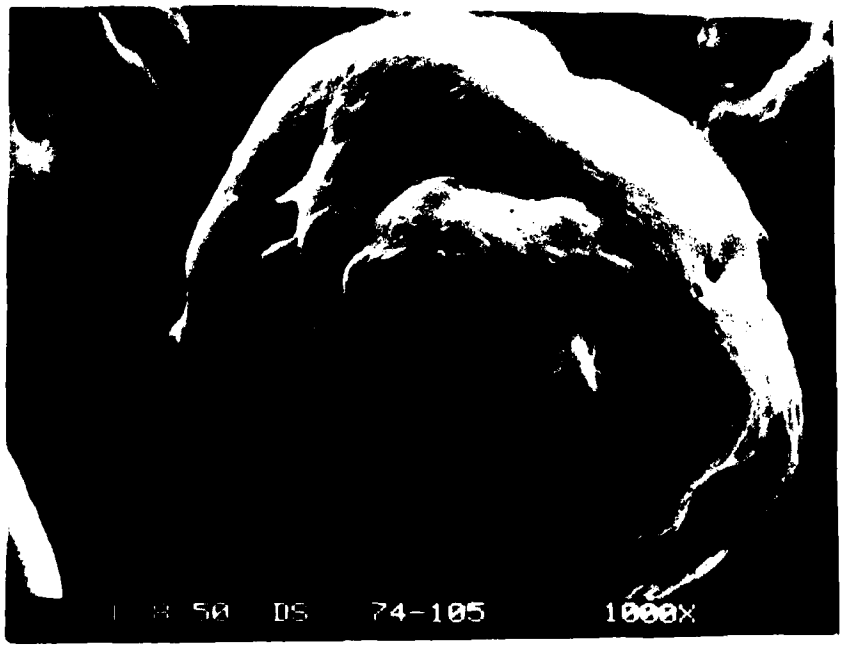


FIGURE 10

Photomicrograph of Lidocaine Microcapsules from Run L-8 After Diffusion Study

Magnification: 1000X

At this point it was noted that large agglomerates were being formed. This apparently occurred because of blockage of the atomizer air flow. The coated material was sieved to remove the large particles; however, so little material remained in the small sieve fraction (<105 microns) that continuation of the run was not warranted.

3. Run L-5

Run L-5 was conducted to investigate the use of higher process air temperatures in coating lidocaine with poly-L(-)lactide.

The use of the highest feasible air temperature in the coating process is desirable in that it provides the most rapid vaporization of the polymer solvent and consequently allows the highest rate of coating solution addition. In order to explore the range of temperatures suitable for lidocaine coating, drug coated to 10 percent with 0.89 dl/g poly-L(-)lactide in Run L-1 was placed in the coating unit and the process air temperature gradually increased (with no addition of polymer). At 130°F (inlet air temperature, as measured in the plenum below the coating chamber), microcapsules began to stick to the coating unit and some agglomeration occurred. It was concluded that an inlet air temperature of up to 130°F may be safely employed. The bed temperature corresponding to this inlet air temperature was about 90-95°F.

4. Runs L-6, L-7, and L-8

This sequence of runs was performed in order to prepare microcapsules similar to those produced in Runs L-2 through L-4, but coated to a higher level. In L-6 and L-7, 440 and 369 grams, respectively, of micronized lidocaine were coated to the 10 percent level. A polymer solution addition rate of 20 ml/min was employed in both runs. The products of these two runs were combined and sieved to obtain the 44-105 micron fraction. In L-8, 300 grams

C. IN VITRO DRUG RELEASE RATES

1. Microcapsules from Run L-4

Release rate studies were conducted on two batches of microcapsules from Run L-4, designated L-4-20 and L-4-26. These batches had nominal coating levels of 20 and 26 percent, respectively. (The nominal coating level is the quantity of polymer added to the coating unit divided by the sum of the weight of the core material and the coating polymer.) Studies were performed on the 74-105 micron sieve fraction in both cases. Both batches exhibited a very fast rate of release. Essentially all the drug was released within one hour. Because of this fast release rate, a new sequence of coating runs was initiated to produce more highly-coated and presumably more slowly-releasing microcapsules.

2. L-8 Microcapsules

Release rates were measured for 50% coated L-8 microcapsules in three size ranges, 74-105, 125-149, and 177-210 microns. The results are shown in Figure 11. Theoretically, it would be expected that the rate of release would decrease with increasing microcapsule size. The 177-210 micron fraction does not obey this rule. Scanning electron photomicrographs of this fraction (Figure 12) showed that it contained aggregates of smaller microcapsules; this accounts for its anomalous behavior.

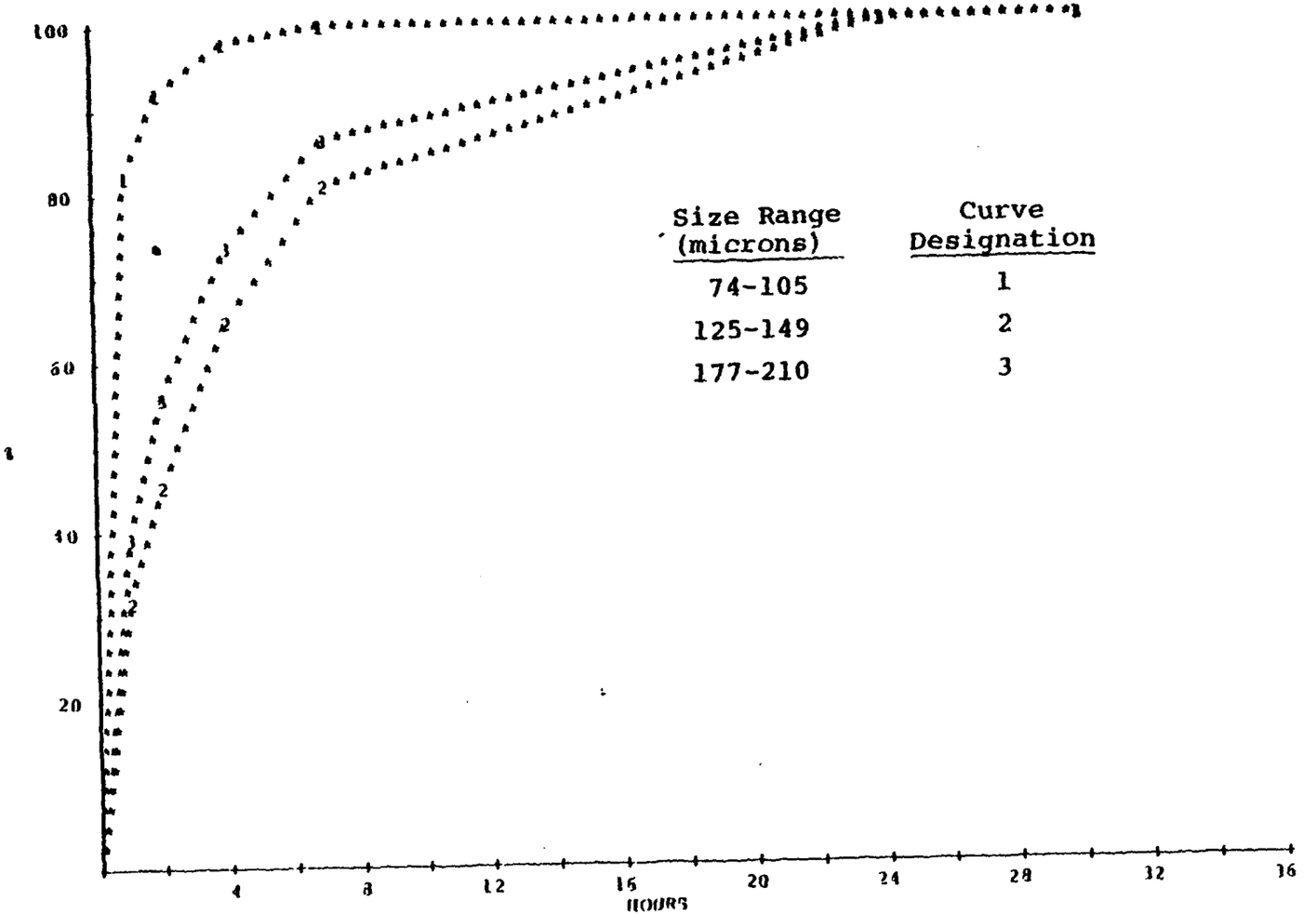


FIGURE 11: Release Curve for Lidocaine Microcapsules from Run L-8
Nominal Coating Level - 50%

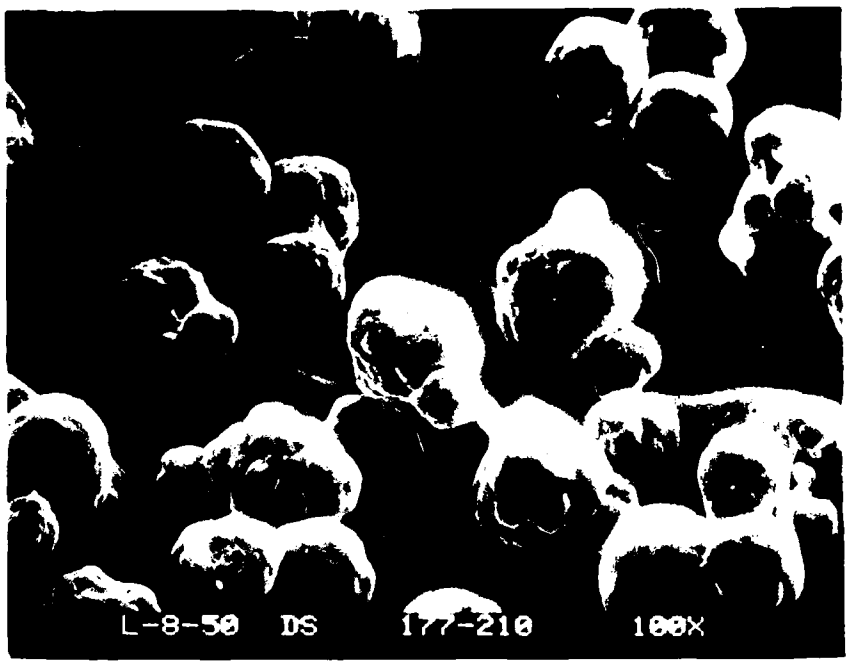


Figure 12

Photomicrograph of microcapsules from Run L-8, 177-210 micron sieve fraction.

Demonstrates that many of the microcapsules in this fraction are in fact aggregates of smaller microcapsules.

D. IN VIVO TEST RESULTS

The sciatic nerve block test in the rat (Camougis and Takman, 1971) was employed for in vivo evaluation of the microencapsulated lidocaine. A sciatic nerve block was induced by injection of the anesthetic agent under the skin at the junction of the biceps femoris and gluteus maximus muscles. Anesthesis is indicated by a characteristic change in stance which occurs when the sciatic nerve is blocked. The time until onset of conduction block, the degree of block (partial or complete), the duration of block, and the frequency (number of rats in group exhibiting block) were recorded.

Sprague-Dawley rats, purchased from Charles River Breeding Laboratories in Wilmington, Massachusetts, were utilized in the test. These animals were housed in individual cages in the controlled environment of the Abcor animal facility. Food and fresh water were supplied ad libitum.

Tests were conducted in a total of 113 animals (controls and microcapsule injections). On the basis of the various preliminary studies, conclusions were made about dosage and the required size of the needle, and these parameters were utilized in the final studies.

During the preliminary rat studies, there were a large number of problems with placement of the dose in the proximity of the sciatic nerve. Some of the problems were solved through practice and the use of a spacer to reduce the effective length of the needle. These studies are summarized in Table V. All microcapsules were from Run L-8-50 and were injected through a 22 gauge needle.

Tests in other contract work had indicated that a 22 gauge needle had a sufficiently large bore that microcapsules would not be significantly held up by the needle during the injection. Our

Table V

Preliminary In Vivo Studies

Material	Vehicle*	Size (μm)	Dose (mg Lidocaine)	Onset (minutes)	Duration (minutes)	Frequency
Lidocaine 1%	Saline/ CMC	----	2	2.2±0.5	44±25	5/8
L-8-50	SSV	125-149	4	---	---	0/6
L-8-50	SSV	125-149	8	3.4	7 (partial)	1/6
L-8-50	SSV	74-105	8	3.7±0.3	57±12	2/6
Lidocaine 1%	Saline	---	2	3.2±1.1 4.4±2.1	28±11.7 , 3± 0.95	8/12 Complete 3/12 Partial
Lidocaine 1%	ASV	---	2	1.5±0.6	47± 8.6	3/6 Complete
L-8-50	ASV	125-149	8	3.5±0.3	41±29	3/6 Complete
Lidocaine 2%	ASV	---	4	4.2±5.7	76±22	11/12
L-8-50	ASV	125-149	20	5.7±5.0	61±33	6/12

* CMC - Carboxymethyl Cellulose

SSV - Steroid Suspending Vehicle (NCI:UT-74-901)

ASV - Aqueous Suspending Vehicle (See Text)

first series of studies showed few blocks in the microcapsule-dosed animals, and short durations for those which did block. Inspection of the hub of the needles after injection showed that a major portion of the sample was remaining in the syringe and needle, causing dose levels to be unreliable.

One interesting observation from the last of these studies is that the microcapsules apparently continue to release for several hours after the block has disappeared. In the last study, six rats were injected at about 9:30 in the morning and the two blocks which occurred had an average duration of 47 minutes. At about 1:15 p.m., the contralateral legs were injected on the same animals giving four blocks with an average duration of 68 minutes (Maximum = 114 minutes). At the same time, one of the legs which had been used in the morning re-achieved a partial block indicating that the microcapsules were still releasing.

On the basis of these results, it was decided not to re-use needles or rats. New needles were ordered which were shorter (1/4") and had a larger bore (20 gauge).

In creating a control solution with which to compare the response to the microcapsule doses, a 1% solution was initially chosen. Since the frequency of block was not sufficient at this dose level, a 2% solution was chosen.

The maximum amount of microcapsules which could be suspended in the vehicle with effective dosing was an amount such that the drug concentration (based on an assay of the microcapsules) was 40 mg/ml. This was the suspension concentration used for all studies and is 4%, as lidocaine. An aqueous microcapsule suspending vehicle (ASV) containing 9 grams sodium chloride, 5 grams sodium carboxymethylcellulose, and 4 ml sorbitan monolaurate (Tween 20) per liter was chosen for further tests.

The first experiment in the final series compared 0.2 ml doses

of two different size range microcapsules (74-105 μm and 125-149 μm) with the control. The results are shown in Table VI.

On the basis of the study using 0.2 ml volumes, it was decided that the 74-105 μm size offered the most promise, but that a larger dose might be needed. A new study was performed using 0.5 ml volumes.

The results of the studies (Table VII) show that the microcapsules were significantly longer-lasting than the 2% control dose ($p<0.001$). Therefore, a final experiment was performed using an equal amount of lidocaine in solution, as was contained in the microcapsules. Unfortunately, the duration of nerve block was slightly longer with the 4% solution than with the microcapsules. The onset of the block with microcapsules, however, requires a considerably longer time (similar to 1% lidocaine solution).

Although no obvious toxic effects were noted in the rat when the 4% solution was used, toxic effects are expected to be more severe with the solutions of lidocaine than with microcapsules. To demonstrate an improved duration of anesthesia as a nerve block, an even larger quantity of lidocaine microcapsules may be required. Unfortunately, this is difficult to inject in the area of the sciatic nerve of the rat.

Comparison of a block of a major nerve trunk with the block of a fine nerve network, such as in the skin (or cornea), is an important consideration in this program. The amount of drug required to block the sciatic nerve is large because of the size of the nerve bundle and the need for the drug to penetrate the nerve sheath. These blocks are therefore also longer-lasting than skin or corneal blocks. Thus a very demanding model was chosen to demonstrate the effect of microencapsulated drug. Larger quantities of these microcapsules would probably be needed at the site than has been injected in this program. These concepts are diagrammed in Figure 13.

TABLE VI

Results of In Vivo Studies Using 0.2 ml of Vehicle (20 gauge needle)

Substance	Total No. Animals	Non-Block	Partial	Complete	Time of Complete Block (in minutes)	
					Onset	Duration
2% Control	6	0	0	6	4.6±3.0	44.6±25.9
74-105 µm Microcapsules	6	2	1	3	6.0±2.7	58.8±39.4
125-149 µm Microcapsules	12	5	1	6	11.2±6.4	32.6±16.5

TABLE VII

Results of In Vivo Studies using 0.5 ml of Vehicle (20 gauge needle)

Substance	Total No. Animals	Non-Block	Partial	Complete	Time of Complete Block (in minutes) Onset	Duration
2% Control	9	0	0	9	1.1±0.3	110±19
74-105 µm Microcapsules	18	0	1	17*	2.8±1.1	168±32
4% Solution	6	0	0	6	0.6±0.2	196±23

* For three of these animals, a large portion of the dose was held up in the syringe and never delivered. This led to shortened duration and consequently was treated as extraneous data, and not included in the calculations.

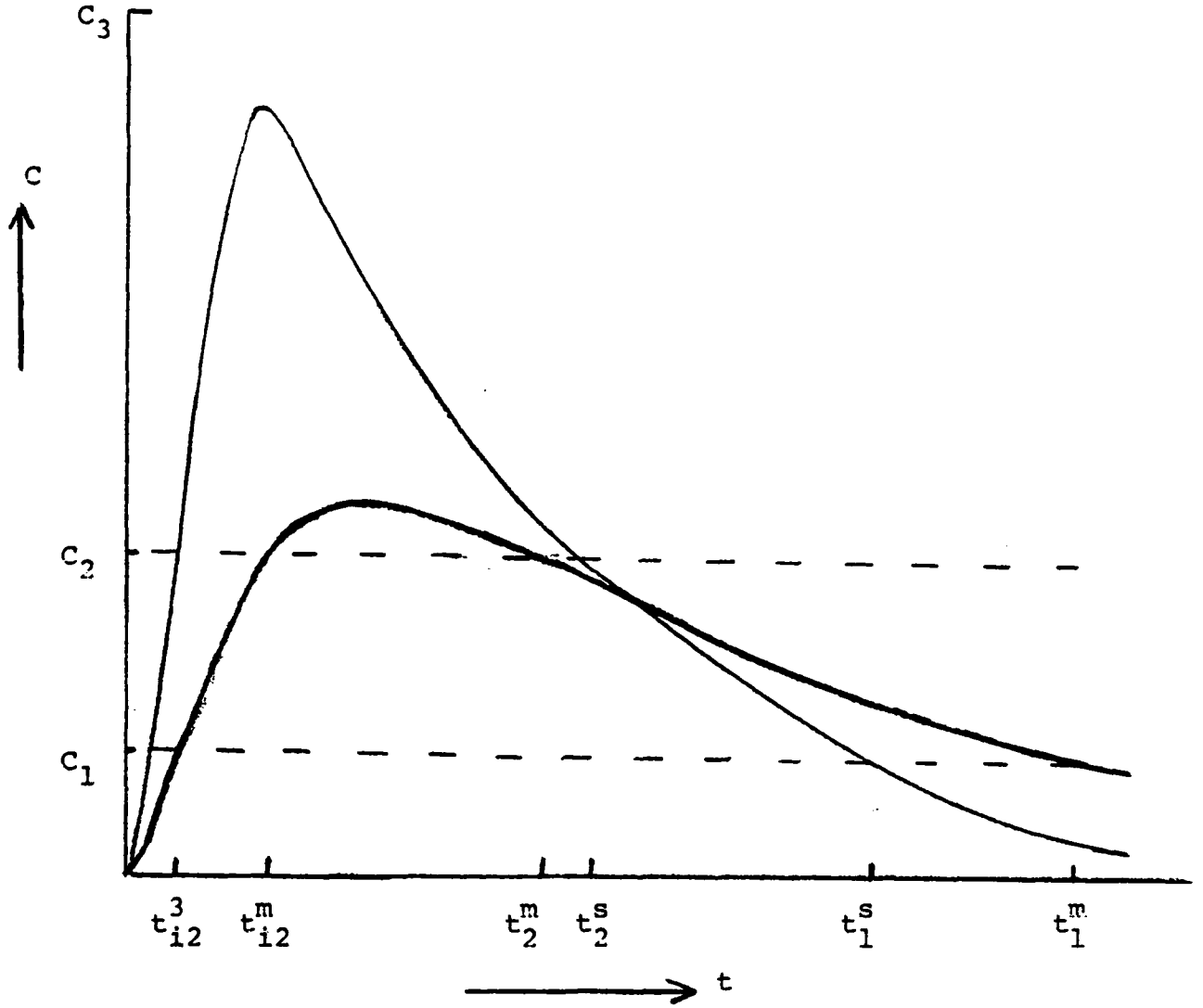


Figure 13 Schematic of Lidocaine Concentration at Nerve Sites

C_2 - Concentration for nerve block (sheathed bundle)

C_1 - Concentration for peripheral nerve end anesthesia

C_3 - Concentration for toxicity

t_{i2} - Time to initiate nerve block
t^s_{i2} - solution
t^m_{i2} - microcapsules

t_2 - Time to end of block of nerve bundle

t_1 - Time to end of peripheral nerve anesthesia

Using the present approach, serum levels of lidocaine could be measured to document the slow release of lidocaine from the injected microcapsules. Other approaches (e.g., using the response of the rabbit cornea or guinea pig wheal, Camougis and Takman, 1971) could use fewer microcapsules, lower solution concentrations and larger needles. It was not possible to pursue these approaches on the present contract.

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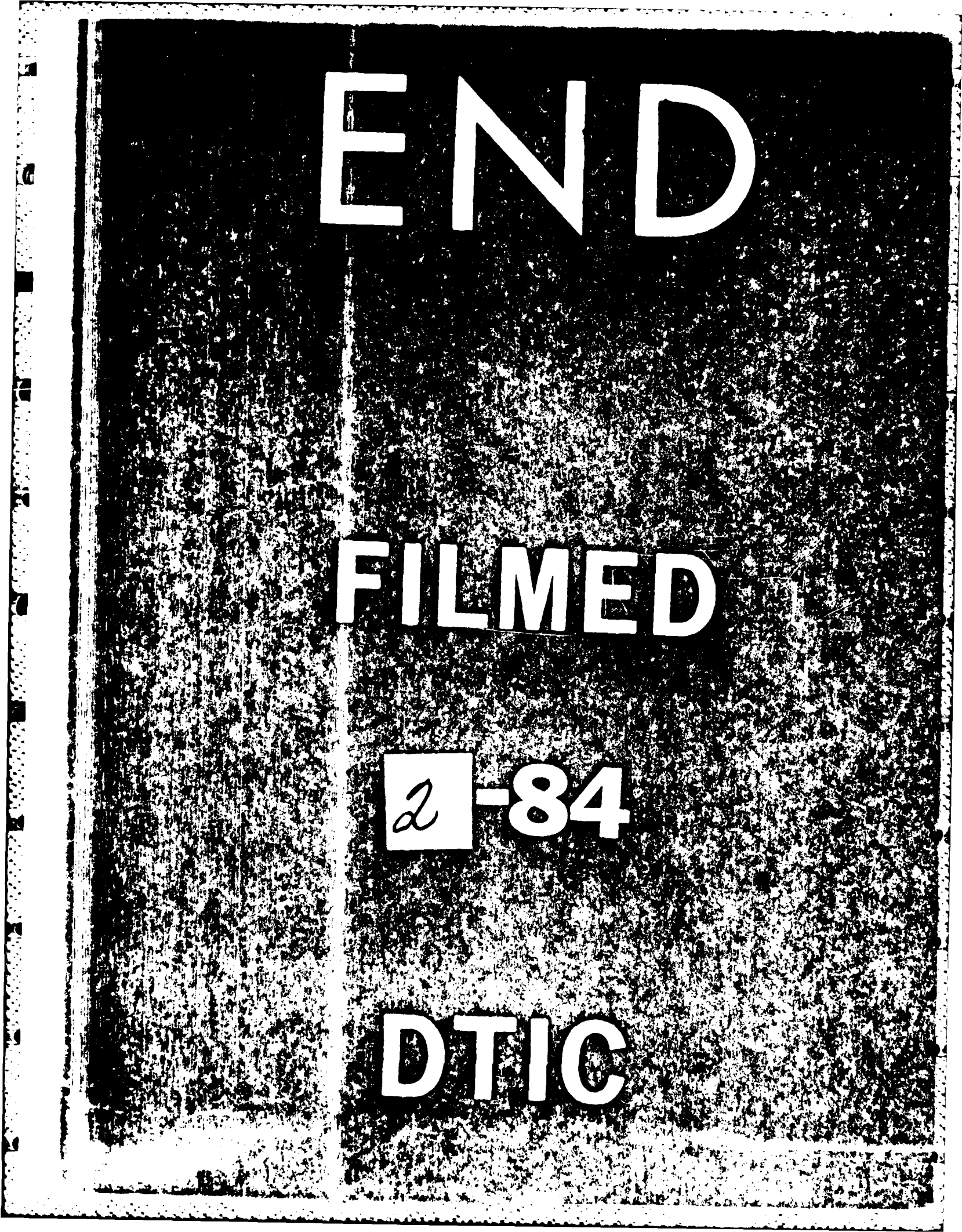
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