An *in vitro* study of a ring-shaped device made from 2-hydroxyethyl methacrylate-methyl methacrylate copolymer for the extended release of tylosin tartrate

by

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INTRODUCTION

Statement of Problem

The eye is a distinctive organ which presents unique problems when pharmacological treatment is required due to disease. An effective drug delivery method should localize the effect of the drug action while providing sufficient action for the duration required for treatment. Prolonging the drug action while reducing the frequency of administration is of particular interest in the field of veterinary medicine. Ophthalmic disorders in livestock are costly both in terms of reduced production and time required for administration of treatments.

Current treatments of bacterially-induced ophthalmic disorders in livestock involve topical applications of antibiotics and sulfonamide in the form of eyedrops, sprays, powders, or ointments over a five- to seven-day period. Since lacrimation rapidly removes these compounds from the eye, multiple daily treatments are required. This repetitious regimen is time-consuming and costly; for this reason a more efficient drug delivery method is of interest.

This work describes the development and evaluation of a ring-shaped system with possible application as a method of administering antibiotic to the eyes of cattle. Such a controlled-release system would maintain a therapeutic level of a suitable drug in the eye and would eliminate the repetition of the current treatments. This system uses biocompatible polymers to regulate the rate and duration of the drug release.

Hydrogels were chosen for these systems, as certain formulations have gained wide use in contact lens applications, and they have controllable water permeability characteristics. Tylosin tartrate, an agricultural antibiotic which effectively eradicates the most common organisms associated with bovine ocular infections, was chosen as the treatment drug.

In addition to having proper in vitro drug release characteristics, an acceptable ocular insert system must also be capable of remaining in the animal's eye for extended periods of time without causing adverse side effects. An important part of this study was an attempt to develop a method of fabricating the devices in an acceptable configuration. A tubular ring-shaped device was made, and several fabrication techniques were evaluated based on their ability to produce proper in vitro drug release rates. These experiments were all directed at determining the feasibility of using a hydrogel-based ocular insert to maintain a therapeutic level of an antibiotic within the bovine eye for an extended period of time.

LITERATURE REVIEW

Nature of the Problem

Bovine Infectious Keratoconjunctivitis is a widespread, bacterially-induced ocular disorder in cattle. The disease is commonly referred to as 'pinkeye'. A strain of Moraxella bovis, a common agricultural bacteria, induces Bovine Infectious Keratoconjunctivitis (BIK). Although the exact mode of natural transmission of the disease is unknown, it can be produced by transferring Moraxella bovis into the conjunctiva of the eye (Pugh and Hughes, 1975). Depending on the progression of the disease prior to treatment, corneal opacities, corneal ulcers, and temporary or permanent blindness may result; in rare cases fatal meningitis develops (Jensen and Mackey, 1965). Deaths in range cattle result from starvation, drowning, and falling from high places due to impaired sight (Baldwin, 1945). The disease also adversely effects the growth and productivity of cattle confined to feed lots (Thrift and Overfield, 1974), and a study on dairy cattle found a 25% decrease in milk production during the course of the disease (Baldwin, 1945).

Present Treatment Methods

Early cases of BIK are treated with topical applications of antibiotic solutions and compounds containing chloramphenicol, oxytetracycline, penicillin-streptomycin (Jensen and Mackey, 1979; Blood and Henderson, 1979), or tylosin (Burger, 1970; Rossoff, 1974). Table 1 summarizes the major treatments and the drug delivery method used for

each. Eyedrops, ointments, sprays, and powders are all common, generally inexpensive, and simple methods for treating ocular infections. Although various additives may prolong their effects, all of these methods suffer relatively low retention times. Gelatt et al. (1979) estimated that 80% of an eyedrop is lost immediately after instillation. However, as systemically administered drugs may cause unwanted side effects, topical applications are generally preferred over parenteral treatments or injections (Chiou and Watanabe, 1982). Soft contact lenses soaked in drug solutions have met with success in human medicine (Podos et al., 1972), but Hughes and Pugh (1975) found that insufficient drug levels were maintained in the bovine eye for treatment of BIK. In addition, movement of the nictitating membrane led to the removal of these devices from the bovine eye within two hours.

Ocular inserts have been studied which would release a therapeutic amount of a biologically active agent for the duration needed to treat BIK. Theodorakis et al. (1983) developed a poly (lactic acid) (PLA)-chloramphenicol sodium succinate (CASS) ocular matrix which was attached to the outer side of the third eyelid by sutures or a spear. Figure 1 shows the release characteristics of three devices with different amounts of the plasticizer tributyl citrate (TBC). Although the devices were able to maintain a therapeutic level of CASS in the eye for two days, the release rate was irregular, and did not follow the square root of time law (rate proportional to $t^{-1/2}$).

Table 1. Summary of current treatment methods for Bovine Infectious Keratoconjunctivitis

Delivery Method	Delivery Medium	Drugs Used: Dose or Concentration
Eyedrops	Water Thickeners	Chloramphenicol: 0.5-1.0% Gentamycin: 3 mg/ml
Ointments	Lanolin Petrolatum Vegetable oil	Tetracyclines: 5 mg/gm
Sprays	Water	Tylosin tartrate: 30 mg
Powders	Boric acid	Tylan®: 2% Neomycin: 0.25%
Oral Administrations	Feed	Sulphadimidine: 100mg/KBW[a] Oxytetracycline Tylosin
Injections	Various liquid bases	Dexamethasone: 5 mg/ml Penecillins Cephalosporins
Soft Contact Lenses	Bionite	Pilocarpine nitrate: 4% solution Tetracycline
Perfusion Systems	Polyethylene tubing	Antibiotic solutions

[a]KBW = kilograms body weight

Advantages	Disadvantages	References
Lowest cost	Low retention time	Chiou and Watanabe, 1982 Gelatt et al., 1979
Base increases corneal penetration	Low retention time	Chiou and Watanabe, 1982
Easiest application	Low retention time	Chiou and Watanabe, 1982 Ellis and Barnes, 1961
Easy to administer	Low retention time	Sampson and Gregory, 1974
Easy to administer	Non-topical	Chiou and Watanabe, 1982 Blood and Henderson, 1979 Hughes, 1981
Effective for treating posterior	High cost per dose	Blood and Henderson, 1979 Blogg, 1980
Significant increase in retention	Insufficient duration Dislodging	Podos et al., 1972
Constant drug flow	Cumbersome Expensive	Chiou and Watanabe, 1982

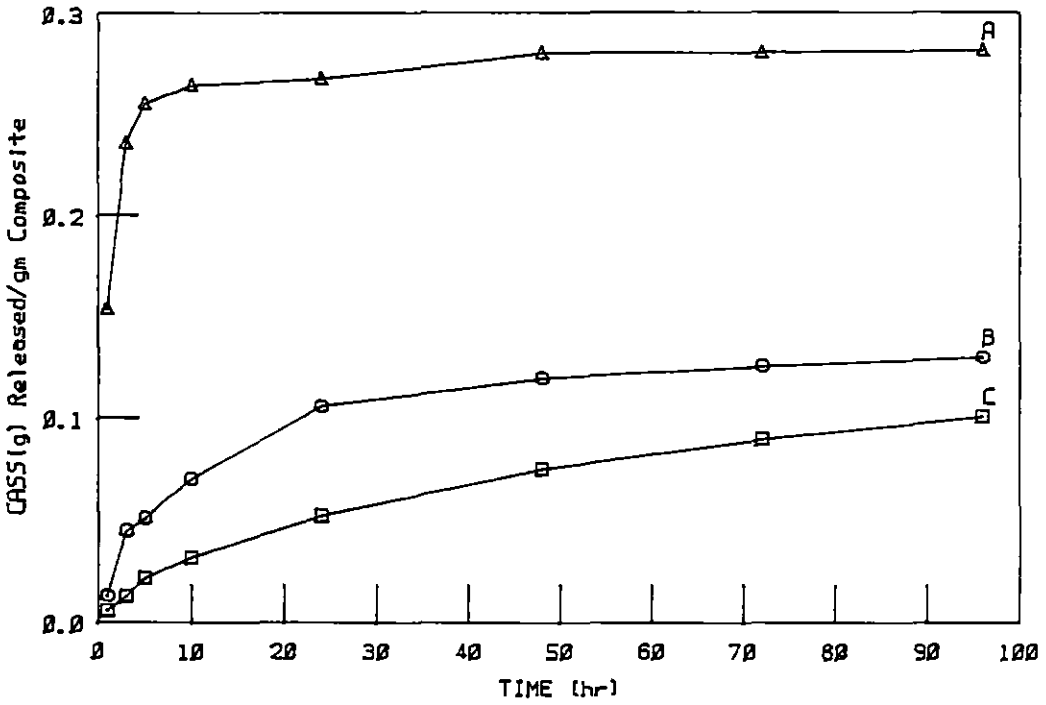

Figure 1. Cumulative amounts of CASS released from composites of PLA (A) 0% TBC; (B) 6.5% TBC; (C) 8.5% TBC (from Theodorakis et al., 1983)

Controlled Release Systems

The only single-treatment method of ocular drug delivery capable of maintaining a therapeutic level of drug for the time required to treat BIK is a controlled release system. Controlled release systems are classified by the release mechanism; diffusion-controlled systems are the most common. There are two types of diffusion-controlled systems: reservoir and monolithic.

In the reservoir system, a permeable film surrounds the drug. Transport through the membrane is governed by Fick's first law, which for

these systems can be expressed as

$$J=DK\Delta C/\ell \qquad (1)$$

where J is the flux in gm/cm^2-sec, DK is the permeability in cm^2/sec, ℓ is the membrane thickness in cm, and ΔC is the concentration gradient (gm/cm^3) between the two sides of the membrane (Baker and Lonsdale, 1974). The permeability (DK) is the product of the diffusion coefficient D (cm^2/sec) and the dimensionless partition coefficient K. The partition coefficient is defined as the ratio of the solubility of the permeant in the polymer to the solubility in the release medium. No good method exists for estimating the value of the partition coefficient (Baker and Lonsdale, 1974); the diffusion coefficient may be estimated for different permeants from the known values of other permeants using the equation

$$\log(D) = -s_M \log(M) + k_M \qquad (2)$$

where M is the molecular weight and s_M and k_M are constants (Lee and Robinson, 1978). This equation provides an estimate of the diffusion coefficient over a limited range of molecular weights, but for molecular weights over 500 the correlation is less predictable. Zero order release in reservoir systems occurs when the design maintains unit thermodynamic activity immediately inside the rate-limiting membrane (Hophenberg and Hsu, 1978). Reservoir systems are not biodegradable and sometimes develop leaks.

In the monolithic system, the drug is uniformly dispersed or

dissolved in a solid, nonbiodegradable matrix. With both dissolved drug and dipersed drug within the matrix, the release rate is inversely proportional to the square root of time (Higuchi, 1963). When only dissolved drug is present, the release can be represented by a two-part curve:

$$dM_t/dt = 2M_\infty[D/\pi\ell^2 t]^{\frac{1}{2}} \qquad M_t/M_\infty < 0.6 \qquad (3)$$

$$dM_t/dt = \{8DM_\infty/\ell^2\}\exp[-\pi^2 Dt/\ell^2] \qquad M_t/M_\infty > 0.6 \qquad (4)$$

where M_t is the cumulative amount of drug released at time t, M_∞ the total mass of drug at time zero, ℓ is the layer thickness, and D is the diffusion coefficient of the permeant in the polymer (Schacht, 1984). Over the first 60% of release, the rate falls off as $t^{-\frac{1}{2}}$ according to Equation 3; after this time the rate decays in an exponential manner as seen in Equation 4 (Baker and Lonsdale, 1974).

It is evident from these equations that constant release cannot be expected from monolithic devices. However, since monolithic devices are not dependent on a rate-limiting barrier, they will not experience rapid loss of drug if broken.

The design of the drug release system depends on the nature of the application. Monolithic devices are generally easier to construct than reservoir devices; in cases where zero-order release is not essential, or where rapid loss may be harmful, monolithic devices may be preferred. In cases where constant release is essential, reservoir systems will be required.

Ocular Insert Materials

The most common polymers used as ophthalmic materials are silicone rubber, poly(methyl methacrylate), and hydrogels. Silicone rubber is used extensively as an insert material and has been studied in great detail; poly(methyl methacrylate) and hydrogels are both used in the contact lens industry.

Poly(methyl methacrylate) (pMMA) (Figure 2) is a lightweight,

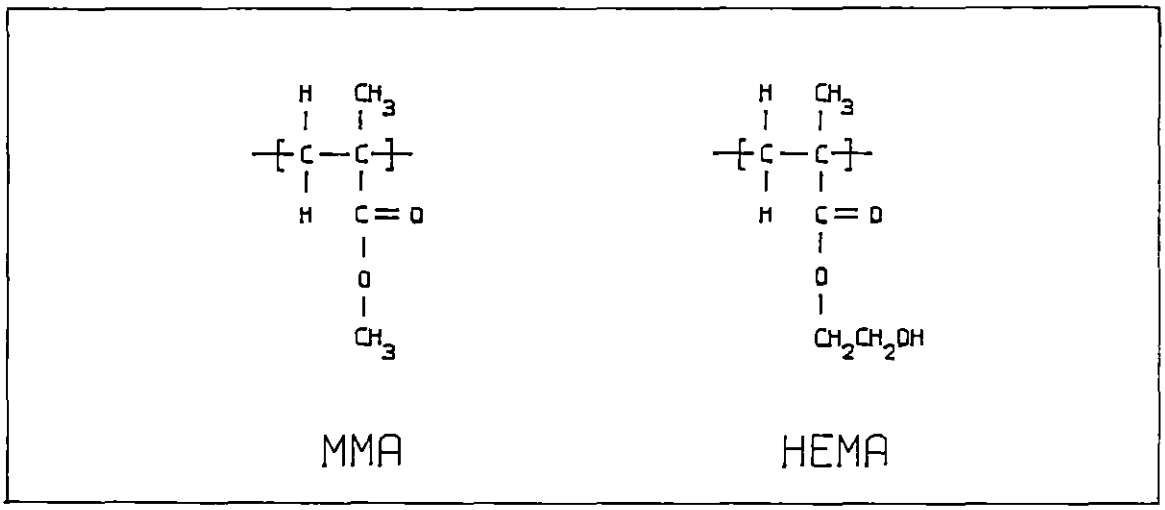

Figure 2. Structures of poly(methyl methacrylate) (pMMA) and poly(hydroxyethyl methacrylate) (pHEMA)

nonirritating material with high optical quality and excellent molding and machining characteristics. It is relatively hydrophobic, and absorbs 1.5% water by weight (Refojo, 1974).

Hydrogels are water-swollen, water-insoluble, polymeric materials with an equilibrium water content of up to 90% (Ratner and Hoffman, 1976;

Pedley et al., 1980). The most frequently used hydrogel material is poly(2-hydroxyethyl methacrylate) (pHEMA) (Figure 2), due to its stability under varying pH, temperature, and toxicity conditions. It is used extensively in the soft contact lens industry and has good biocompatibility in addition to excellent molding and machining characteristics. Poly(2-hydroxyethyl methacrylate) has an equilibrium water content of 40% that can be reduced by copolymerization with MMA (Pedley et al., 1980). Figure 3 illustrates the variation of equilibrium water content of MMA-HEMA copolymers.

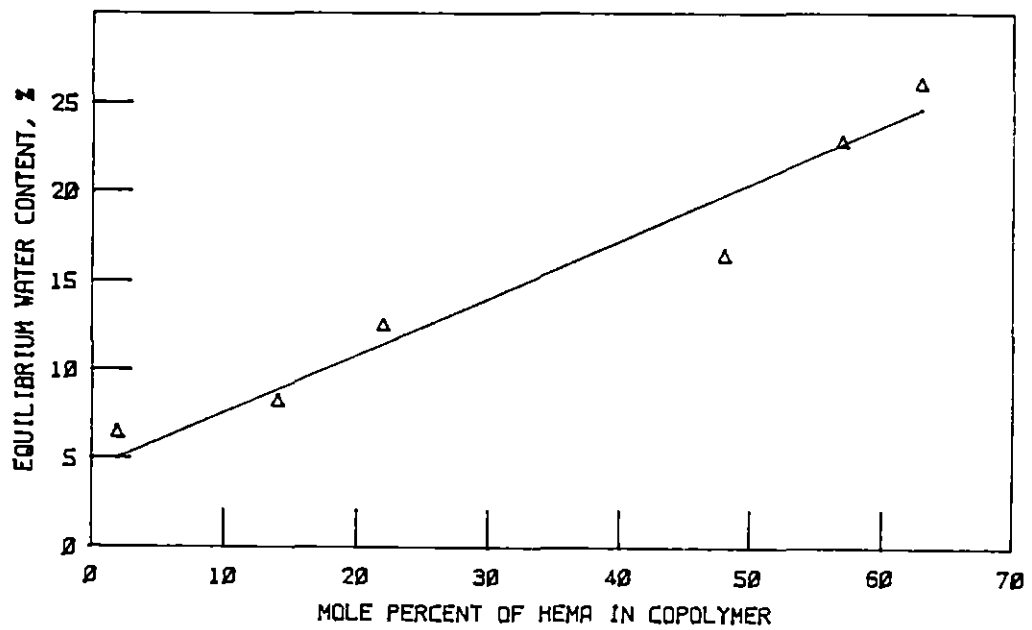

Figure 3. Variation of equilibrium water content with copolymer composition (from Olanoff et al., 1979)

Pedley et al. (1980) report that hydrogel drug delivery systems are effective for antibiotic release into areas with primary or secondary infection, and numerous reports support this claim. Abrahams and Ronel (1975) constructed hollow cylinders of pHEMA 2.54 cm long, 3 mm inside diameter, with 1 mm wall thickness. These were filled with a polymer blend containing cyclazocine (M.W. 271.39), and placed in a 37 C, agitated, phosphate-buffered solution. The authors found that the devices released cyclazocine at a rate of 1 mg/day for five months. In a similar experiment, Cardinal et al. (1980) filled pHEMA tubes (2.85 cm long, 1.2 mm wall thickness) with a silicone oil blend containing 100 mg of progesterone (M.W. 314.45). The initial release varied between 0.04 mg/day and 0.15 mg/day for the first 20 days, then remained constant at 0.04 mg/day for 30 days.

Cowsar et al. (1976) loaded 50:50 MMA:HEMA copolymer slabs with 62-80% by weight of sodium fluoride, and dip coated the slabs with a layer of 70:30 MMA:HEMA copolymer 0.11-0.28 mm thick. Constant release of 0.02-1.0 mg/day of sodium fluoride was obtained into a constant-flow synthetic saliva apparatus. The copolymer-drug mixure ensures a fixed-geometry core, and would prevent rapid release of sodium fluoride if the control membrane were ruptured.

Olanoff et al. (1979) fabricated trilaminate disks from various MMA-HEMA copolymers to release tetracycline (M.W. 444.43). Hydrophilic cores of 67:37 HEMA:MMA loaded with 0.02-0.2 mg of tetracycline/mg of core were coated with 0.053-0.147 mm of relatively hydrophobic 2:98 HEMA:MMA copolymer and cut into disks with a surface area of

0.709-1.33 cm^2. The steady state release of tetracycline from these devices varied from 0.54-23.9 ug/day. The authors concluded that the release of tetracycline was dependent on the size of the device, the release area, and the thickness and composition of the rate-controlling membrane. Membranes with a greater equilibrium water content resulted in higher drug release.

Other studies have used hydrogels in monolithic release systems. Ebert et al. (1980) fabricated devices from HEMA containing 1% prostaglandin E_1 (PGE_1) and 10% sodium heparin. These devices released PGE_1 and heparin at a rate which could effectively reduce surface thrombosis for 72 hours. This rate approximately followed the square root of time law (rate proportional to $t^{-\frac{1}{2}}$).

Studies specifically using hydrogels in ophthalmalogical controlled release systems have focused mainly on soft contact lenses soaked in drug solutions. These systems prolong the effect of the drug, but not for the duration required for treating BIK (Hughes and Pugh, 1975). However, the above studies demonstrate the effectiveness of hydrogel-based drug delivery systems. This property, combined with their acceptance in the contact lens industry, makes hydrogels good candidate materials for ocular drug delivery.

PROPOSED TREATMENT METHOD

Design Criteria

Three important criteria must be met in order to obtain a successful ocular drug-release insert. The drug must be effective in treating the disorder under consideration, the device must be a shape which will stay in the eye without causing adverse physical side effects, and the materials from which the device is made must be biocompatible and capable of sustaining the drug release.

Treatment drug

An effective treatment drug must eradicate causative and contributory organisms associated with bovine ocular infections. Penicillin and streptomycin are widely used to treat BIK, yet they are not as effective as some other drugs. Tetracycline, erythromycin, and tylosin are all effective against *Moraxella bovis* infections; of these, tylosin has a reported inhibitory concentration as low as 0.63 ug/ml (R. F. Rosenbusch, as cited in Leytem (1984)), although the actual inhibitory concentration varies according to the specific strain of *Moraxella bovis*. Webber et al. (1982) report a minimum inhibitory concentration for tylosin of 6.69 ug/ml for the hemolytic strains of *Moraxella bovis* associated with BIK.

Tylosin (see structure, Figure 4) is a macrolide antibiotic that forms several soluble salts and ester compounds, one of which is tylosin tartrate, a commercially available agricultural antibiotic (Burger,

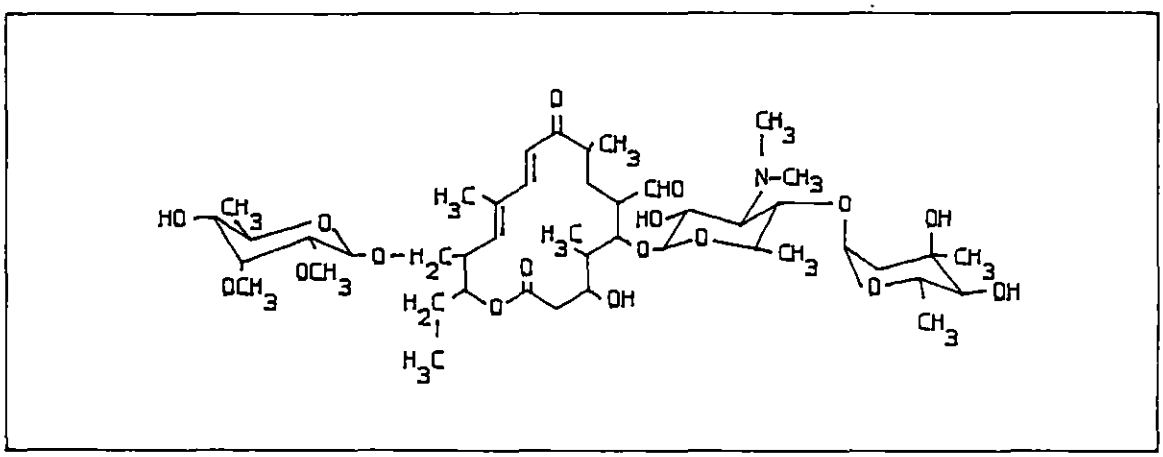

Figure 4. Structure of tylosin (from Windholz et al., 1976)

1970). Tylosin tartrate is soluble in water at concentrations greater than 300 mg/ml and forms aqueous solutions which are stable (pH 4-9) for at least one month (Ose and Barnes, 1960). The antibiotic is essentially nontoxic and nonirritating to the eye and conjunctival sac (Ellis and Barnes, 1961; Johnston, 1982); for these reasons, tylosin tartrate was chosen as the treatment drug.

Device shape

As stated above, the insert must be made in a shape which will stay in the eye for extended periods of time without causing unwanted side effects. Hughes and Pugh (1975) constructed inert ring-shaped devices for insertion into the bovine eye. Using polyethylene, vinyl, or nylon tubing with an outside diameter of 0.91-3.61 mm, they made rings with diameters ranging from 31.5-47.1 mm. Although an optimal cross section was not determined, rings with a circumference of 135-140 mm were found to stay in the eyes of 4-10 month old dairy calves for up to 19 days. Of

12 rings within this size range, six stayed in the eye for six days or more. Local reaction to the rings was minimal. Using the technique developed by Draize (1965), ocular irritation was a zero for all four factors on all 20 cattle tested in the experiment; a score of zero represents little or no adverse reaction. The proper size depends on the actual size of the eye; the ring should have a circumference smaller than that of the conjunctival sac but larger than that of the globe of the eye. This ring shape was chosen as the configuration of the proposed treatment device.

Materials

The final factor under consideration is the material from which the device is to be fabricated. As mentioned above, poly(methyl methacrylate) and poly(2-hydroxyethyl methacrylate) are widely used as contact lens materials and have been used in drug-delivery systems. For these reasons, MMA and HEMA were chosen as the fabrication materials.

Design Parameters

The desired drug release system must maintain the minimum inhibitory concentration of tylosin tartrate within the eye for the duration needed to treat bovine ocular infections. The required duration for tylosin is not clear, and may depend in part on the progress of the disease (Blogg, 1980). Although Sampson and Gregory (1974) reported curing BIK with a single application of Tylan® Plus Neomycin Eye Powder[1], Aronson et

[1]Elanco Products, Indianapolis, Indiana.

al. (1983) recommends seven daily applications of this powder for treating Moraxella bovis infections. Ellis and Barnes (1961) applied 30 mg doses of tylosin tartrate as a 50 mg/ml spray twice daily for five days to treat BIK. To ensure sufficient duration, the proposed device should release tylosin for at least the seven day period recommended by Aronson et al. (1983).

The required drug release rate from the device is a function of the minimum inhibitory concentration and the lachrymal flow rate. The minimum inhibitory concentration will be the value obtained by Rosenbusch (as cited in Leytem (1984)), as this is the lowest value which was reported to be effective. Hoffman and Spadbrow (1978) obtained mean lachrymal flow rates in cattle of 0.18-1.86 ml/hr using a catheterization method; Slatter and Edwards (1982) obtained mean flow rates of 1.96±1.84 ml/hr (±s.d.). Lachrymal flow rates may vary due to disease or other conditions, but the range of interest is known to be around 2 ml/hr. Based on these conditions, the required drug release rate from the insert system can be calculated as 2.54 ug/hr of tylosin tartrate. However, the spray used by Ellis and Barnes (1961) had a concentration of 50 mg/ml of tylosin tartrate, and would have introduced an instantaneous concentration of over 10 mg/ml to the eye; no adverse effects were reported from this. Although the overall effect of a sustained concentration this high is unknown, it can be estimated from this that the maximum safe concentration of tylosin tartrate in the eye is on the order of 10 mg/ml. On this basis, a device with a release rate between 2.5-10,000 ug/hr of tylosin tartrate could be expected to effectively and

safely treat bacterially-induced bovine ocular infections.

Olanoff et al. (1979) concluded from their study on tetracycline release that that the rate of drug release depended in part on the composition of the rate-controlling polymer membrane. Using data from their study, the release characteristics from the proposed treatment device can be estimated. Leytem (1984) produced trilaminate devices from tylosin tartrate and HEMA-MMA copolymers made using the procedure of Olanoff et al. (1979). Leytem (1984) concluded that a 2:98 HEMA:MMA copolymer trilaminate disk with a diameter of 17.9 mm and a membrane thickness of 0.223 mm enclosing 50 mg of tylosin tartrate released the drug at a rate of 1.0-33.3 ug/hr for up to 84 hours. The proposed device will be made from this copolymer formulation.

For calculation purposes, a reservoir device in the shape of a ring can be treated as a hollow, drug-filled cylinder. Fick's law (Equation 1) for a cylinder can be expressed as:

$$dM_t/dt = 2\pi hDK\Delta C/\ln(r_o/r_i) \qquad (5)$$

where r_i and r_o are the inside and outside radii of the cylinder, respectively, and h is the height of the cylinder (Schacht, 1984). Hughes and Pugh (1975) reported that rings with a circumference of 140 mm stayed in the eyes of dairy calves without causing irritation, so the value for the height (h) in Equation 5 will be set at 14 cm. The cross section was not determined, so for the purpose of calculation r_o and r_i will be set at 0.11 and 0.10 cm, respectively. The concentration gradient ΔC depends on the interior volume and the amount of drug in the

device. If the interior is filled with dry drug, the concentration can be assumed to be near the saturation level of 300 mg/ml; since the exterior concentration will be low, it can be neglected and the concentration gradient will be equal to the saturation concentration of 300 mg/ml. Olanoff et al. (1979) reported the diffusion coefficient and partition coefficient values for tetracycline in the 2:98 HEMA:MMA copolymer. The relationship between the diffusion coefficient and molecular weight is given by Equation 2. Using the value for the diffusion coefficient of 8.0×10^{-9} cm^2/sec for tetracycline in 2:98 HEMA:MMA copolymer given by Olanoff et al. (1979), Equation 2 gives a value for the diffusion coefficient of tylosin tartrate in the copolymer of 5.5×10^{-10} cm^2/sec, assuming that k_M in Equation 2 is equal to zero. This value is only an estimate, as many other factors will affect the permeation rates of high molecular weight compound such as tylosin tartrate. The partition coefficient, K, will be assumed to be the value of 6.8×10^{-3} given for tetracycline (Olanoff et al., 1979), as no good method exists for estimating this value (Baker and Lonsdale, 1974). Using all these values in Equation 5, the steady state release of tylosin tartrate can be estimated as approximately 4 ug/hr. Therefore, the proposed treatment device, with a 14 cm circumference, 2 mm inside diameter, and 0.1 mm wall thickness should release tylosin tartrate at a rate which will maintain a therapeutic level of the drug within the bovine eye for the required duration.

As stated earlier, the maximum safe concentration for tylosin tartrate in the eye has not been determined. Since a wide range of

concentrations has been used without adverse side effects, constant release is not essential, and a monolithic controlled-release system may be considered. Monolithic devices do not give constant release rates, but are generally easier to fabricate and will not release large amounts of drug if broken. The monolithic system would be made in the same shape as the reservoir system described above, but with drug dispersed throughout the polymer. These devices would be expected to release drug at a rate inversely proportional to the square root of time; if the initial rate maintains a drug concentration within the eye below 10 mg/ml and the rate after seven days maintains a level above the minimum inhibitory concentration, then the device could be expected to be effective in treating bovine ocular infections.

Equation 3 can be used to estimate the release characteristics of this device. The release depends on the diffusion coefficient of the copolymer, the amount of drug in the matrix, and the thickness of the layer. For the purpose of calculations, this device can be treated as a slab of copolymer covering a ring-shaped core. The amount of drug in the slab will be set at 50 mg; the thickness of this slab, based on the physical properties of the copolymer found by Olanoff et al. (1979), will be assumed to be approximately 0.02 cm. Using the value of $5.5 \times 10^{-10} cm^2/sec$ for the diffusion coefficient from above, the rate of release from the device after seven days can be estimated as approximately 300 ug/hr. Although this is much higher than the value of 4 ug/hr estimated for the reservoir device, it is still within the range of interest. In addition, the rate can be adjusted by modifying the

value of M_∞, the total amount of drug initially present.

Both reservoir and monolithic controlled-release systems were fabricated and tested in this study. The fabrication methods were evaluated based on their ability to produce devices with predictable release rates in the range necessary to treat ocular infections in cattle.

PROCEDURES AND MATERIALS

Production of 90:10 MMA:HEMA Copolymer

The devices tested in these experiments were fabricated from a copolymer of methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA). This copolymer was made in a batchwise process from commercially available monomers.

The following materials were added in the order listed to a one-liter Erlenmeyer flask: 570 ml of absolute ethanol, 380 ml of water[1], 6.1 ml of HEMA[2], 46.6 ml of MMA[3], 0.25 gm of sodium persulfate[4], and 0.125 gm of potassium persulfate[5]. The flask was sealed with a rubber stopper and the liquid contents were bubbled with nitrogen for thirty minutes. After the initial thirty minutes, slight positive pressure was maintained on the system for the ten day reaction time; the reaction was carried out at room temperature (20-22°C). On the tenth

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[1] All water used in these experiments was type-one purified water according to the American Society for Testing Materials definition; 0.1 mg/l maximum total matter, 0.06 microohm/cm maximum electrical conductivity at 25 C, 16.67 megaohm/cm minimum electrical resistivity at 25 C, 60 minutes minimum color retention time of potassium permanganate, no detectable soluble silica.

[2] Polysciences Inc., Lot #2-2405, Ophthalmic Grade, Warrington, PA.

[3] Aldrich Chemical Co. Inc., Lot #041557, Milwaukee, Wisc.

[4] Aldrich Chemical Co. Inc., Lot #0608HK, Milwaukee, Wisc.

[5] Fisher Scientific Co., Lot #714237, Fair Lawn, New Jersey.

day, the white copolymer precipitate and solvent were added to a four-liter beaker containing three liters of water. The precipitate and liquid were filtered through 1-qualitative filter paper[6] in a 7 cm Buchner funnel with the aid of a low vacuum. Each time the funnel was full, the filtered precipitate was washed four times with 75 ml amounts of water, and the washed copolymer was collected in a 190 x 100 mm Pyrex dish. When all the copolymer had been filtered and washed, the Pyrex dish was covered with filter paper[7] and the copolymer was dried in an oven[8] at 50°C for five days under a 25 in Hg vacuum[9].

Production of Ring-Shaped Devices

Two types of devices were fabricated for these studies. Reservoir-type devices were made from copolymer tubing filled with drug and formed into a ring. Monolithic devices were made by mixing drug with a polymer solution and applying the mixure to a ring-shaped inert core.

All the devices were made from a solution of the copolymer from the above process dissolved in dimethyl formamide[10]. One gram of the copolymer powder was added to 20 ml of dimethyl formamide and mixed and

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[6] Whatman Limited, London, England.

[7] Whatman Limited, 18.5 cm 1-quantitative, London, England.

[8] Chicago Apparatus, Model 524A, Chicago, Illinois.

[9] The Welch Scientific Co., Duo Seal Vacuum Pump, Model 1402, Skokie, Ill.

[10] Fisher Scientific Co., Lot #745395, Fair Lawn, New Jersey.

heated[11] at 50°C for six hours to assure complete solution of the copolymer. The solution was kept in a short, wide-mouthed jar and sealed when not in use.

Production of reservoir devices

Production of mandrels All the devices used in this study were fabricated on ring-shaped mandrels. The mandrels were made by inserting a solid copper wire[12] through a piece of Teflon®[13] or Silastic® (silicone rubber) tubing[14], wrapping it around a cylinder of the correct diameter (35-50 mm), and twisting the ends of the wire together (see Figure 5). A finished mandrel is shown in Figure 6; the dimensions of the mandrel are shown in Figure 7. The mandrels were cleaned by soaking them overnight in a soap solution[15], then dried in air without rinsing. The presence of a soap film on the rings fascilitated the application of the intitial coat of copolymer solution.

Coating of mandrels The mandrels were coated with the copolymer solution using two different techniques. In the first method, the mandrels were held with small alligator clips onto which had been added a

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[11] Corning Glass Works, Model PC-351 hot plate-stirrer, Corning, NY.

[12] Belden Wire Co., #26AWG copper wire, Geneva, Ill.

[13] Cole-Parmer, Cat. # P/N 6417-21, Chicago, Illinois.

[14] Dow Corning, Silastic® Medical Grade Tubing, Midland, Michigan.

[15] Proctor and Gamble, Ivory Snow flakes, Cincinnati, Ohio.

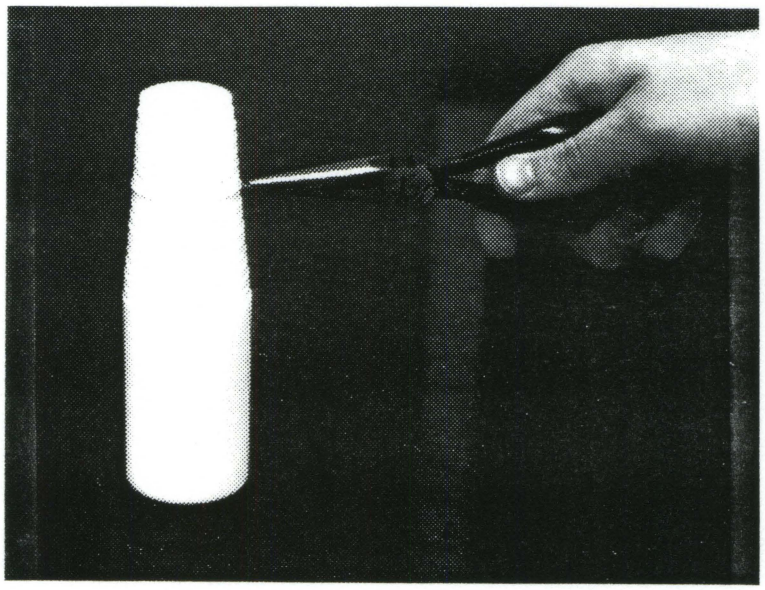

Figure 5. Producing a round mandrel

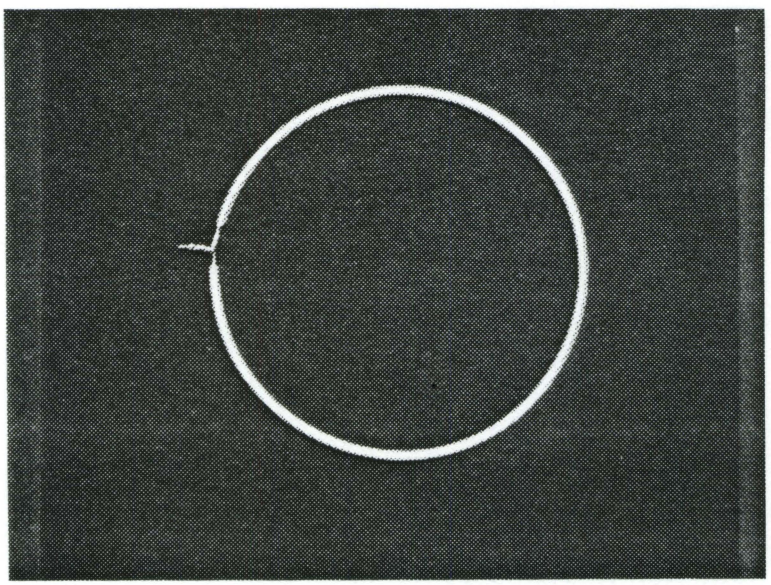

Figure 6. Finished mandrel

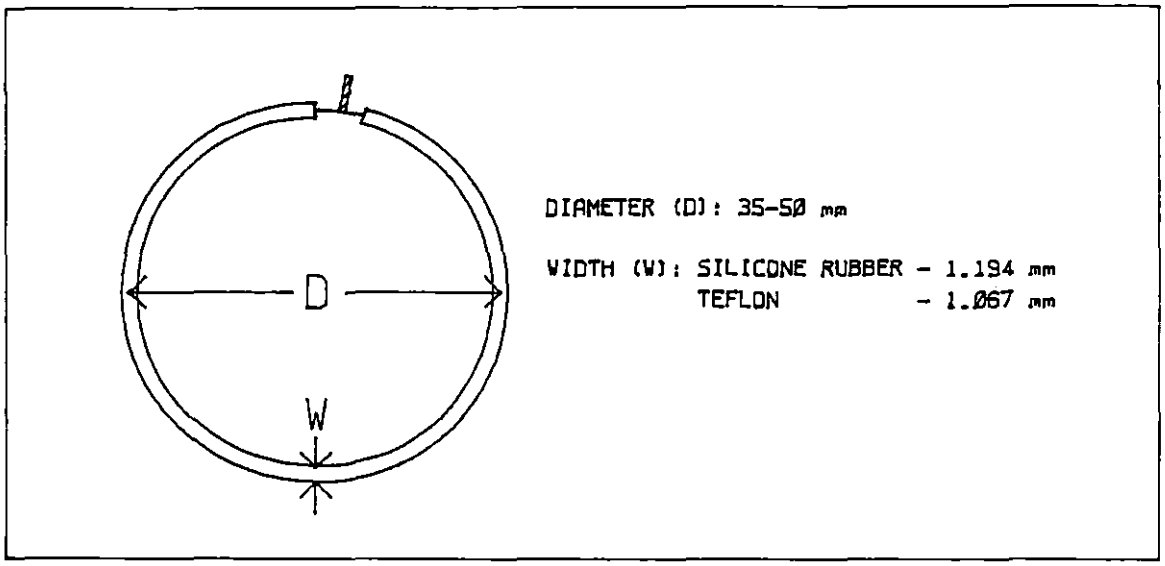

Figure 7. Dimensions for the finished mandrels

length of heavy copper wire[16] bent into a hook (Figure 8). Using the hook as a handle, the mandrel was dipped into the jar of copolymer solution for several seconds, and was hung by a support to dry. Large lumps or drops were smoothed out using a brush; thin spots were supplemented using a brush or a syringe[17] with a 20 gauge needle[18] filled with copolymer solution. Each coat deposited 5-10 mg of copolymer on the mandrel. A thickness of 0.15-0.25 mm was desired, and the thickness was checked after each application using a micrometer[19]. Since the lower

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[16] Belden Wire Co., #18AWG copper wire, Geneva, Illinois.

[17] Sherwood Medical, 5 cc Monoject syringe, St. Louis, MO.

[18] Becton, Dickinson and Co., Yale hypodermic needle #20G1, Rutherford, Massachusetts.

[19] L. S. Starret Co., cat. #T230P, Athol, Massachusetts.

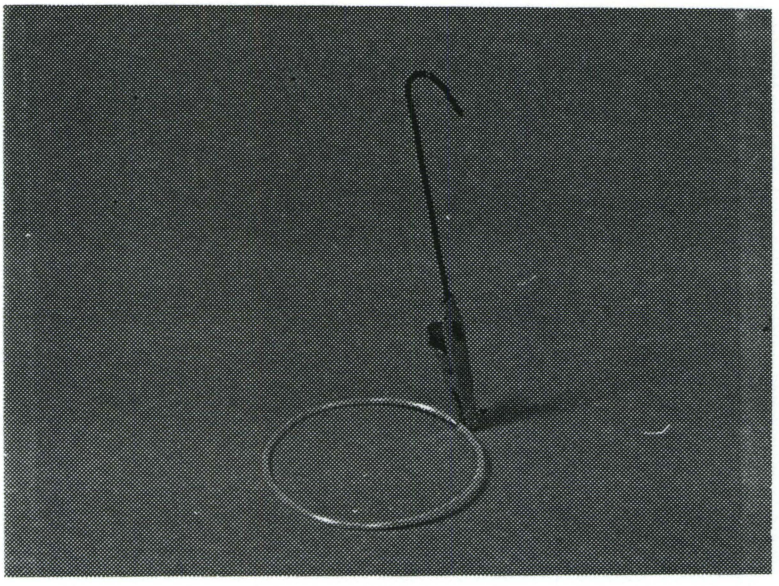

Figure 8. Hook for holding mandrels during dip coating process

side of the ring tended to become thicker due to gravity, the rings were inverted between coats by removing the clips and replacing them in the opposite direction. When the desired thickness was acheived, the rings were allowed to dry for three days. The entire coating process was carried out in a glove bag[20] over an active desiccant[21], as moisture in the air adversely affected the formation of the copolymer layer.

In the second method, the mandrels were attached to a hub and rotated through a shallow copolymer bath (Figure 9). The rings were coated by rotating them at a speed of approximately 5 rpm while allowing

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[20] Instruments for Research and Industry, Model X-27-27, Cheltenham, PA.

[21] W. A. Drierite Co., Drierite ($CaSO_4$), Xenia, Ohio.

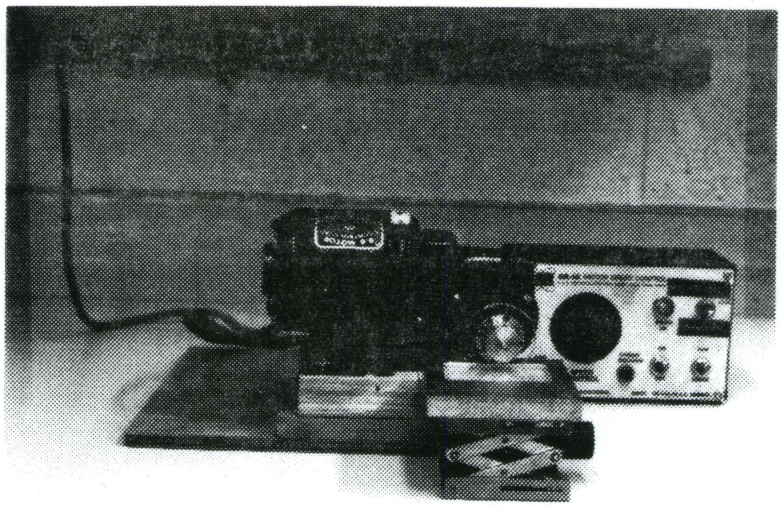

Figure 9. Apparatus for rotation coating of mandrels

the bottom of the mandrel to pass through the copolymer solution for two minutes. After removal from the solution, the rings were rotated continuously for four hours, the thickness checked, and the procedure repeated as necessary. This procedure was also carried out over a desiccant.

Production of finished rings After complete drying, the copolymer tube was removed from the mandrel. To accomplish this, the ring was first cut in half with a sharp wire cutter; the cuts were made at the wire joint and directly opposite. The mandrel tubing was grasped with tweezers and carefully pulled out of the formed copolymer tube. The copolymer tube was inspected for cracks, holes, or obvious weaknesses; if no flaws were found, the tube was washed in water for five days, then

dried in a desiccator. The formed tubes are shown in Figure 10.

The copolymer tubes were each weighed, then filled with tylosin tartrate[22]. To fill the tubes, a low vacuum was applied to one end of the tube and the drug was drawn in by the resulting air current (Figure 11). A piece of cotton on the end where the vacuum was applied kept the drug from being drawn all the way through the tube.

The two halves were then affixed in a ring using two short pieces of a connecting tube, either polyethylene[23] (PE) or poly(vinyl chloride)[24] (PVC). When PE was used for the connecting tube, Silastic® adhesive[25] was used to cement the tubes together. A small bead of the adhesive was applied to the PE tube, and the end of the PE tube was inserted into the copolymer tube section. The same procedure was used on all four joints; a finished ring is shown in Figure 12. Devices made with PVC were done in a similar manner, only the copolymer solution was used to cement the pieces together instead of the Silastic® adhesive. In addition, five of the devices made with PVC (numbers 25A-29A) had the PVC segment encased in a segment of copolymer tubing. Twenty-nine reservoir-type devices were made; the materials and procedures used for producing these devices are listed in Table 2.

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[22] Sigma Chemical Co., Lot #89C-0315, St. Louis, Mo.

[23] Clay Adams, Intramedic PE tubing, Parsippany, NJ.

[24] Becton, Dickinson and Co., cat. #6109, Rutherford, NJ.

[25] Dow Corning, Type A medical grade adhesive, Midland, Michigan.

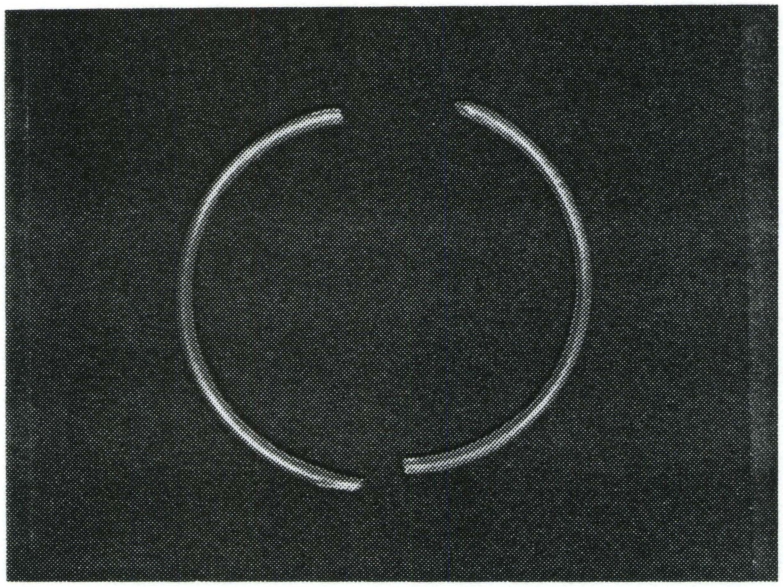

Figure 10. Copolymer tubes after removal from mandrel

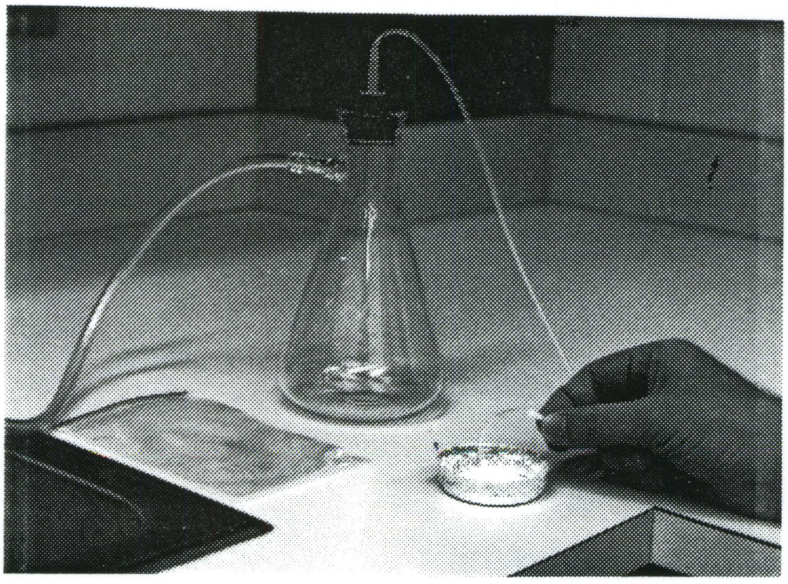

Figure 11. Apparatus used to fill tubes with drug

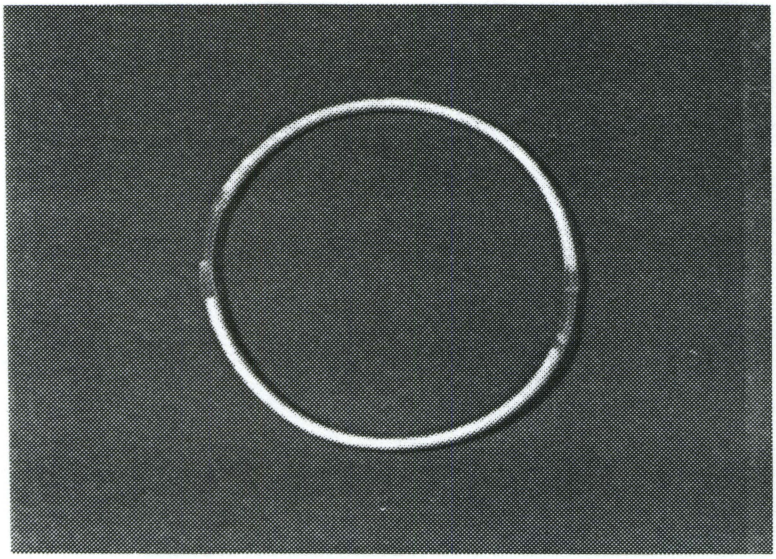

Figure 12. Finished reservoir device

Table 2. Materials and procedures used for producing the reservoir devices

Device Number	Mandrel Material	Mandrel Diameter (mm)	Coating Method	Desired Thickness (mm)	Connecting Tube Material
1A	Teflon®	48	Rotation	0.175	PE
2A-6A	Teflon®	48	Rotation	0.225-0.280	PE
7A-11A	Silastic®	42	Dip	0.125-0.150	PE
12A-15A	Silastic®	45	Rotation	0.240	PE
16A-19A	Teflon®	35	Dip	0.175	PE
20A-25A	Teflon®	38	Dip	0.175	PVC
26A-29A	Teflon®	40	Dip	0.175	PVC

Production of monolithic devices

The monolithic devices were fabricated in a manner similar to the method already described for the reservoir devices. The copolymer/drug mixure was made by adding 0.5 gm of tylosin tartrate to the solution of 1.0 gm of copolymer in 20 ml of dimethyl formamide and mixing thoroughly without heating. Silicone rubber mandrels were utilized and coated using the dip coating technique. The mandrels were weighed before applying any copolymer solution, and weighed between applications. Successive coats of the copolymer/drug mixure were added until approximately 150 mg of material had been deposited on the mandrel. Ten rings were made in this manner; five of these rings were coated two additional times in copolymer solution without drug. The devices were tested without removing the copolymer layer from the mandrel; a completed monolithic device is shown in Figure 13.

Tylosin Tartrate Release Determination

Release experiments

The rate at which the devices released tylosin tartrate was determined through in vitro experiments. Each device was placed in the bottom of a wide jar, covered with a small amount of liquid (water, physiological saline[26], or mammalian Ringer's solution[27]), and placed in

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[26] 0.9% w/w NaCl in water.

[27] 8.60 g NaCl, 0.30 g KCl, 0.33 g $CaCl_2$ in 1.00 l aq. solution.

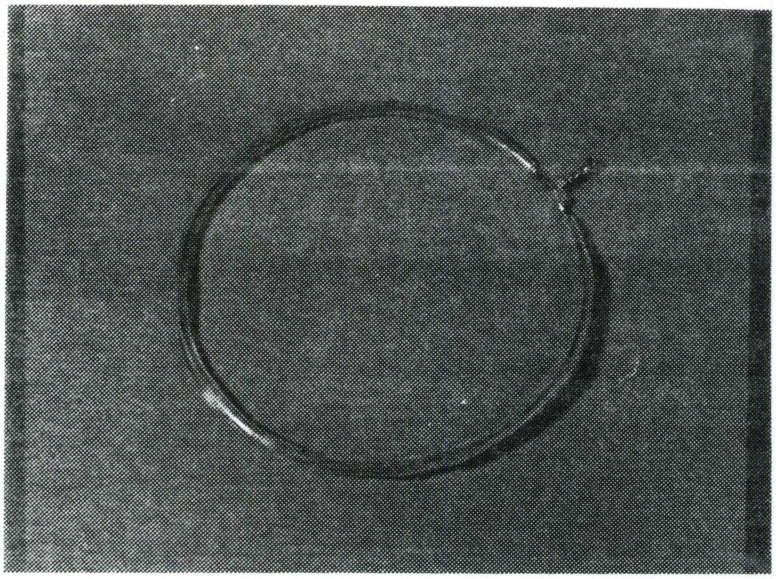

Figure 13. Finished monolithic device

a shaking water bath[28] at 37°C. At specific time intervals, the liquid was collected in a small vial, labeled, and saved for analysis. Fresh liquid was added to the sample jar and the jar was returned to the water bath. Table 3 gives the conditions of the release experiments.

Quantitative analysis of tylosin tartrate using TLC

The amount of tylosin tartrate in the samples was determined with thin-layer chromatography (TLC). Leytem (1984) determined that this method is effective for determining the presence of tylosin tartrate even in very small amounts (<1 ug). Tylosin tartrate produces a pattern with a primary dark spot with an R_f value of approximately 0.58 where R_f is

[28] Fisher Scientific Co., Model 127, Fair Lawn, NJ.

Table 3. Conditions for release experiments

Device Number[a]	Release Medium	Volume of Release Medium	Sample Collection: Time from start of experiment	collection frequency during time period
1A	Water	10 ml	0-120 hours	8 hours
2A	Saline	10 ml	0-120 hours	8 hours
3A,4A	Water	10 ml	0-16 hours	8 hours
			20-48 hours	4 hours
			48-120 hours	8 hours
			120-168 hours	24 hours
5A,6A	Saline	10 ml	0-16 hours	8 hours
			20-48 hours	4 hours
			48-120 hours	8 hours
			120-168 hours	24 hours
7A-11A	Water	10 ml	1-120 hours	8 hours
			120-148 hours	24 hours
12A-29A	Saline	2 ml	0-120 hours	8 hours
			120-148 hours	24 hours
1B-10B	Ringer's	2 ml	0-120 hours	8 hours
			120-216 hours	24 hours

[a]Devices designated with an 'A' are reservoir devices; those with a 'B' are monolithic devices.

defined as

$$\frac{\text{center-of-sample distance from zero reference}}{\text{developing-solvent-front distance from zero reference}}$$

where the preadsorbent layer-stationary phase interface is the zero reference. Leytem (1984) found that the relationship between the amount of drug in the spot and the spot intensity was nearly linear for spots with an amount of drug between 0.8 ug and 10.0 ug. Spots within this range fit a linear regression calculation with a coefficient of determination between 0.967-0.995.

The samples collected during the release experiments were dried in an oven[29] at 50°C for approximately three days. The dried samples were then redissolved in a volume of water equal to one tenth of the original sample volume.

Whatman LKC18F 20 x 20 cm TLC plates[30] were fully developed in a standard developing chamber[31] containing methanol[32]. These plates were air-dried at least three days prior to use. A volume of 2.5 ul of each redissolved sample was applied onto the preadsorbent layer of a TLC plate using a 0-10 ul pipette[33]. Samples containing a known amount of tylosin tartrate in the range of 0.5-4.0 ug were also applied to the plate. The

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[29] GCA/Precision Scientific, cat. #31543, Chicago, Illinois.

[30] Whatman Chemical Separation Inc., Clifton, NJ.

[31] Whatman Chemical Separation Inc., Type CDC-12, Clifton, NJ.

[32] Fisher Scientific, Lot 734176, Fair Lawn, NJ.

[33] Drummond Scientific Co., 0-10 ul Micropipette, Broomall, Pa.

standards contained an amount of tylosin tartrate such that the 2.5 ul volume contained the standard amount of drug. The amount of other salts (NaCl, KCl, $CaCl_2$) in the samples affects the spot size and R_f value (Leytem, 1984); for this reason, the standards were made with a concentration of salts to approximately match the redissolved samples. The samples were redissolved to one tenth their original volume; therefore, the standards were made with a salt concentration equal to ten times that in the original release medium. Ten spots were applied to each plate: four standards and six samples. When the spots were completely dry, each plate was developed a distance of 8 cm in the developing chamber in a solution of 85% methanol and 15% water (by volume). Fresh developing solution was produced in 100 ml amounts only as needed. The chamber was lined on one side with filter paper[34] and equilibrated for one hour before use. The gel side of the plate was placed facing the filter paper during developing. Developed plates were dried at room temperature before being visualized. When dry, the spots could be viewed using an ultraviolet light source[35] to give an indication of their intensity. If any sample spots were noticibly darker than all of the standards on the plate, those samples were diluted and spotted onto another plate. As noted earlier, the salt concentration affects the spot size and R_f value. When samples required dilution, this was accomplished by adding a solution with a salt concentration ten times

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[34] Whatman Limited, 18.5 cm type 1-qualitative, London, England.

[35] UVP, Inc., Model UVGL-25 Mineralight®, San Gabriel, Ca.

that in the original release medium.

Visualization of the spots on the developed plates was accomplished by spraying[36] ten percent (by volume) sulphuric acid[37] in methanol onto the developed area of the plate at a rate of 15 ml/min for approximately 15 seconds. The sprayed plate was placed in a 100°C oven for five minutes, then allowed to sit for 15 minutes at room temperature. Densitometric analysis was carried out within two hours after the visualization procedure; fading of the spots was noticed within three hours after the visualization procedure.

A Kontes fiber optic scanner, Model 800[38], was utilized to measure the tylosin tartrate dark-spot intensity by cross-scanning the TLC plate (perpendicular to the direction of development). The plates were placed with the gel side up, facing the scanning heads. The densitometer operated in the dual-beam mode and measured the values for the absorbance of both reflected and transmitted light; the light source was filtered and emitted light at 615 nm. The scanning speed was set at 2 cm/min, and the attenuation was adjusted to produce output peaks with a ratio of peak height to width at half height of one to ten. The output signal of the densitometer was recorded on a chart recorder[39] which produced a series of peaks for area determination.

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[36] Kontes, Model K-422550, Vineland, NJ.

[37] Fisher Scientific, Lot #732068, Fair Lawn, NJ.

[38] Kontes Scientific Instrument Group, Vineland, NJ.

[39] Linear Instruments Corp., Model 255/MM, Irvine, Ca.

The output peaks were weighed[40], and the standards plotted on a curve of peak weight versus micrograms of drug in the spot. (A standard curve was obtained for each plate). The amount of tylosin tartrate in the samples was determined by comparing the weight of the sample peak to the standard curve and using linear interpolation. Extrapolation was avoided, and spots which were darker than any of the standards were not used; the samples producing darker spots were diluted and re-spotted onto another plate (with new standards). The amount of tylosin tartrate in spots which were less intense than the 0.5 ug standard spot was estimated using the origin (0,0) as a point; however, these values are less reliable (Leytem, 1984). The amount of tylosin tartrate in each sample was calculated based on the amount of drug in the TLC spot and the total redissolved volume of the sample.

Physical Examination of the Devices

Each device was physically examined before and after the release experiments to check for structural irregularities such as poor seals or cracks. A Nikon 90783 stereomicroscope was used for a 40X stereomicroscopic examination. Selected devices were viewed with a JSM-U3 scanning electron microscope (SEM) to check for incomplete sealing or structural defects. A thin film conductive coating of gold (300Å) was applied to the specimens using a Polaron sputter coater to prevent sample charging during the SEM analysis.

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[40] Mettler Instrument Corp., Model H31AR, Princeton, NJ.

RESULTS

Production of Release Systems

Reservoir devices

Copolymer solution The copolymer dissolved slowly in the dimethyl formamide; with low heat, the copolymer required four to six hours to dissolve. After this time, a cloudy, viscous solution with some gel material resulted. This solution was stored in a short, wide-mouthed jar. The solution was produced in 60 to 80 ml amounts, and could be used to produce a series of devices, as the solution kept well in the jar for a period of several weeks.

Mandrel coating techniques Devices 1A-6A and 12A-15A were produced using the rotation-coating method. The rings were turned at the lowest speed possible on the motor which was used for this procedure, which was approximately 5 revolutions/minute. Despite the low speed, the viscosity of the solution prevented uniform coating of the rings; the inside portion of the ring was incompletely immersed in the copolymer solution bath. This caused the copolymer layer to be thicker on the outside wall of the formed tube. This can be seen in Figure 14, which is a cross-sectional view of a segment of copolymer tubing prepared using this method. Note the curvature of the tube, and that the outside wall is approximately twice as thick as the inside wall (0.35 mm compared to 0.18 mm).

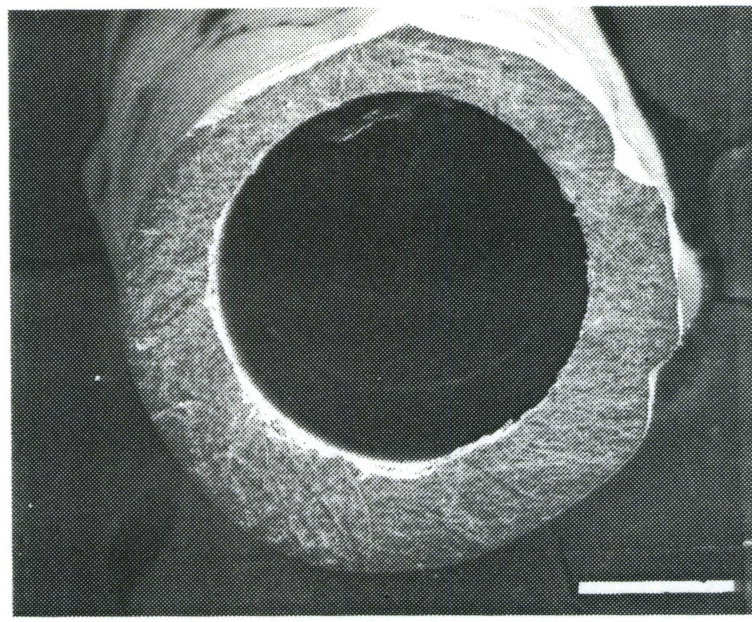

Figure 14. Scanning electron micrograph showing non-uniform wall thickness obtained with rotation coating procedure. Segment from device 14A. Scale bar = 0.5 mm. 25 keV

Devices 7A-11A and 16A-29A were produced using the dip coating procedure. The mandrels were suspended in a horizontal plane using the hooks described in the previous section. The entire mandrel was immersed in the jar of copolymer solution, then hung to dry. The solution initially covered the surface of the mandrel uniformly, but within several minutes drops would form on the lower side of the suspended ring. These drops would rapidly thicken, and could be smoothed out using a brush or syringe with a needle. Because of this effect from gravity, the copolymer layer on the lower side of the ring would become thicker than the top layer. To overcome this, the rings were inverted at least once during the coating process. Ten coats were normally required to build up the desired thickness of copolymer, so the rings were inverted after five

coats had been applied. This procedure produced a copolymer tube with a more uniform wall thickness than the rotation coating method.

The two coating methods were each tried using both Silastic® and Teflon® mandrels. The effect of the mandrel material was the same for both coating methods. The initial coat of copolymer solution produced a less uniform layer on the Teflon® than on the Silastic® tube; the presence of a soap film on the mandrels decreased this effect. After the initial coat had been applied, the subsequent coats formed uniform layers on either mandrel material. The most noticeable difference between the two mandrel materials was the ease with which the copolymer could be removed from the mandrel; the Teflon® could easily be pulled out of the formed copolymer tube, but the Silastic® tube frequently broke, leaving fragments in the copolymer tube. No differences in the integrity of the copolymer tubes were noted based on the mandrel material on which they were formed.

Both the rotation and dip coating procedures produced stiff, transparent, colorless copolymer tubes. The tubes were brittle, and broke abruptly when flexed. The presence of moisture in the air during the coating process caused the copolymer to become an opaque white; these tubes were much weaker and broke more easily than the transparent tubes. Copolymer tubes which turned white due to water in the atmosphere were not used for making any of the 29 reservoir devices which were tested. The relative ease of production and uniformity of the formed tubes favored the dip coating procedure, and the later devices, 16A-29A, were all fabricated using this method. Because of the relative

difficulty of removing the copolymer from the Silastic® mandrels, the Teflon® mandrels were preferred, and were used for making reservoir devices 1A-6A and 16A-29A.

Filling the tubes The method for filling the tubes with tylosin tartrate worked well; by grinding the drug thoroughly and filling the tubes slowly, dead space within the tubes could be minimized. Rings with a diameter greater than 40 mm could hold 50 mg of drug; smaller rings (35 mm-38 mm) held less, and were filled with only 40 mg of drug.

Final assembly Silastic® adhesive was chosen to cement the rings together as it is a medical grade adhesive, and would not require any pretreatment before being placed in the eye. However, this adhesive did not effectively bond to either the copolymer or the polyethylene tube. Figures 15 and 16 show the incomplete bonding obtained with Silastic® adhesive. These devices could be pulled apart with only a moderate amount of force. The polyethylene tubes separated from the Silastic® adhesive without tearing or breaking the copolymer segment. For the later devices (20A-29A), PVC tubing and copolymer solution were used to assemble the finished rings. This method afforded much more complete bonding, as shown by Figures 17 and 18; the dimethyl formamide in the copolymer solution gave complete bonding to the PVC tube. The small cracks visible in Figure 18 were compensated for by brushing an additional coat of copolymer over the seam. Subsequent examination of seams treated in this manner indicated complete sealing of the joint. Seals produced in this manner were very strong, and could not be pulled apart without breaking the PVC tube or the copolymer tube segment.

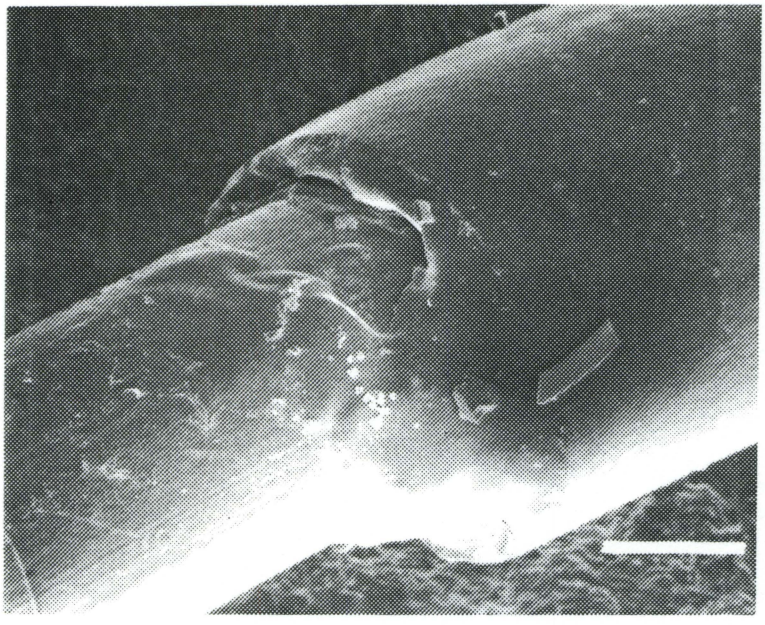

Figure 15. Scanning electron micrograph showing incomplete bonding obtained with Silastic® adhesive. Segment from device 16A. Scale bar = 0.5 mm. 25 keV

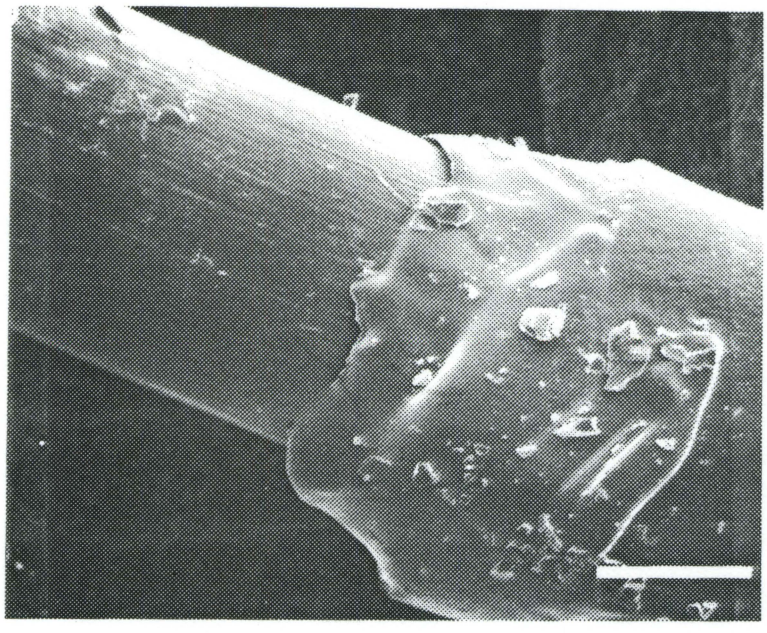

Figure 16. Scanning electron micrograph showing incomplete bonding obtained with Silastic® adhesive. Segment from device 18A. Scale bar = 0.5 mm. 25 keV

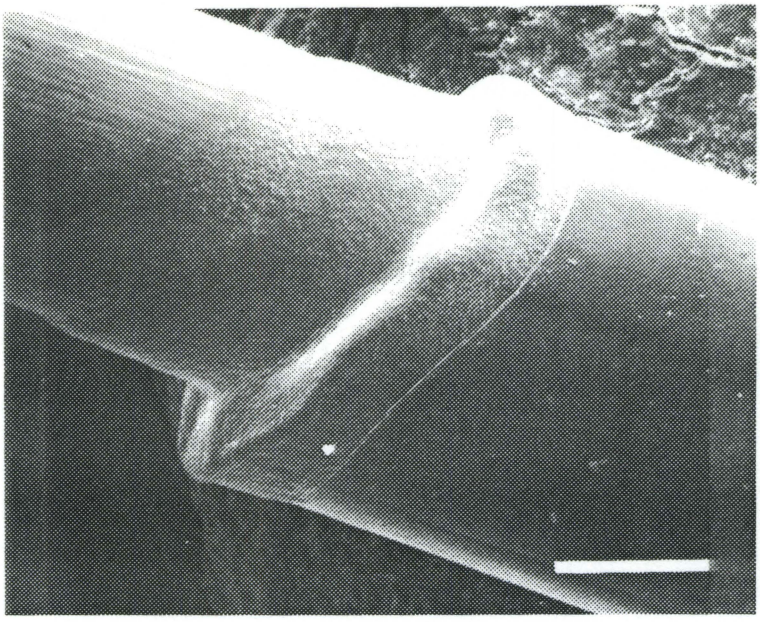

Figure 17. Scanning electron micrograph showing complete seal obtained with copolymer solution and PVC tubing. Scale bar = 0.5 mm. 25 keV

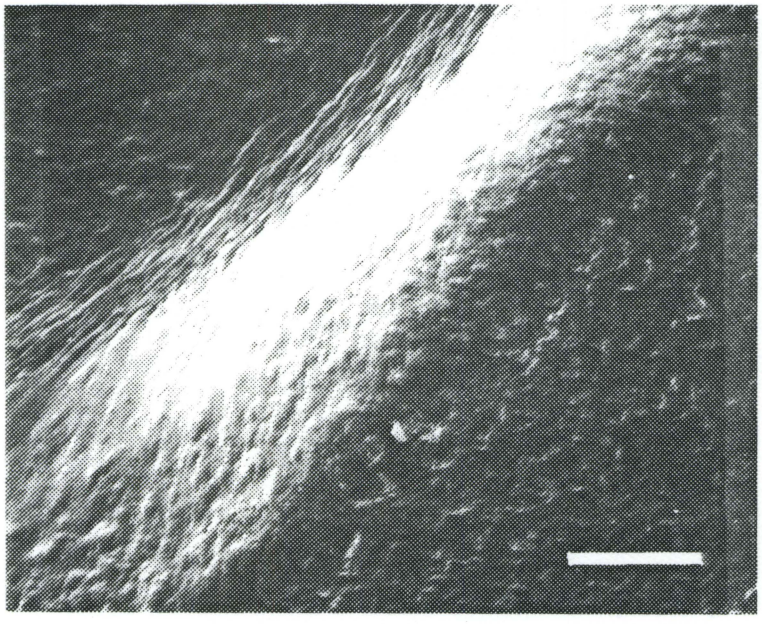

Figure 18. Higher magnification of Figure 17. Scale bar = 0.1 mm. 25 keV

Table 4 lists the measurements for the ring diameter, wall thickness, and drug loading for the 29 reservoir devices. In addition, each device was examined with a stereomicroscope (40X) before the release experiment was conducted; any irregularities are noted in Table 4.

Monolithic devices

Copolymer/drug solution Tylosin tartrate dissolved easily in the solution of one gram of coplymer in 20 ml of dimethyl formamide. Crushing or grinding the particles was not necessary, but reduced the time required for complete dissolution. A yellow solution resulted, and no undissolved particles of drug were noticed in the solution at any time during the procedure. The presence of the drug had no apparent affect on the other physical properties of the solution.

Production of the devices The dip coating method worked satisfactorily for applying the the solution to the Silastic® mandrels. Silastic® mandrels were chosen for these devices as the first coat of solution formed a more uniform layer, and the copolymer would not be removed from the mandrels. The drug had no obvious affect on the formation of the copolymer layer; the layer which formed was transparent and rigid, with a yellow tint. The drug appeared to stay mixed with the formed copolymer, as no drug was observed to recrystallize upon evaporation of the solvent, and the devices retained the characteristic yellow color of the tylosin tartrate. Ten to twelve coats of solution were required to deposit the desired 150 mg of material on the rings. Ten rings with a diameter of 40 mm were produced in this manner; five of

Table 4. Physical characteristics of the reservoir devices

Device Number	Diameter of Ring (mm)	Wall Thickness (mm)	Drug Loading (mg)	Comments
1A	48	0.178	50.0	Devices 1A-6A were produced
2A	47	0.229	51.0	on Teflon® mandrels using
3A	48	0.279	51.0	rotation coating. Device 5A
4A	48	0.216	51.0	had visible holes in one
5A	48	0.279	51.0	segment; these were coated
6A	48	0.254	51.0	using copolymer solution.
7A	42	0.127	50.2	Dip coating technique
8A	42	0.127	50.2	produced more uniform layer
9A	42	0.135	50.0	of copolymer. All devices
10A	42	0.152	50.0	appeared uniform under
11A	42	0.160	50.1	stereomicroscopic view.
12A	45	0.241	50.0	Greater wall thickness
13A	45	0.236	50.0	made devices stiffer. All
14A	45	0.241	50.0	appeared uniform and strong.
15A	45	0.246	50.0	
16A	35	0.165	40.3	Devices held less drug due
17A	35	0.178	40.4	to smaller diameter. Despite
18A	36	0.211	40.0	similar process, thickness
19A	36	0.183	41.0	varied among devices.
20A	38	0.180	40.2	Copolymer/PVC assembly
21A	38	0.183	40.4	produced noticably stronger
22A	38	0.191	40.2	joint. No defects found
23A	38	0.191	40.3	under stereomicroscopic
24A	38	0.178	40.1	examination.
25A	38	0.178	40.6	PVC encased in copolymer
26A	40	0.163	40.2	tube segments. Copolymer
27A	40	0.173	39.9	solution formed complete bond
28A	40	0.183	40.1	between segments. No defects
29A	40	0.185	40.4	found in examination.

these rings were coated two additional times with copolymer solution without drug. Table 5 lists the physical characteristics of the monolithic devices.

Table 5. Physical characteristics of the monolithic devices

Device Number	Weight of Copolymer/drug (mg)	Total Weight (mg)	Weight of Drug (mg)	Weight of Copolymer (mg)
1B	149.2	149.2	49.7	99.5
2B	163.3	163.3	54.4	108.9
3B	148.3	148.3	49.4	98.9
4B	152.0	152.0	50.7	101.3
5B	151.9	151.9	50.6	101.3
6B	187.8	225.0	62.6	162.4
7B	194.8	231.0	64.9	166.1
8B	195.6	231.8	65.2	166.6
9B	193.9	234.2	64.6	169.6
10B	195.6	232.7	65.2	167.5

Tylosin Tartrate Release Experiments

Reservoir devices

The release experiments were conducted as described in the previous section. Due to equipment considerations, devices 1A and 2A were placed in a standing water bath during the test instead of the shaking water bath.

At the beginning of the experiment, each device appeared dry, but after time the liquid medium filled the devices. As liquid entered the devices, the tylosin tartrate dissolved to form a viscous, amber-colored

solution. A wide variation existed in the time required for the liquid to fill the devices; these times are given in Table 6. After 24 hours in the release medium the copolymer softened slightly and became more flexible than the dry material, but would still break abruptly if bent. The devices in water remained transparent, while the copolymer became cloudy or opaque in saline solutions. These devices returned to their original tranparent appearance again when dried at the conclusion of the experiment.

Table 6. Time required for liquid to fill reservoir devices

Device Number	Time required to fill device (hr)	Device Number	Time required to fill device (hr)
1A	8	16A	24
2A	8	17A	24
3A	8	18A	24
4A	8	19A	24
5A	8	20A	24
6A	8	21A	24
7A	24	22A	24
8A	24	23A	24
9A	24	24A	24
10A	24	25A	16
11A	24	26A	16
12A	32	27A	16
13A	24	28A	16
14A	24	29A	16
15A	32		

Monolithic devices

The release experiments were conducted as previously described. The devices began the experiment with a yellow, translucent appearance, but within eight hours the release medium caused the rings to swell slightly and become white and opaque. This swelling increased the flexibility of the rings, but they would still break if bent sharply. Upon removal from the release medium at the end of the experiment, longitudinal cracks were seen to have developed in the copolymer layer. As the copolymer layer dried, it appeared to shrink, as these cracks expanded until they spanned the entire circumference of the ring.

Release Characterization

The samples of release medium required 3-4 days for complete evaporation. The samples were redissolved with a volume of water equal to one tenth the original sample volume; smaller amounts of water often resulted in incomplete dissolution of the dried material. The redissolved samples were analyzed using thin-layer chromatography (TLC) to detect and quantify the tylosin tartrate present in each sample. The dark spot of the tylosin tartrate pattern was utilized for the densitometric scan. Because the size and R_f value of the dark spot vary depending on the amount of salt in the sample, the salt concentration of the standards was matched as closely as possible to the salt concentration of the samples. The samples were redissolved in one tenth their original volume of water; therefore, the standards were prepared in

solutions containing ten times the concentration of the salts found in the original release medium.

The dark spot produced by tylosin tartrate on the TLC plate was sensitive to the amount of drug present; elongation, or tailing, of the spot occurred when an amount of drug greater than 5 ug was present in the spot. For this reason, the dilution of the samples was adjusted so that a 2.5 ul aliquot deposited less than 4 ug of drug onto the plate.

Reservoir devices

Appendix A contains the data from the results of the TLC analysis for the release experiments for the reservoir devices. The data include the collection time, redissolved sample volume, weight of the dark spot densitometer peak, the number of the standard curve data, the amount of drug in the spot, the average release rate over the time period, and the cumulative drug release at the end of the time period. The standard curve number refers to the set of peak weights for the standard drug amounts which were spotted on the same plate as the samples; these data are listed in Appendix C.

Figures 19-39 show the release characteristics for devices 1A-20A and 23A; devices 21A, 22A, and 24A-29A gave no detectable drug release, so their release characteristics were not plotted. The straight lines on the plots of total release versus time for devices 1A-4A, 6A-10A, and 13A-18A indicate the period of time over which the drug release from the devices was approximately constant. Devices 5A, 11A, 12A, and 19A had no range of time over which the release could be considered approximately

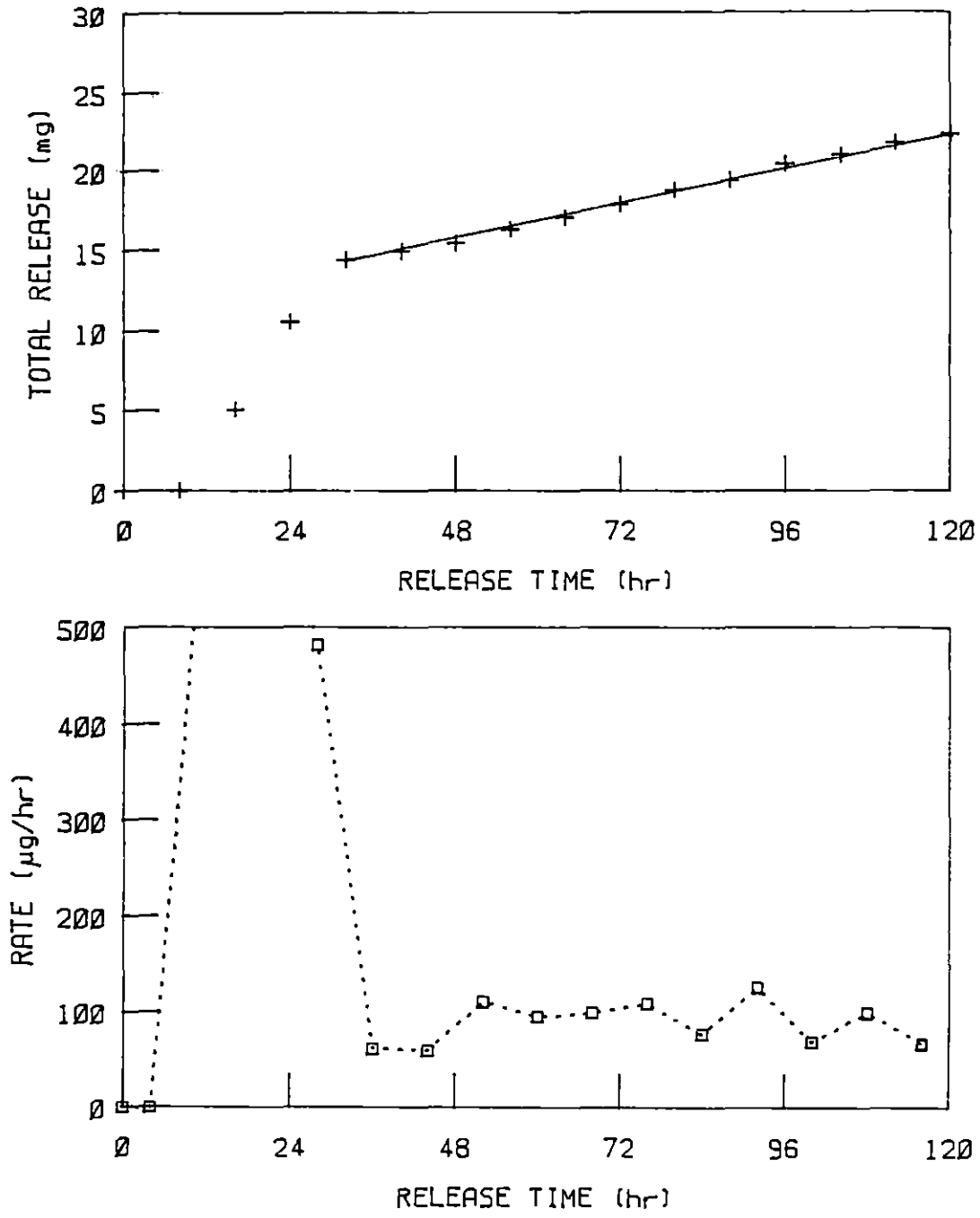

Figure 19. Release characteristics for device 1A. Top: total release vs. time; bottom: rate vs. time

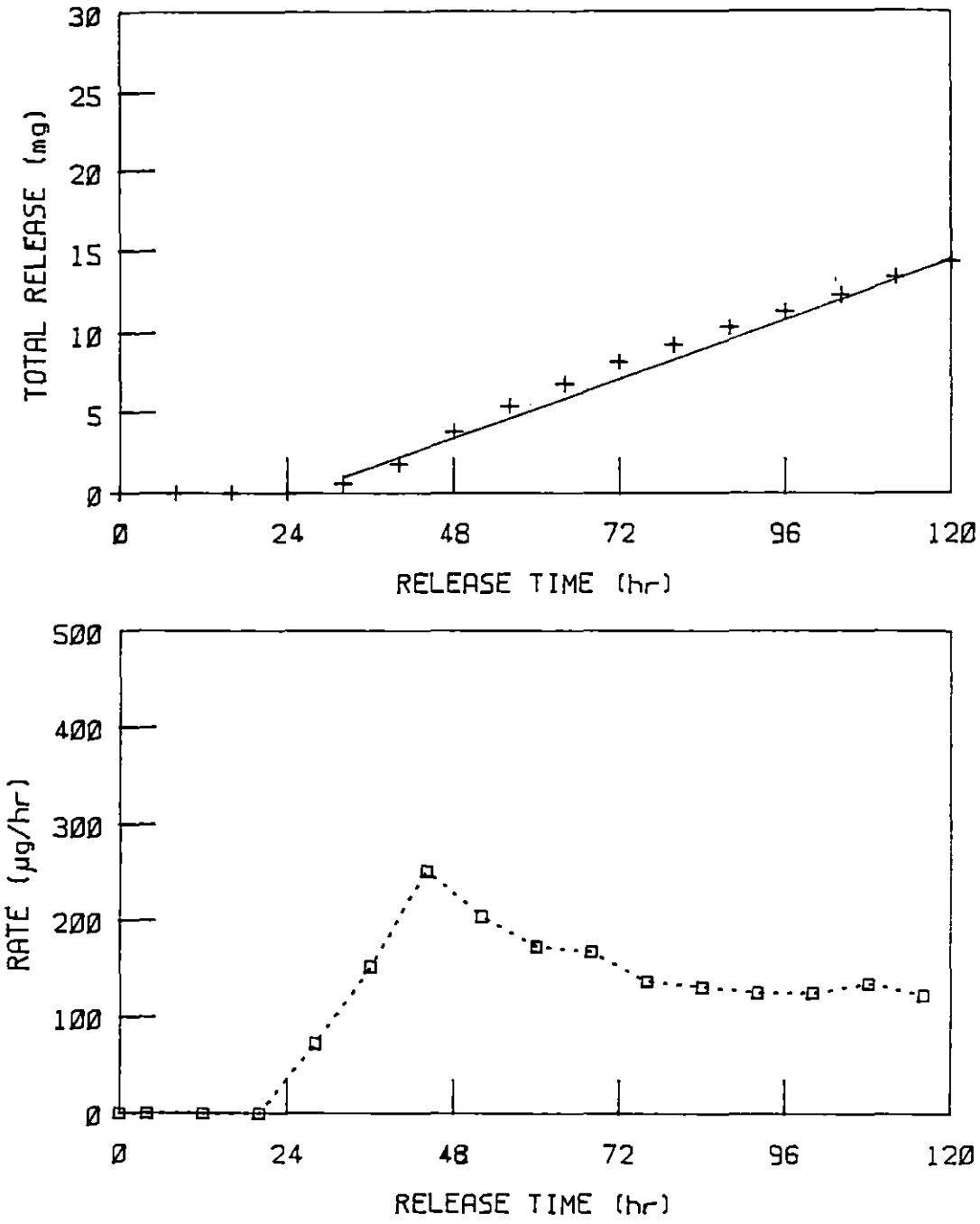

Figure 20. Release characteristics for device 2A. Top: total release vs. time; bottom: rate vs. time

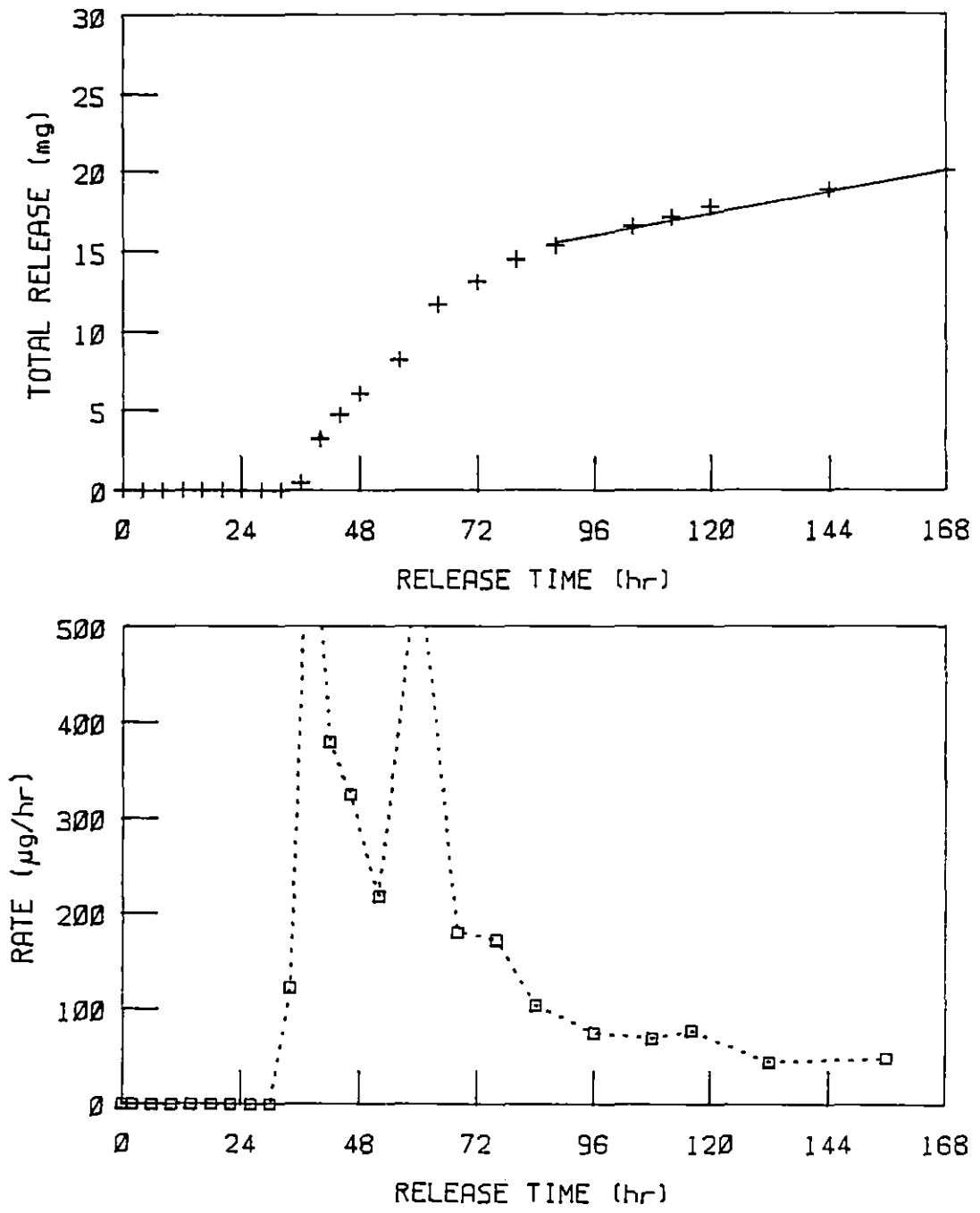

Figure 21. Release characteristics for device 3A. Top: total release vs. time; bottom: rate vs. time

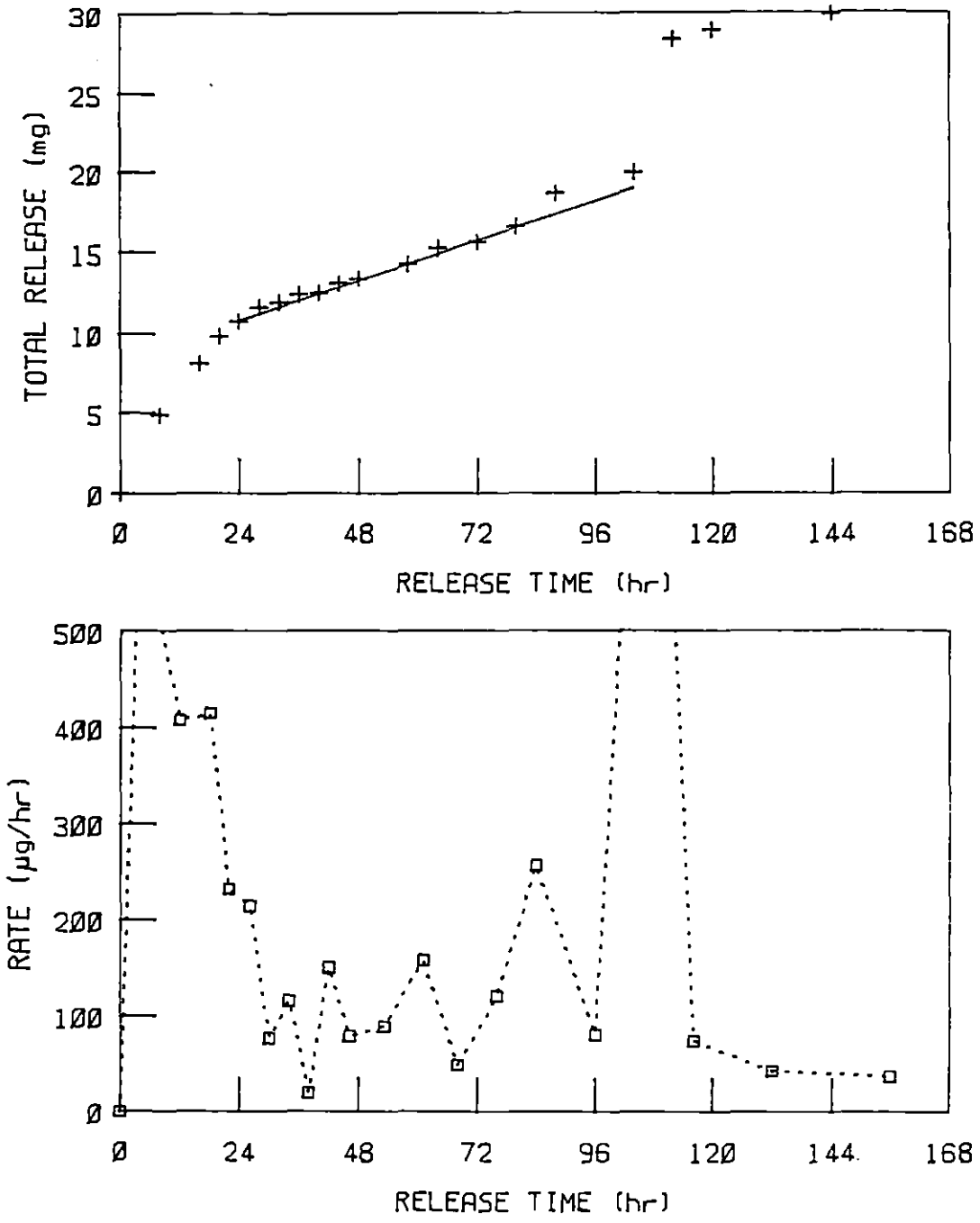

Figure 22. Release characteristics for device 4A. Top: total release vs. time; bottom: rate vs. time

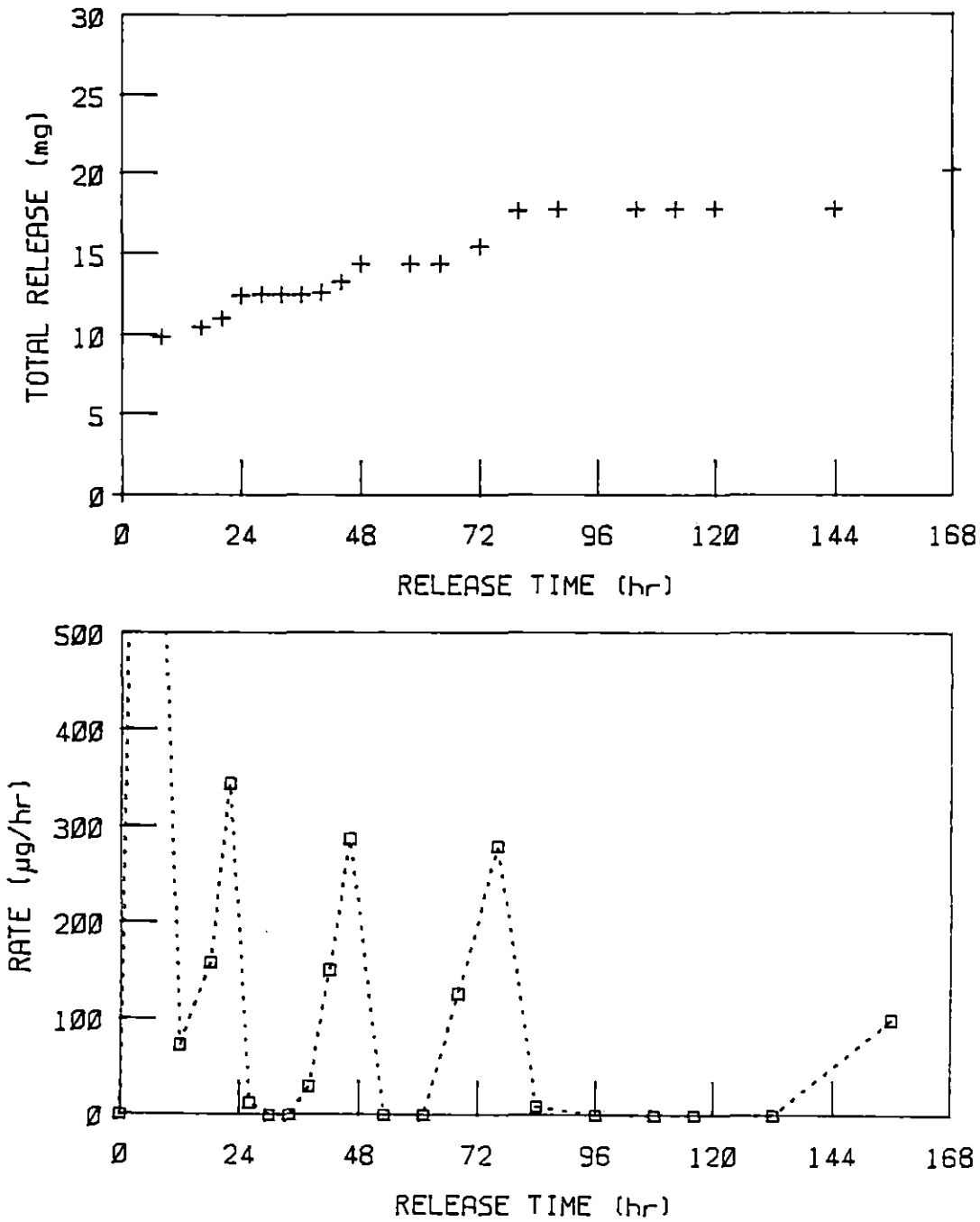

Figure 23. Release characteristics for device 5A. Top: total release vs. time; bottom: rate vs. time

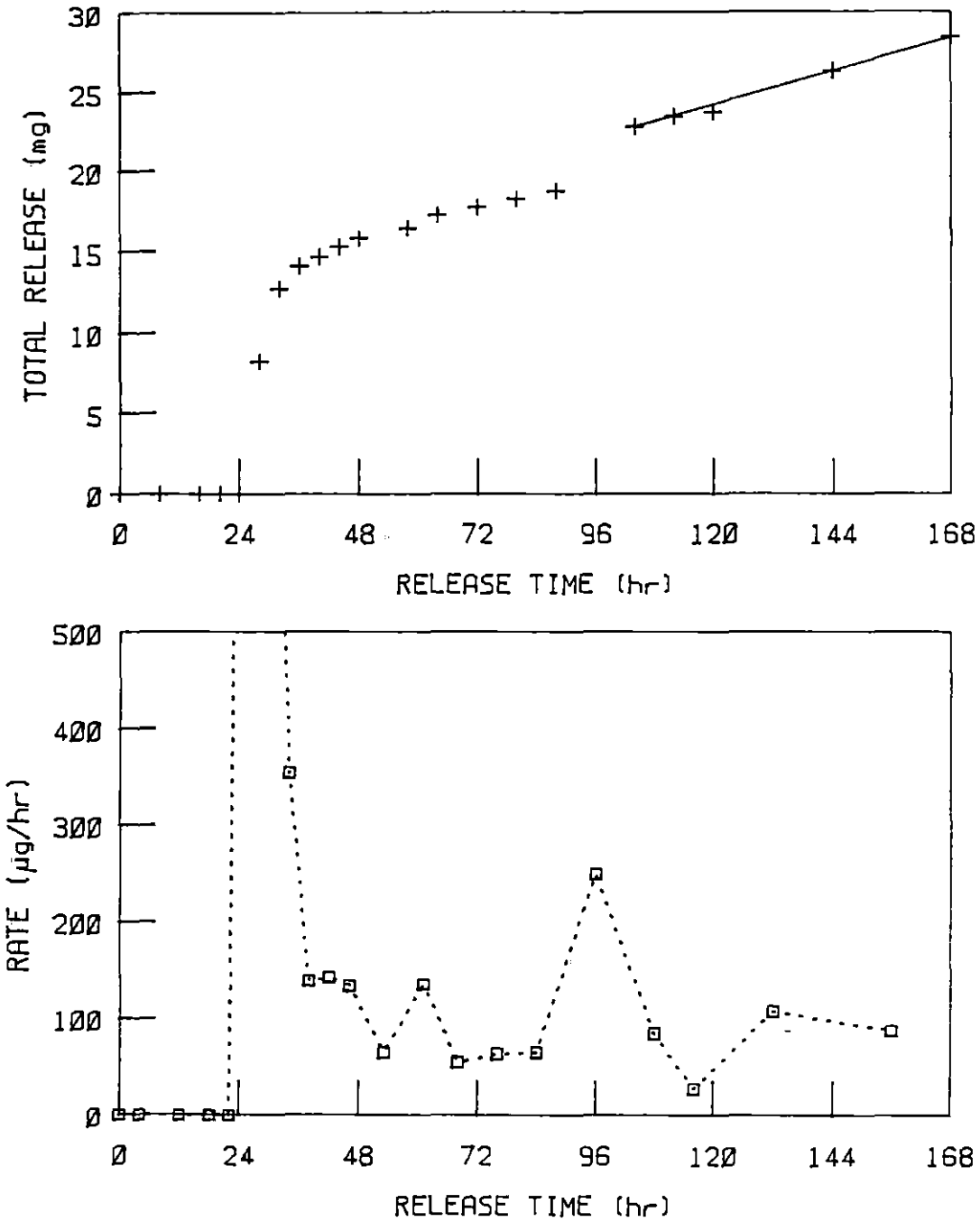

Figure 24. Release characteristics for device 6A. Top: total release vs. time; bottom: rate vs. time

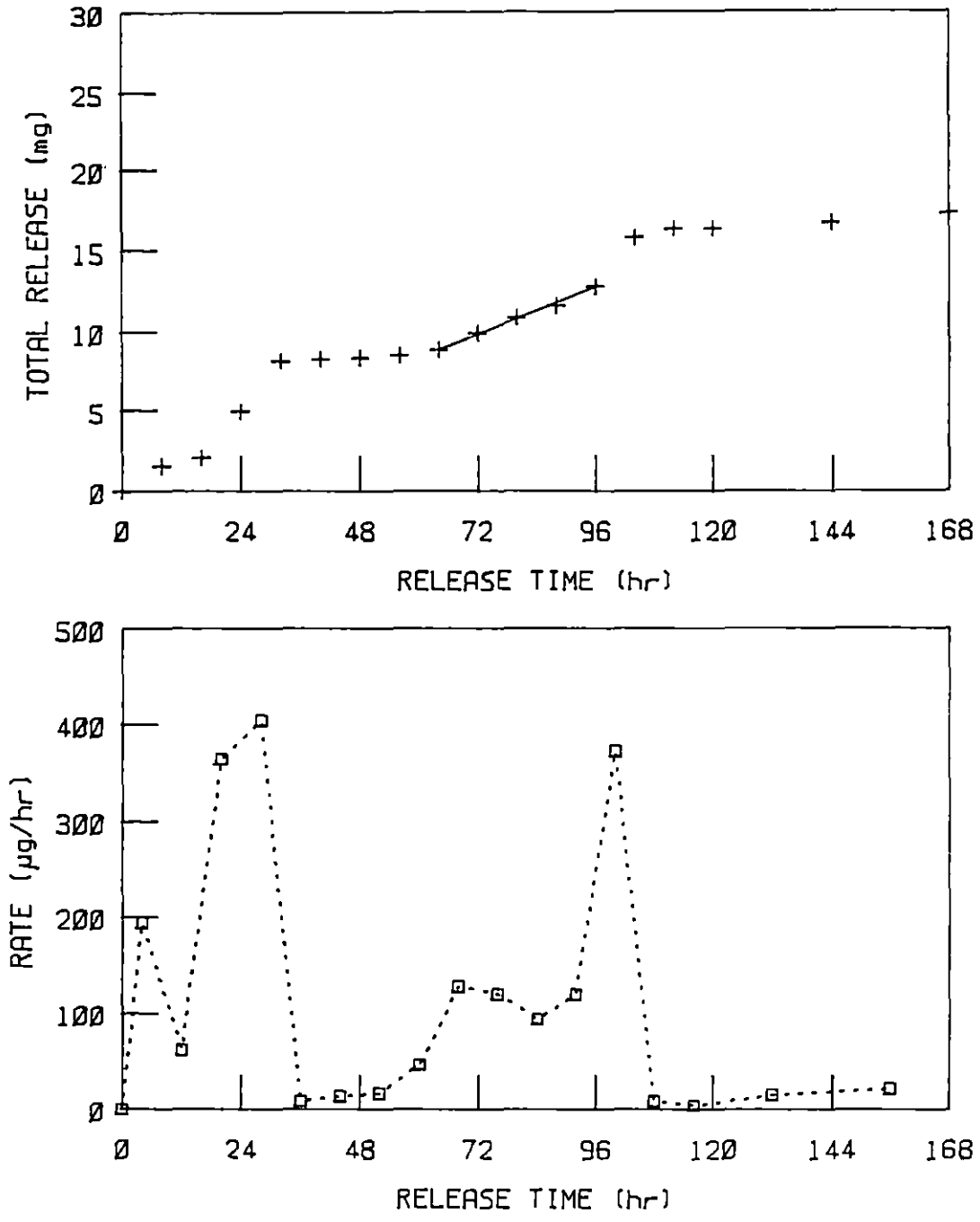

Figure 25. Release characteristics for device 7A. Top: total release vs. time; bottom: rate vs. time

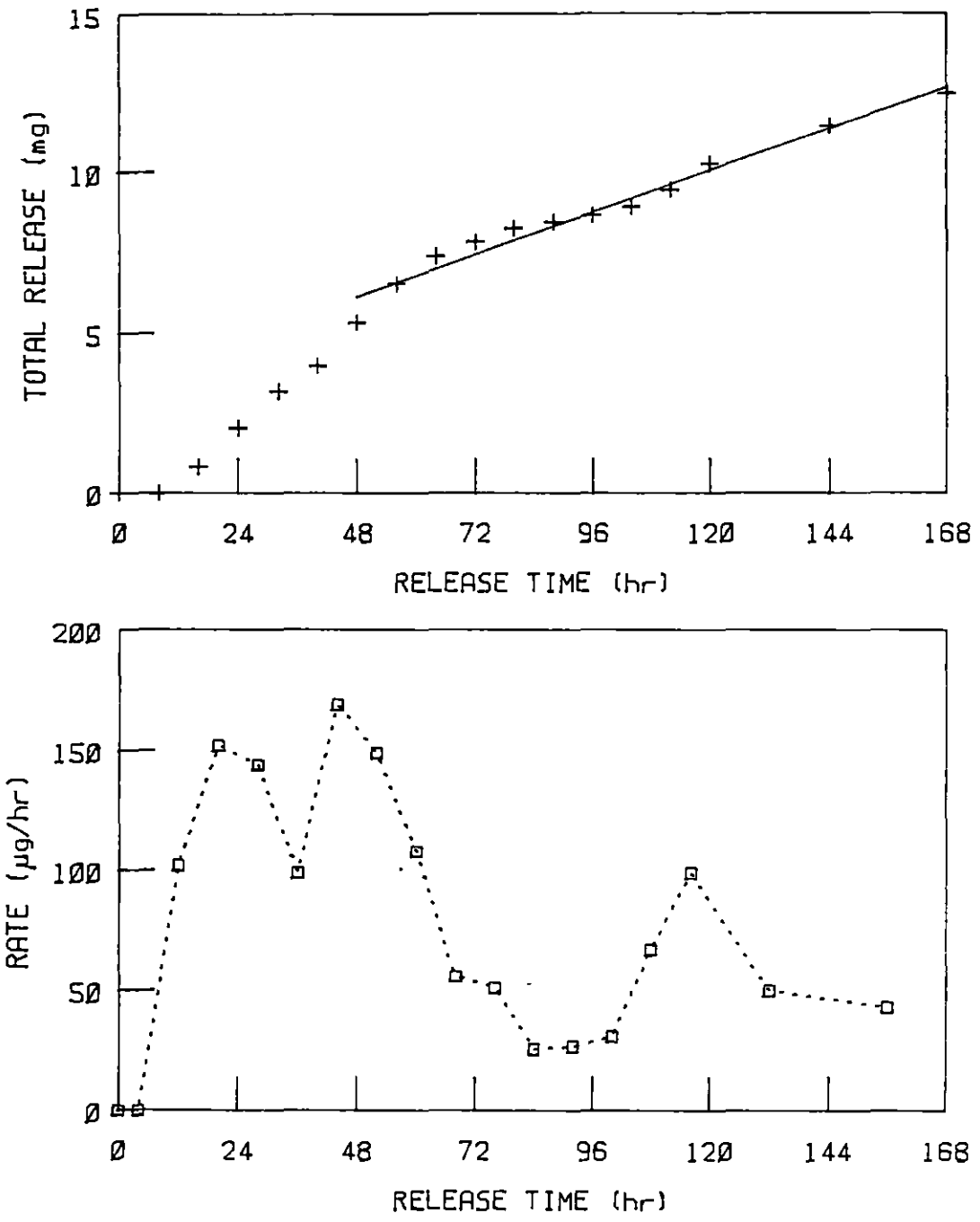

Figure 26. Release characteristics for device 8A. Top: total release vs. time; bottom: rate vs. time

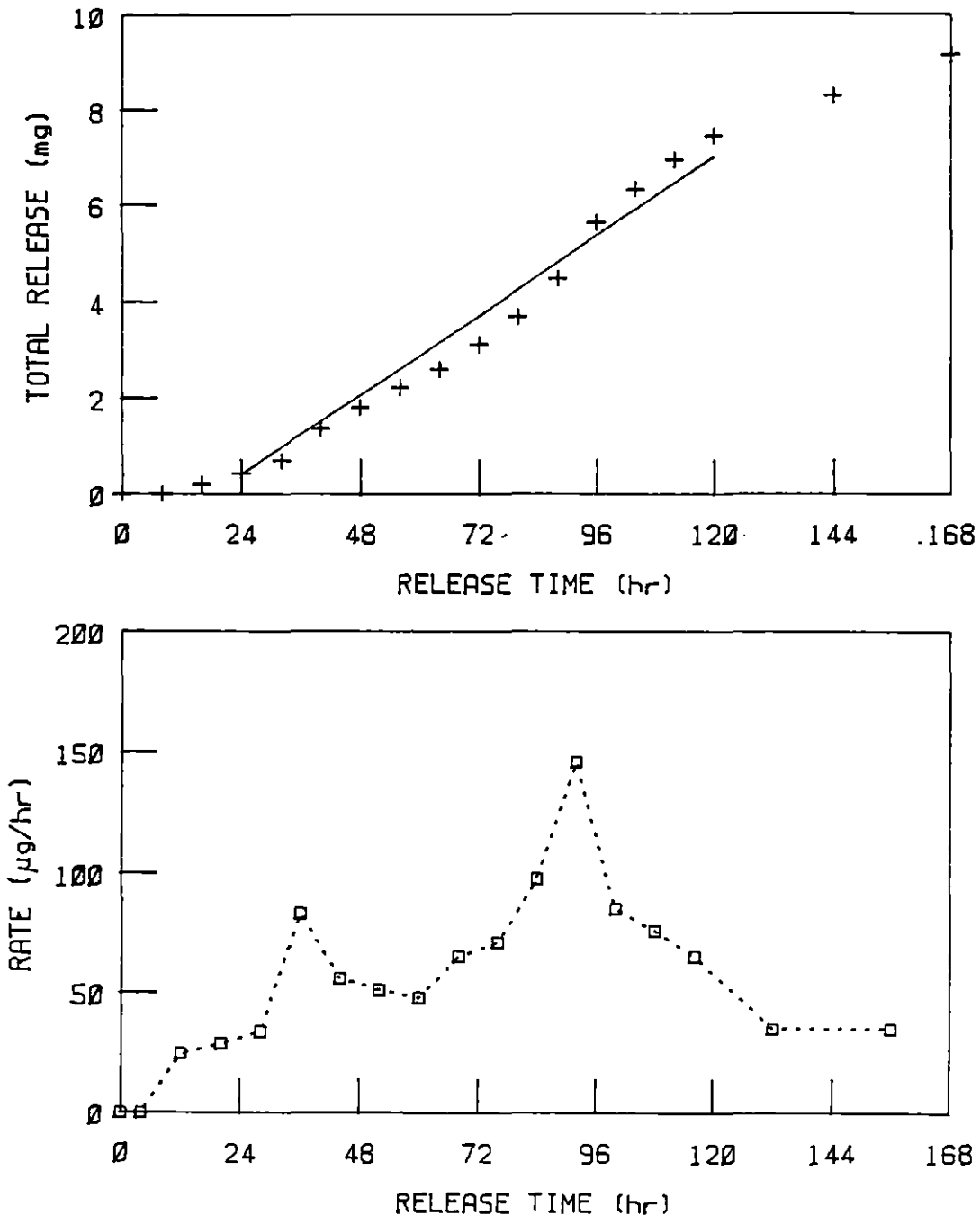

Figure 27. Release characteristics for device 9A. Top: total release vs. time; bottom: rate vs. time

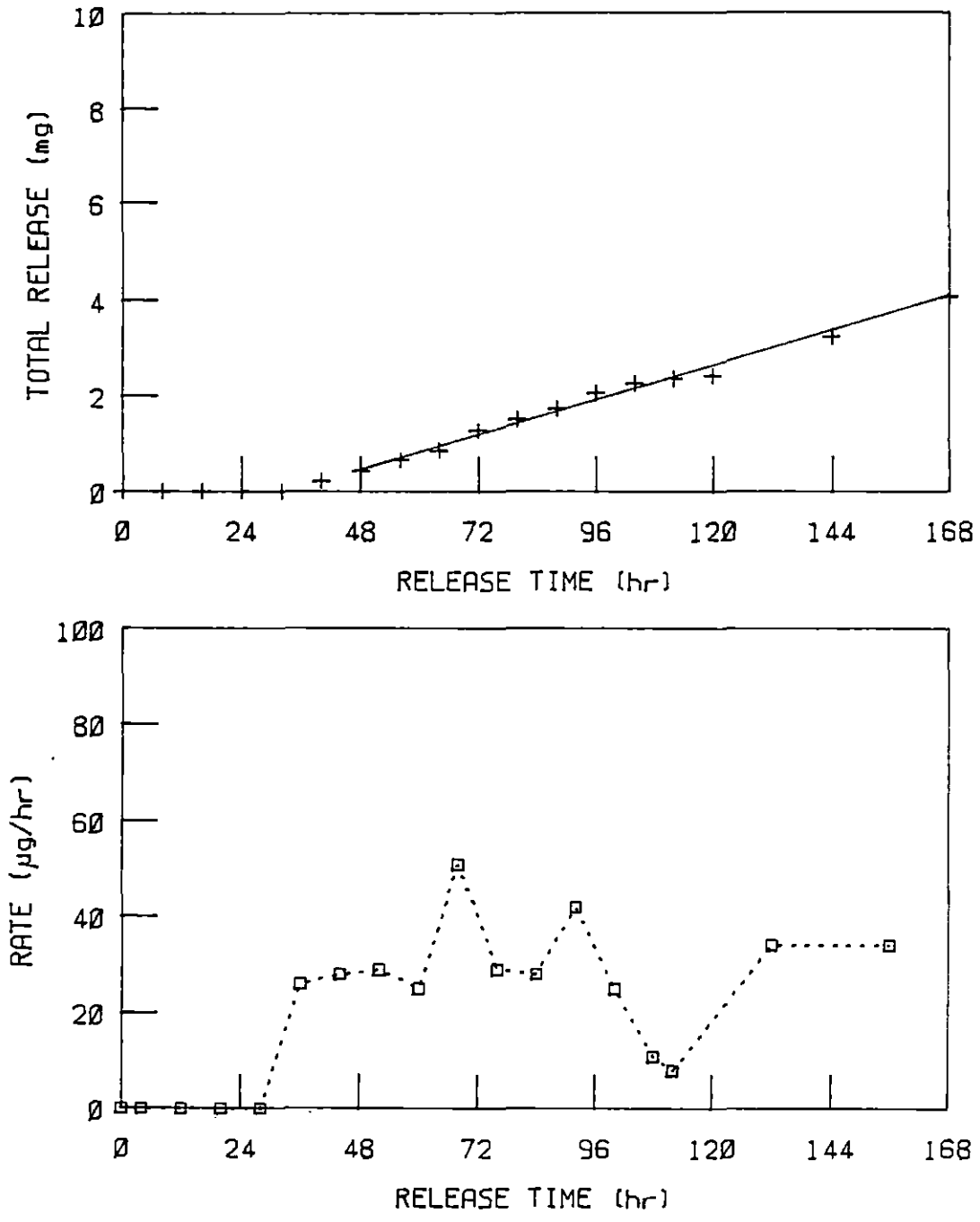

Figure 28. Release characteristics for device 10A. Top: total release vs. time; bottom: rate vs. time

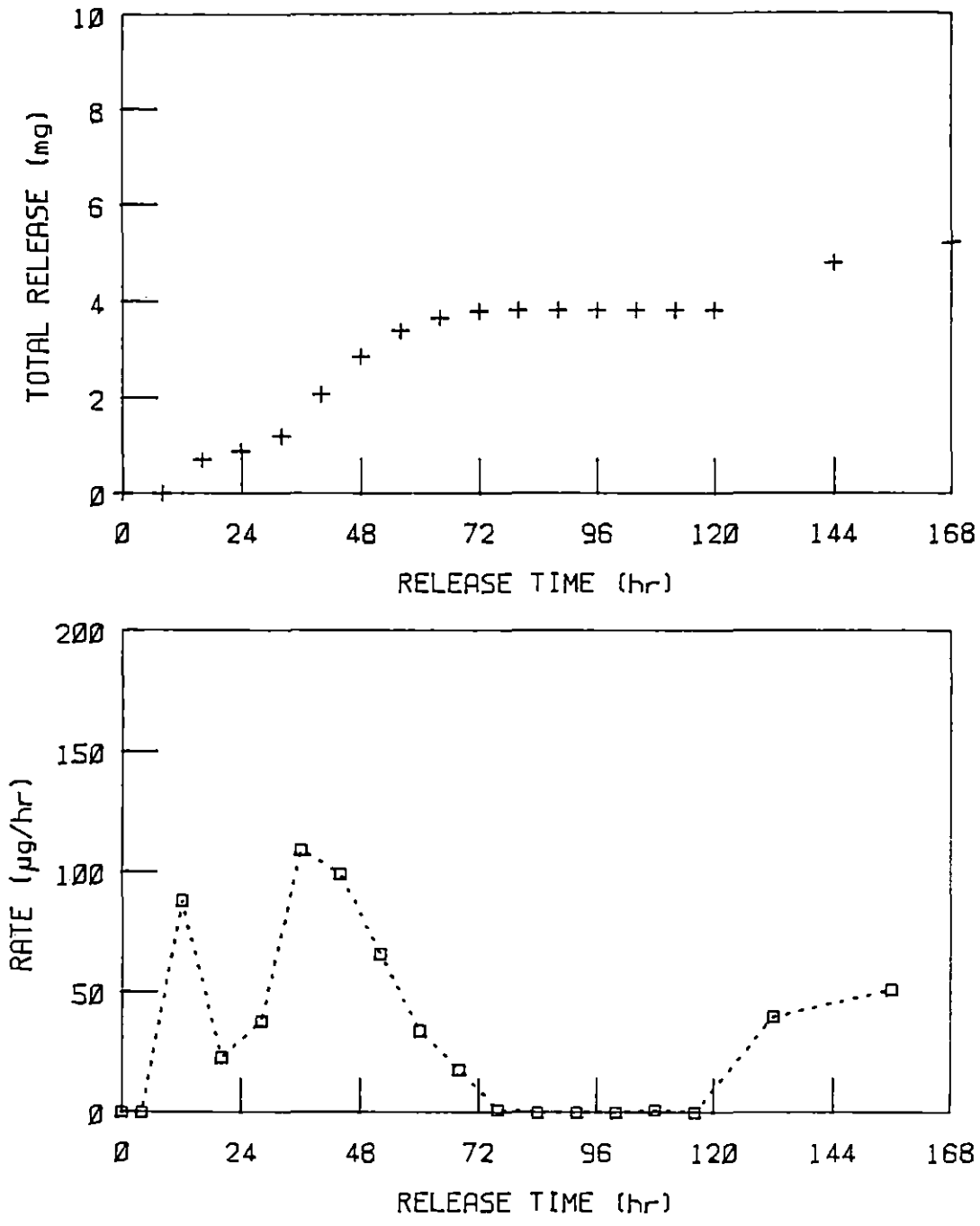

Figure 29. Release characteristics for device 11A. Top: total release vs. time; bottom: rate vs. time

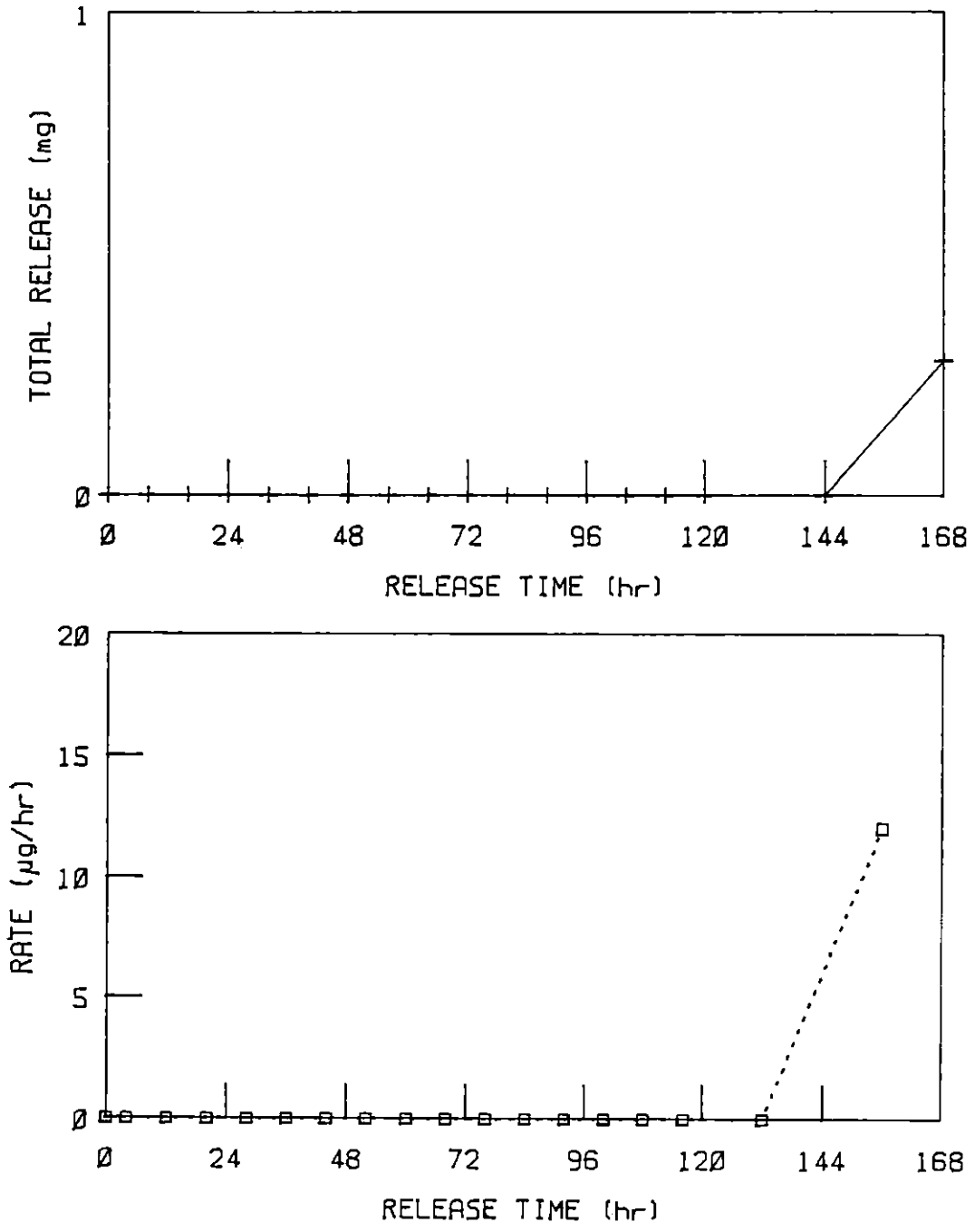

Figure 30. Release characteristics for device 12A. Top: total release vs. time; bottom: rate vs. time

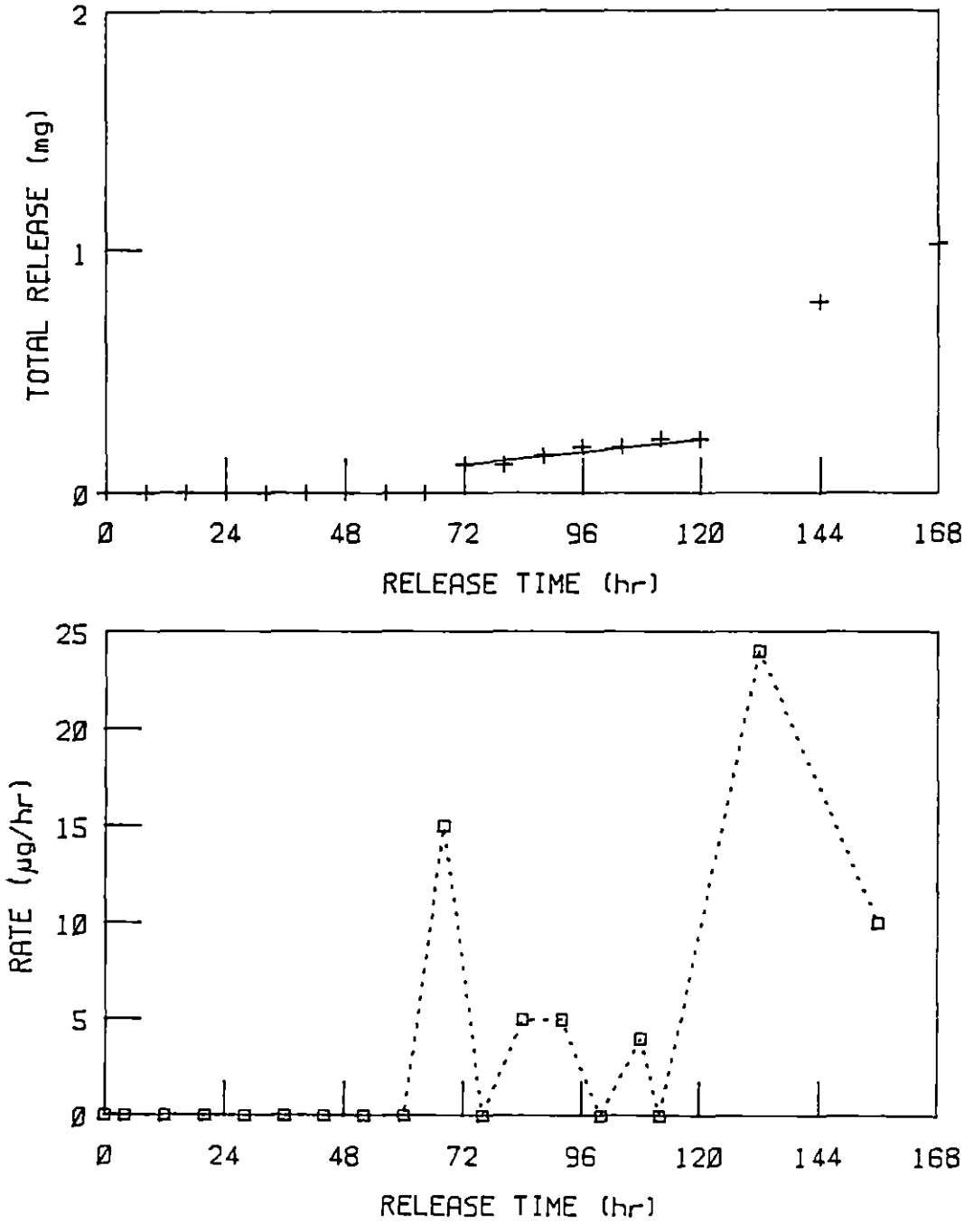

Figure 31. Release characteristics for device 13A. Top: total release vs. time; bottom: rate vs. time

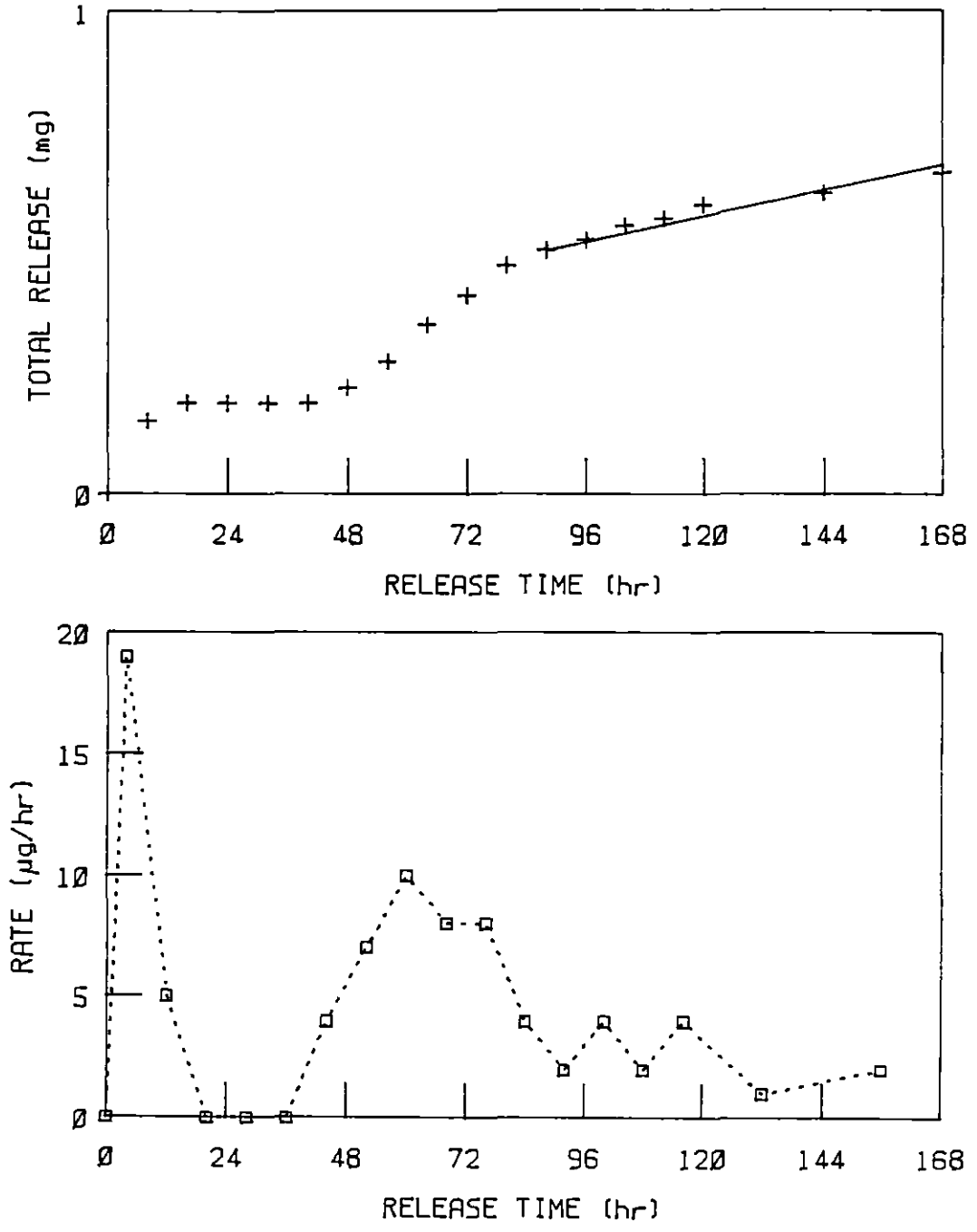

Figure 32. Release characteristics for device 14A. Top: total release vs. time; bottom: rate vs. time

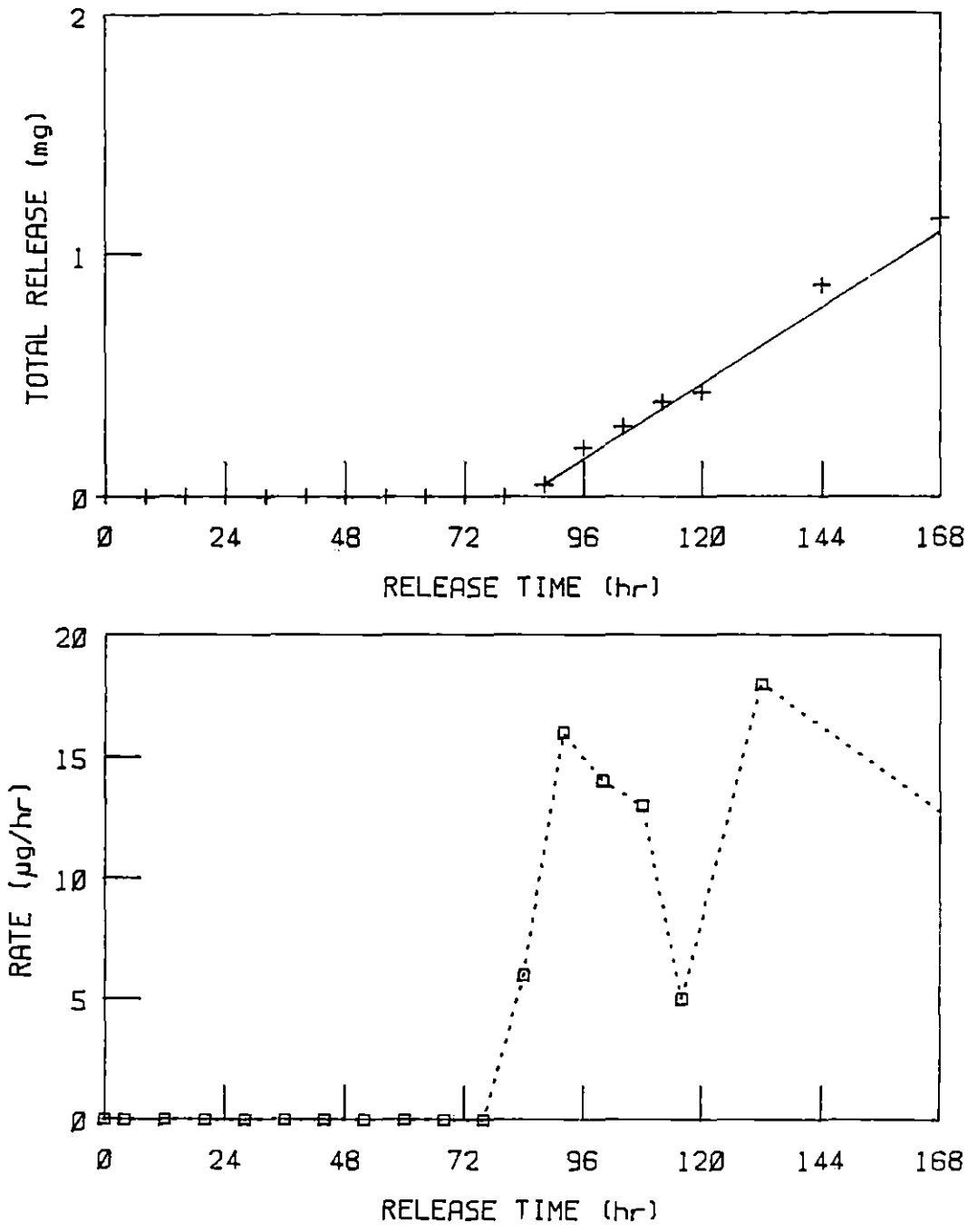

Figure 33. Release characteristics for device 15A. Top: total release vs. time; bottom: rate vs. time

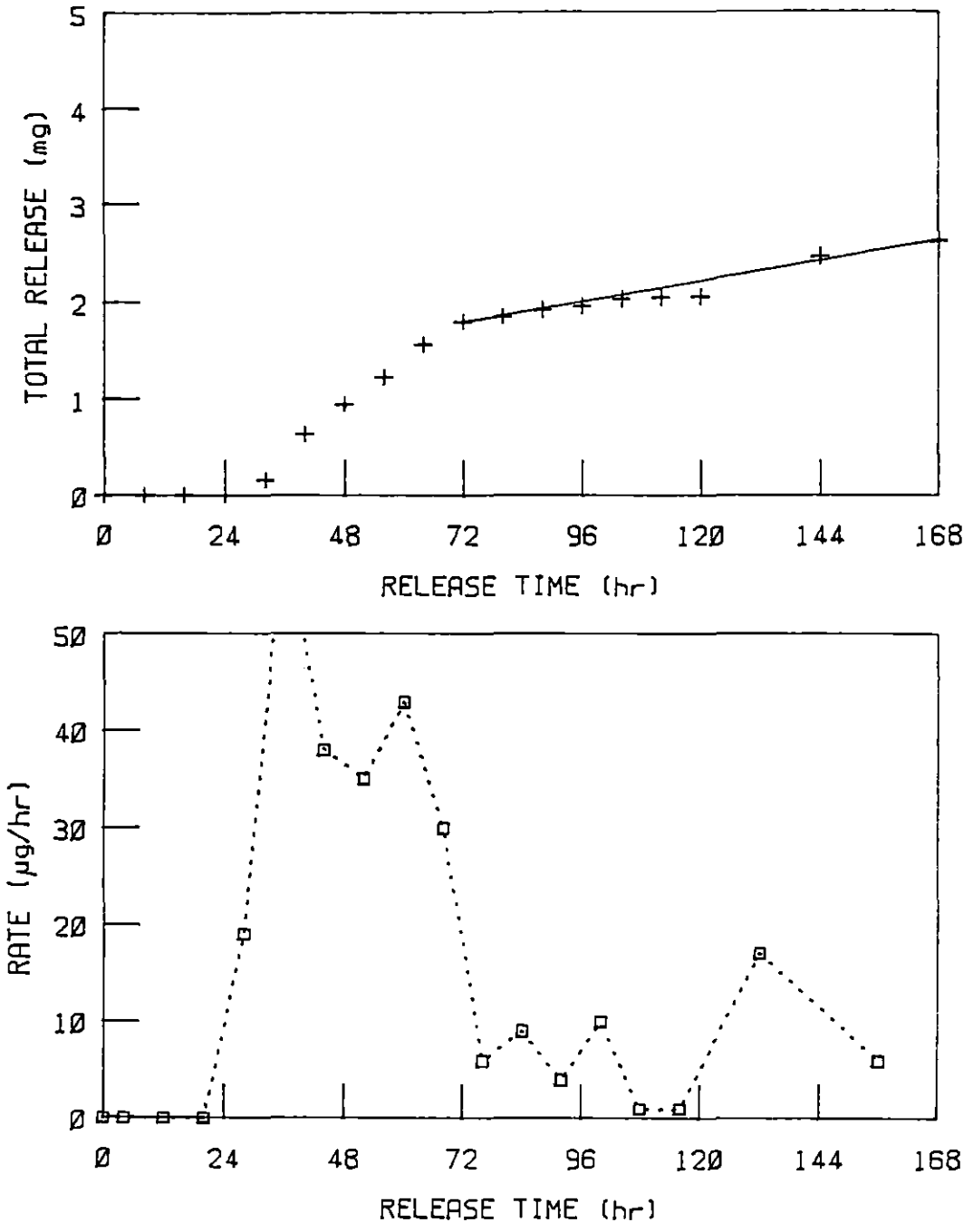

Figure 34. Release characteristics for device 16A. Top: total release vs. time; bottom: rate vs. time

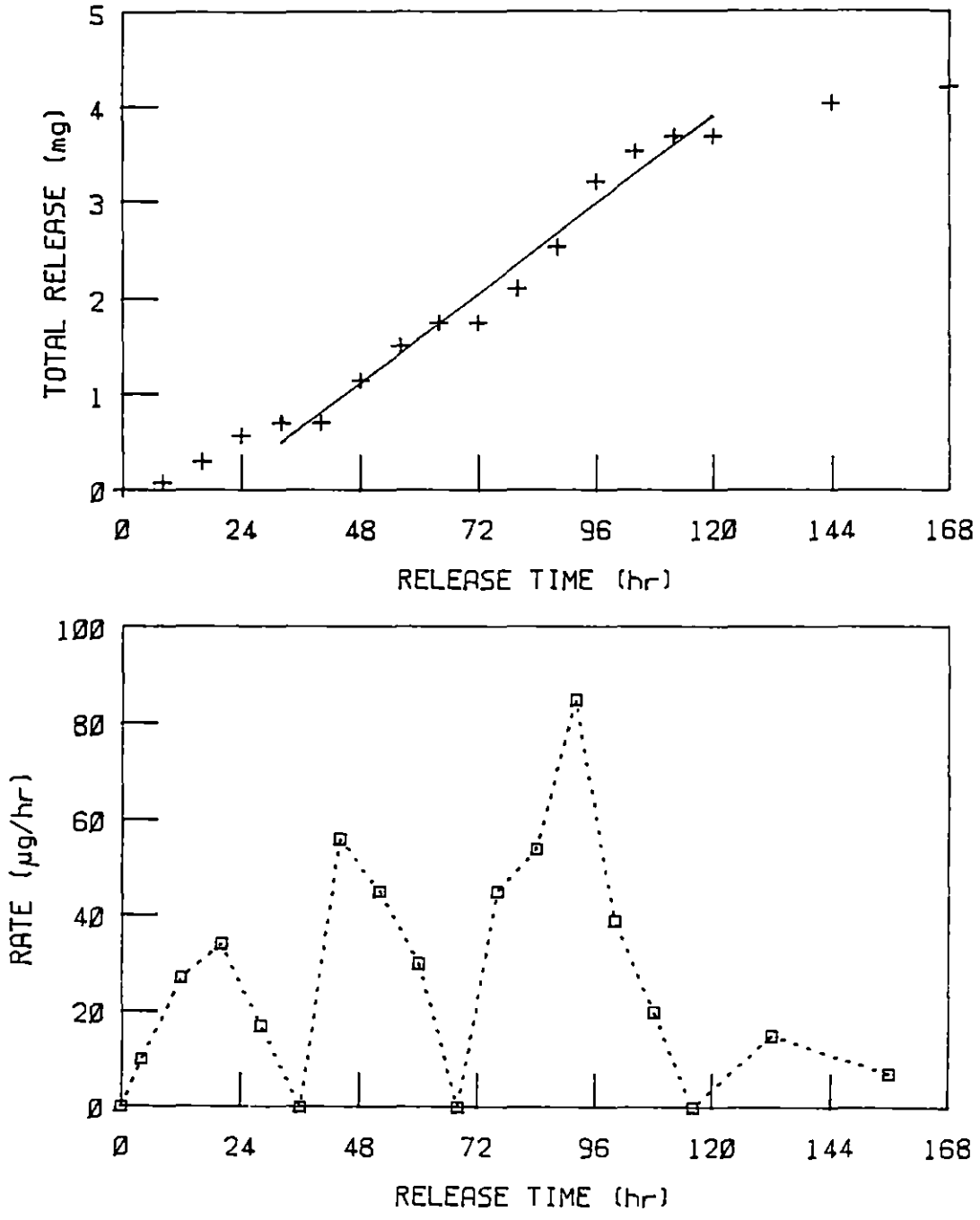

Figure 35. Release characteristics for device 17A. Top: total release vs. time; bottom: rate vs. time.

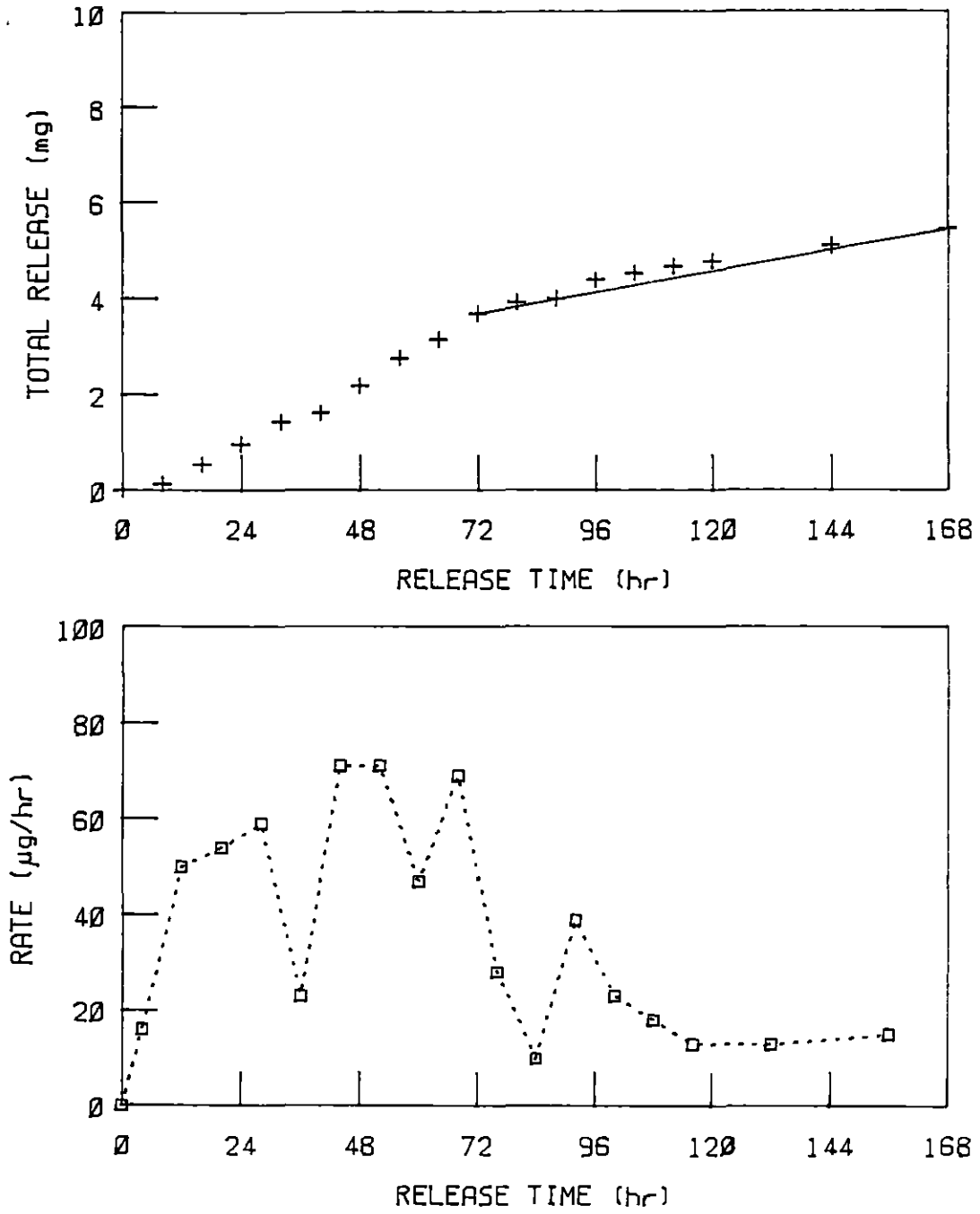

Figure 36. Release characteristics for device 18A. Top: total release vs. time; bottom: rate vs. time

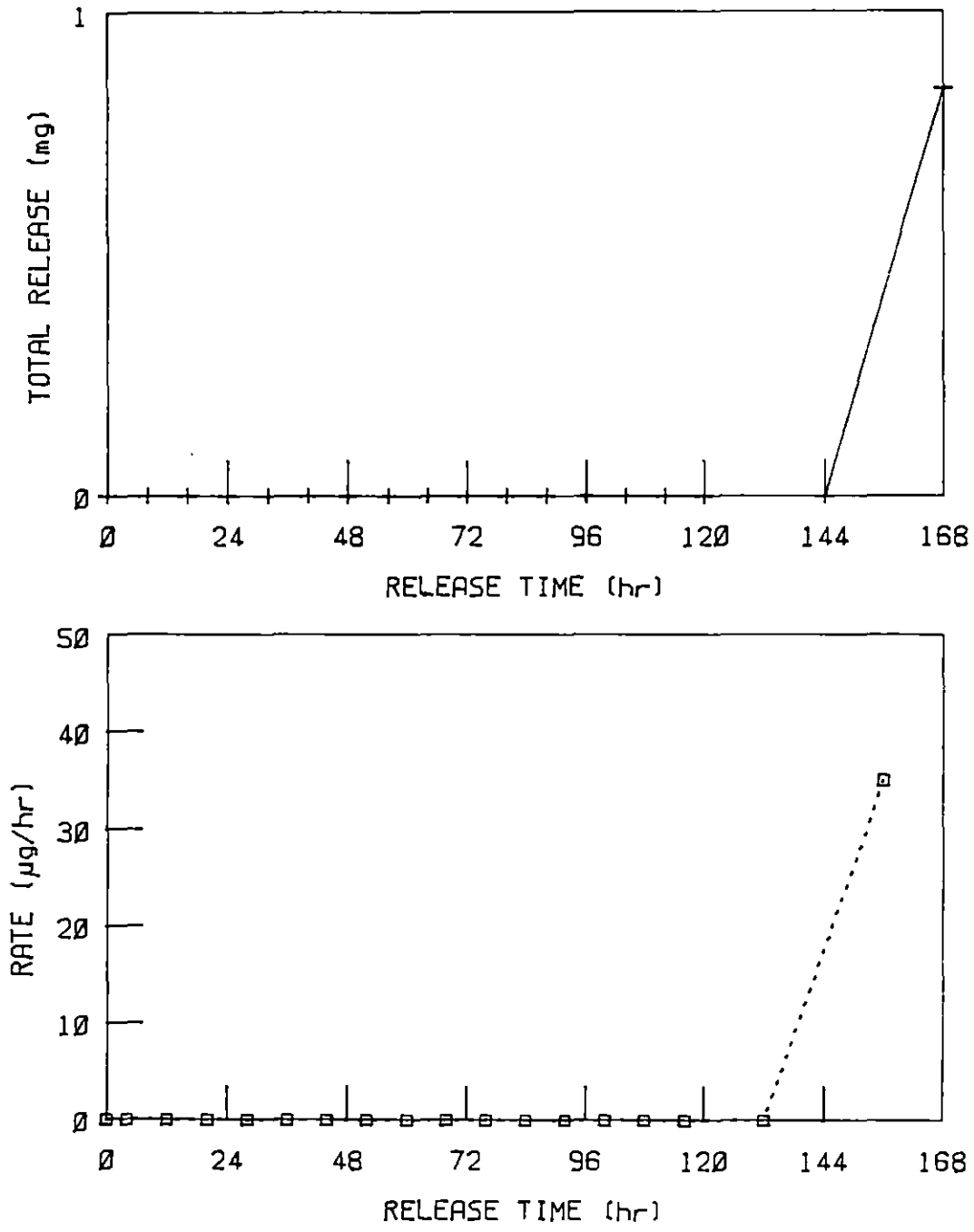

Figure 37. Release characteristics for device 19A. Top: total release vs. time; bottom: rate vs. time

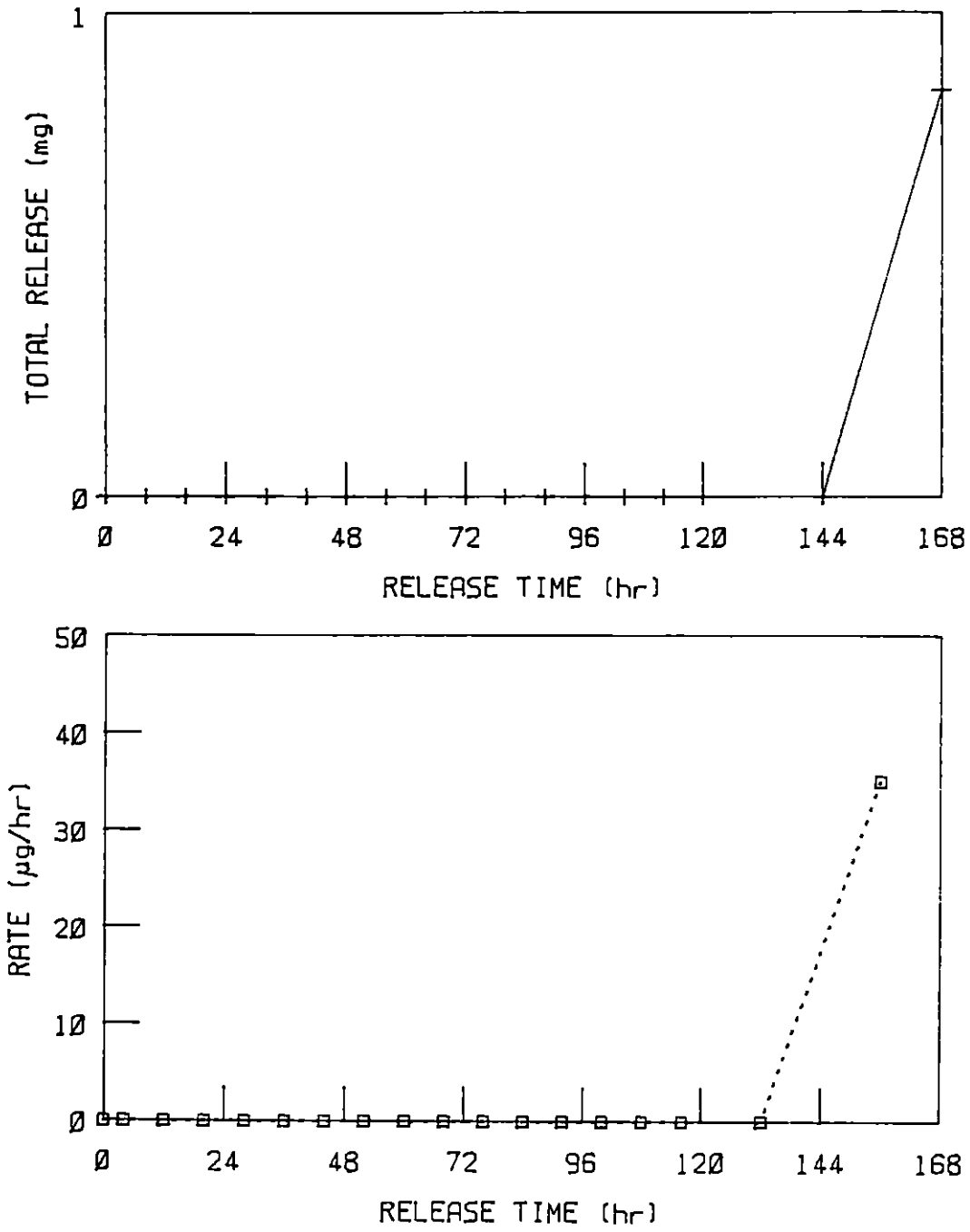

Figure 38. Release characteristics for device 20A. Top: total release vs. time; bottom: rate vs. time

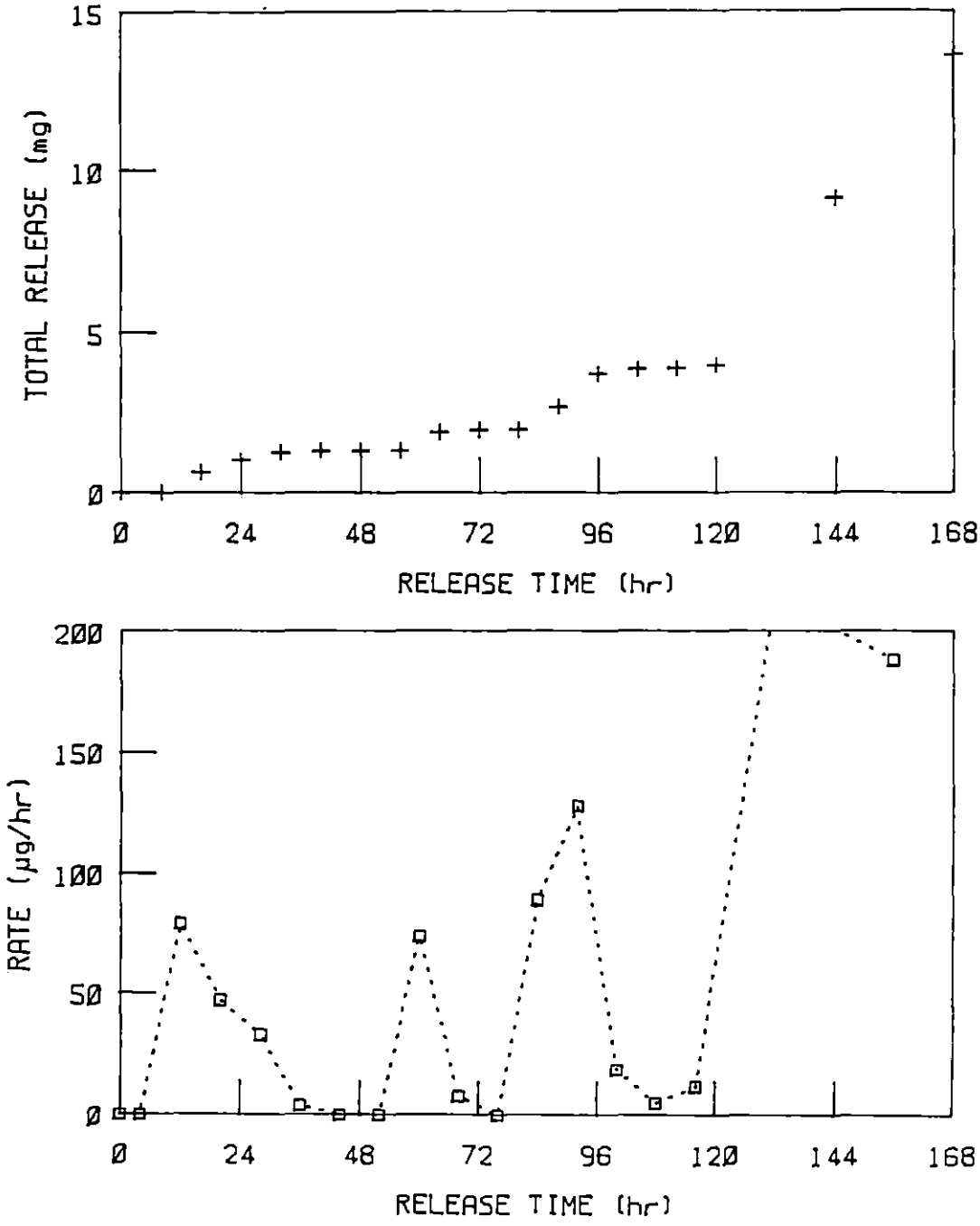

Figure 39. Release characteristics for device 23A. Top: total release vs. time; bottom: rate vs. time

constant, so no lines are included on these plots. Samples that failed to produce spots are designated as having a release rate of 0 ug/hr; due to the sensitivity of the detection method, this represents an actual rate below 1 ug/hr. Table 7 summarizes the release characteristics for the reservoir systems; Table 8 summarizes the results of a post-experiment physical examination of the devices, including a 40X stereomicroscopic examination.

Devices 1A-19A are grouped according to the method used to produce the copolymer segments, and were all assembled using polyethylene connecting tubes and Silastic® adhesive. Despite the similar assembly technique, a wide variation in the drug release characteristics was seen.

Devices 1A and 2A exhibited approximately constant release over the 88 hours between 32 and 120 hours. However, device 1A released drug at a fast rate (>400 ug/hr) during the time between eight and 32 hours, then continued to release drug at an average rate of 93.4 ug/hr for the remainder of the 120 hour experiment, whereas device 2A released no drug before 32 hours, then released drug at an average rate of 155.0 ug/hr. As noted earlier, both of these devices were tested in a standing water bath, instead of the shaking water bath.

Devices 3A-19A, which were all assembled with polyethylene and Silastic® adhesive and tested in a shaking water bath, failed to exhibit any consistent release characteristics. Although periods of constant release are indicated by the straight lines on the plots of cumulative release versus time for devices 3A, 4A, 6A-10A, and 13A-18A, it must be noted that these constant release periods vary greatly with respect to

Table 7. Summary of the release characteristics for the reservoir devices

Device Number	Range of Constant Release (hr)	Length of Time of Constant Release (hr)	Average Release Rate (ug/hr)	Total Release (ug)
1A	32-120	88	93.4	22272
2A	32-120	88	155.0	14376
3A	80-168	88	56.7	19990
4A	24-104	80	98.7	30822
5A	. . .	. .	. .	20084
6A	104-168	64	89.5	28388
7A	64-96	32	116.0	17254
8A	48-168	120	54.6	12472
9A	24-120	96	69.6	9136
10A	32-168	136	30.6	4048
11A	. . .	. .	. .	5187
12A	. . .	. .	. .	280
13A	72-120	48	2.6	1023
14A	88-168	80	1.9	657
15A	88-168	80	13.8	1142
16A	80-168	88	7.8	2600
17A	32-120	88	38.2	4198
18A	80-168	88	19.7	5429
19A	. . .	. .	. .	840
20A	32-88	56	33.7	1755
21A	. . .	. .	. .	0
22A	. . .	. .	. .	0
23A	16-120	104	34.1	13614
24A	. . .	. .	. .	0
25A-29A	. . .	. .	. .	0

Table 8. Post-experiment physical characteristics of the reservoir devices

Device Number	Comments
1A,2A	Both devices appeared intact; copolymer was strong and transparent when dry
3A	Device appeared intact; copolymer was strong and transparent
4A	Obvious holes in one copolymer segment, apparently due to coating problem; bad segment nearly depleted of drug.
5A	Device intact; copolymer transparent and strong. One side virtually depleted of drug; long cracks possible in this side, but stereomicroscopic examination is inconclusive.
6A	Device intact; copolymer transparent and strong. Both sides depleted of drug.
7A-11A	All devices flattened and became somewhat oval, but were transparent and strong when dried. Device 7A was found to have a small hole (break). None of these devices (even 7A) showed large regions of drug depletion.
12A-19A	These devices did not flatten as much as devices 7A-11A. All devices appeared intact, transparent, and strong at the conclusion of the experiments.
20A-24A	PVC tube, which became opaque during the experiments, returned to pre-experiment appearance. Device 20A pulled apart easily, but other four devices were strong, and would break before joints could be separated. All devices were intact; copolymer was transparent and strong.
25A-29A	Joint between copolymer segments remained intact; all devices were intact; copolymer segments were transparent and strong.

the time of onset from the beginning of the experiment, duration, average release rate, and range of release rates included within these periods. The duration of these constant release periods varied from 32 to 136 hours, and the average constant release rate varied from 1.9 ug/hr for device 14A to 116.0 ug/hr for device 7A. The total drug release from the devices ranged from 30,754 ug for device 4A to only 280 ug for device 12A. As seen in Table 8, few of the devices were found to have obvious physical defects. Device 4A was the only one of the 19 reservoir devices assembled with polyethylene and Silastic® which had definite holes in the wall of one of the copolymer segments. Although other devices were suspected of having physical defects, none could be found conclusively using the stereomicroscope.

Only two of the ten devices assembled with PVC connecting tubes exhibited any detectable drug release. Device 20A released drug erratically at rates from 0-66 ug/hr. As noted in Table 8, this device did not appear to be cemented together properly, as it could easily be pulled apart after the experiment. Device 23A also released drug erratically at rates from 0-213 ug/hr, but no obvious defects were found in the post-experiment physical examination. None of devices 25A-29A, which were prepared with the PVC segment encased in copolymer, gave any detectable release of tylosin tartrate.

Monolithic devices

Appendix B contains the data from the results of the TLC analysis for the release experiments on the monolithic devices. The data include the collection time, redissolved sample volume, weight of the dark spot densitometer peak, the number of the standard curve data, the amount of drug in the spot, the average release rate over the time period, and the cumulative drug release at the end of the time period. The standard curve number refers to the set of peak weights for the standard drug amounts which were spotted on the same plate as the samples; these data are listed in Appendix C.

Figures 40-49 show the release characteristics for devices 1B-10B. All ten of the devices showed similiar release characteristics; the initial release rate was very large (1236 ug/hr to 1716 ug/hr), then the rate decreased as a power function until it reached a level of 5-22 ug/hr after nine days. The relationship between the release rate and time was evaluated mathematically by assuming that the rate was proportional to some power of time:

$$\text{release rate} = a(\text{time})^{b} \qquad (6)$$

The values of a and b were determined by performing a linear regression on the plot of the log of the release rate versus the log of the time; these values are in Table 9. For devices 1B-5B, the values of (a) were between 8576 and 21,390; the values of (b) varied from -1.18 to -1.44. For devices 6B-10B, the values for (a) were from 10,452 to 14,167; the values of (b) varied from -1.16 to -1.25. All of the monolithic devices

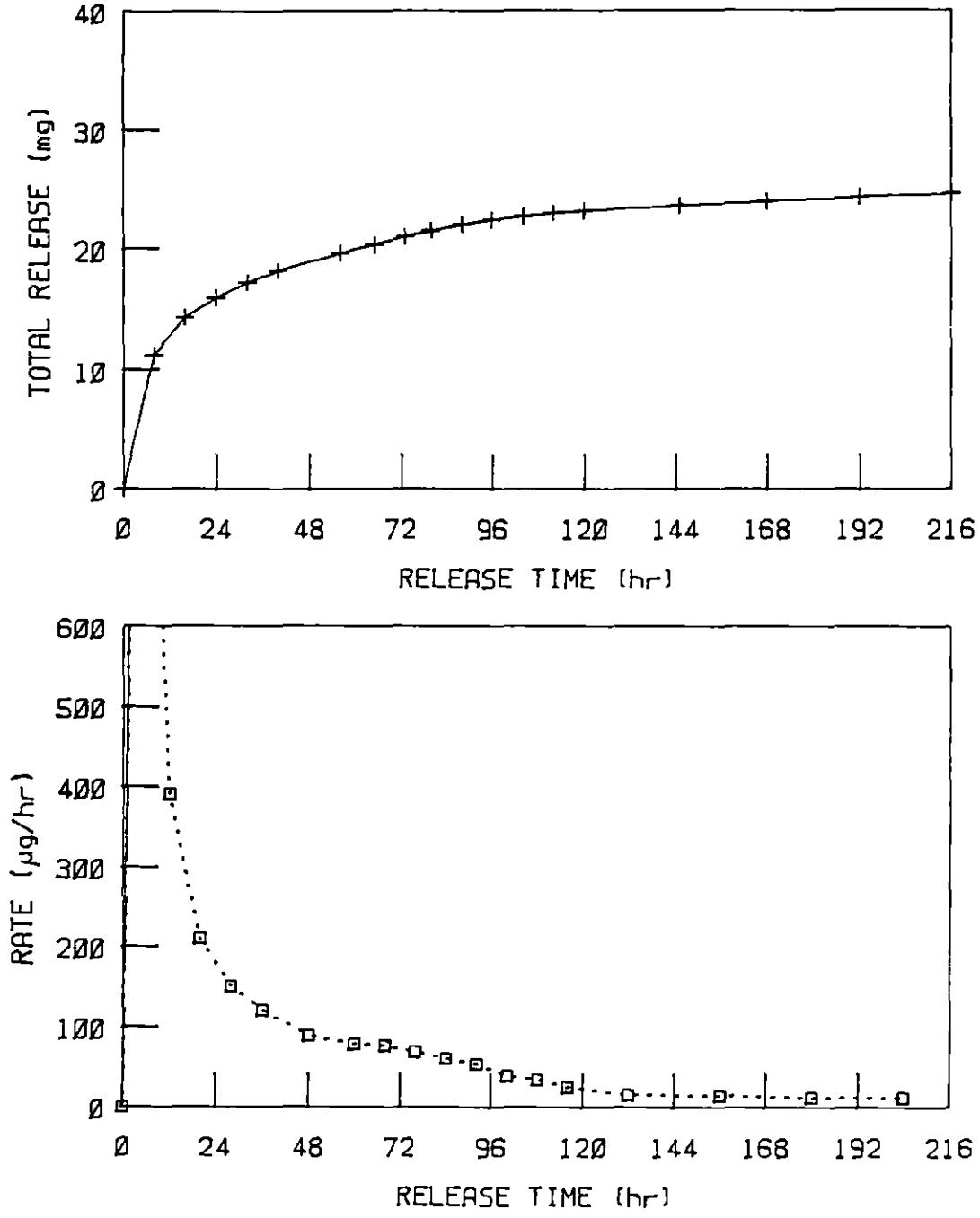

Figure 40. Release characteristics for device 1B. Top: total release vs. time; bottom: rate vs. time

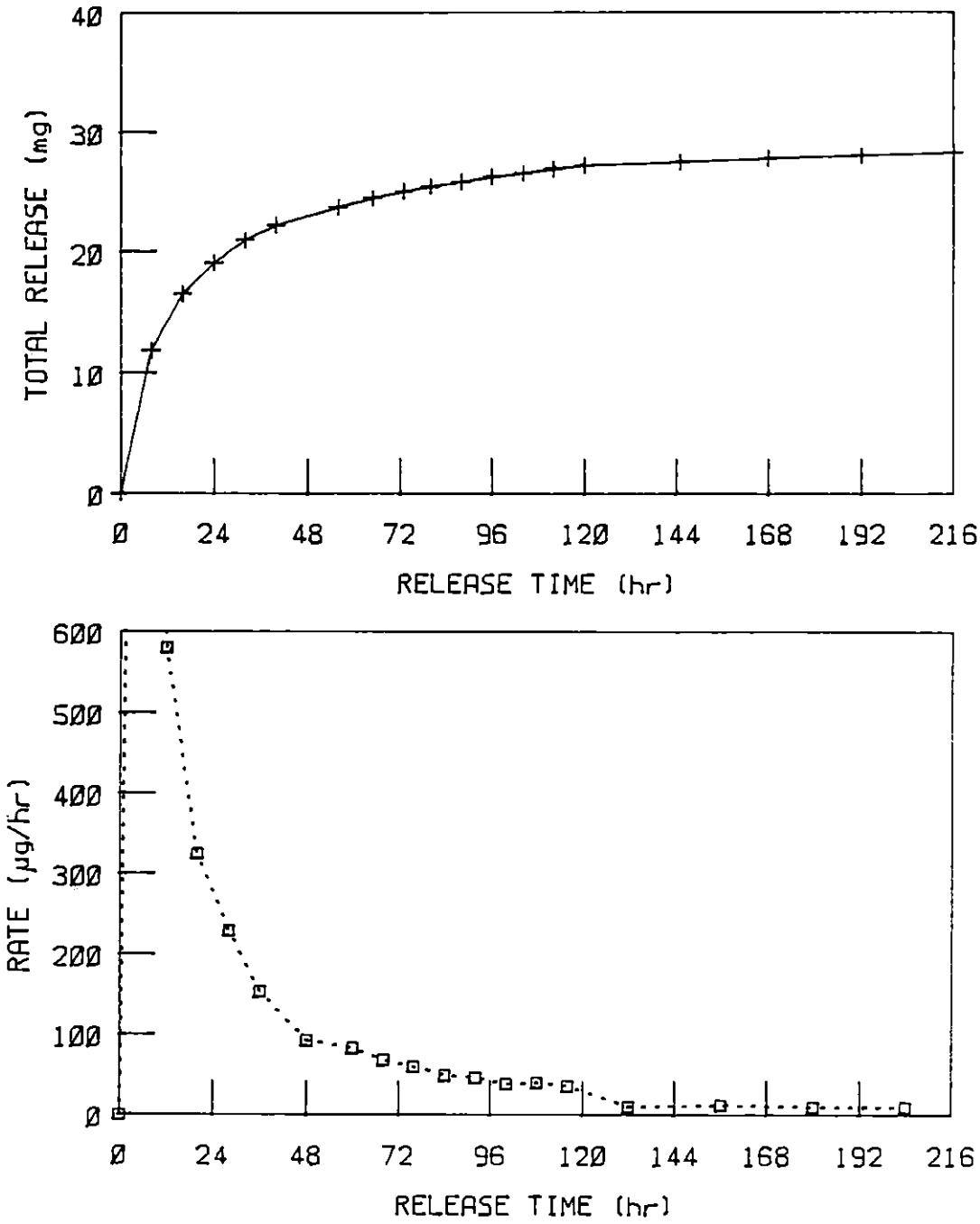

Figure 41. Release characteristics for device 2B. Top: total release vs. time; bottom: rate vs. time

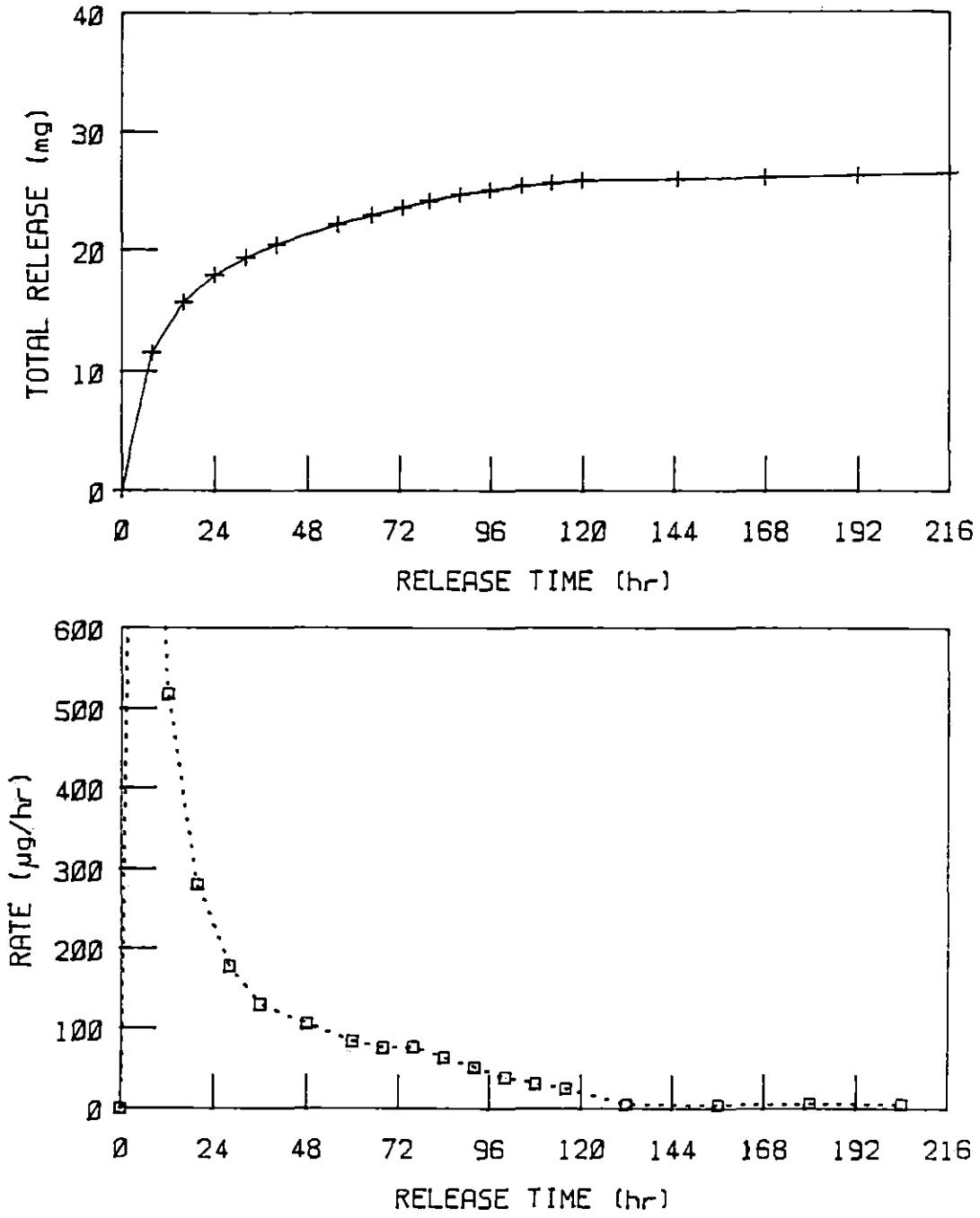

Figure 42. Release characteristics for device 3B. Top: total release vs. time; bottom: rate vs. time

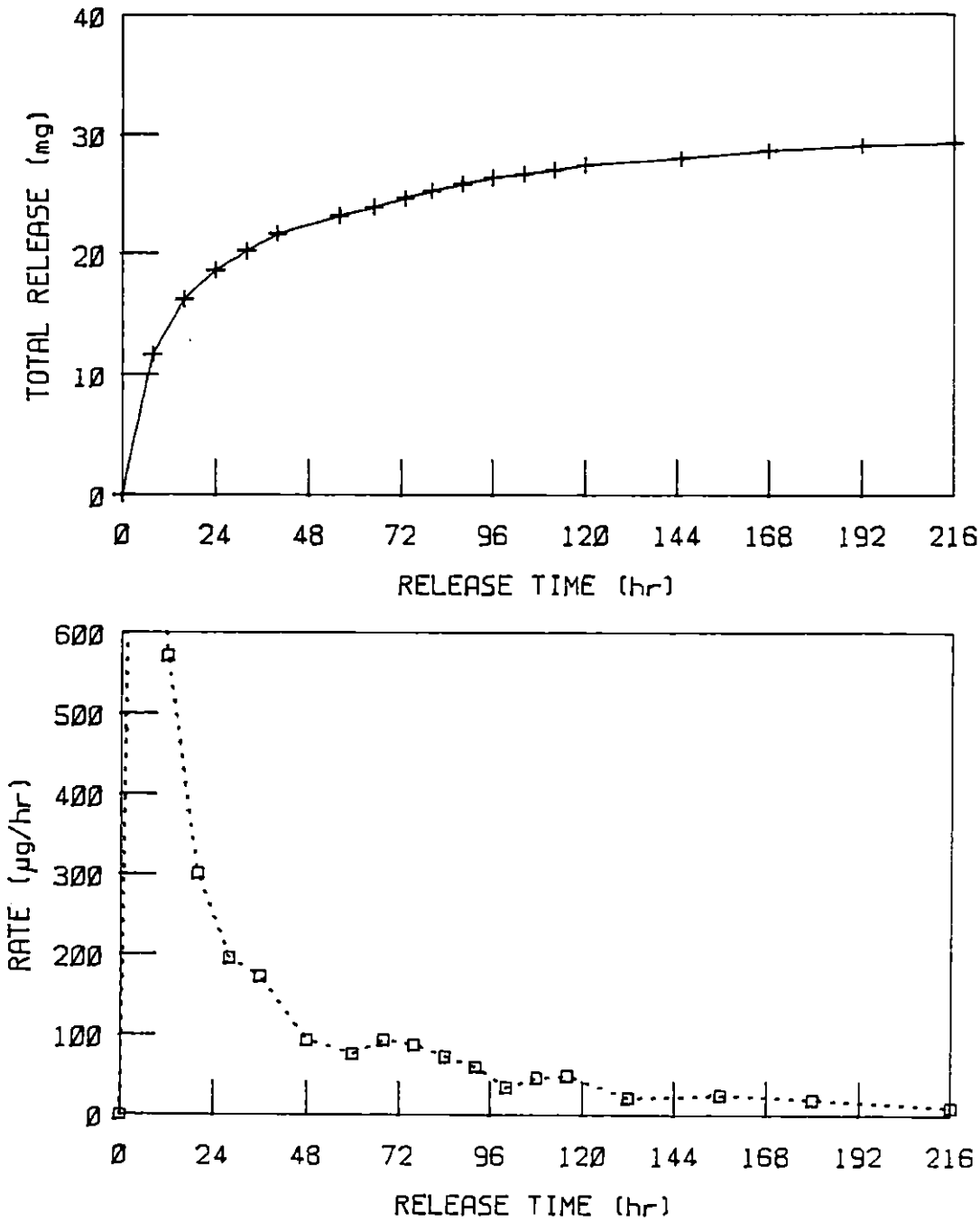

Figure 43. Release characteristics for device 4B. Top: total release vs. time; bottom: rate vs. time

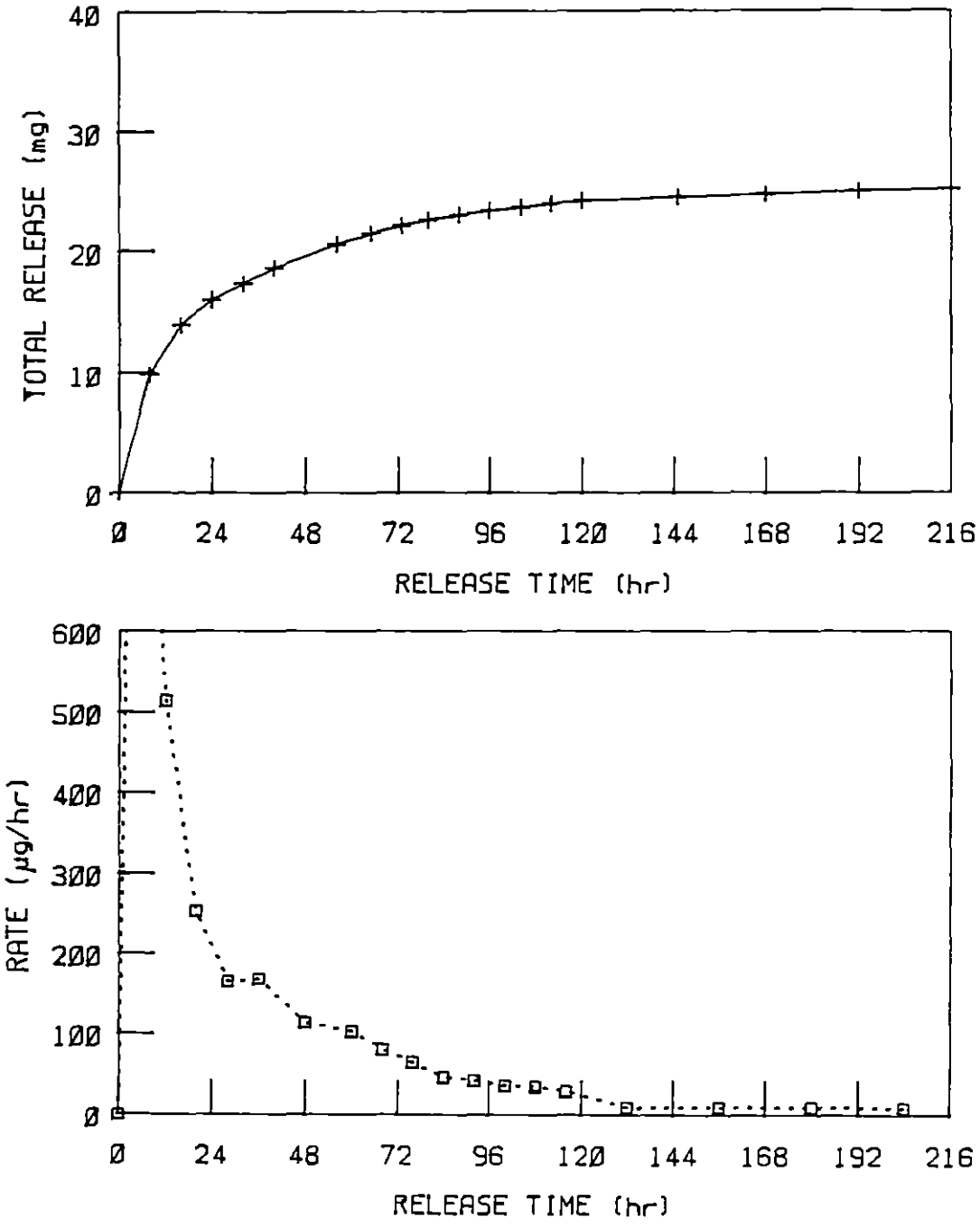

Figure 44. Release characteristics for device 5B. Top: total release vs. time; bottom: rate vs. time

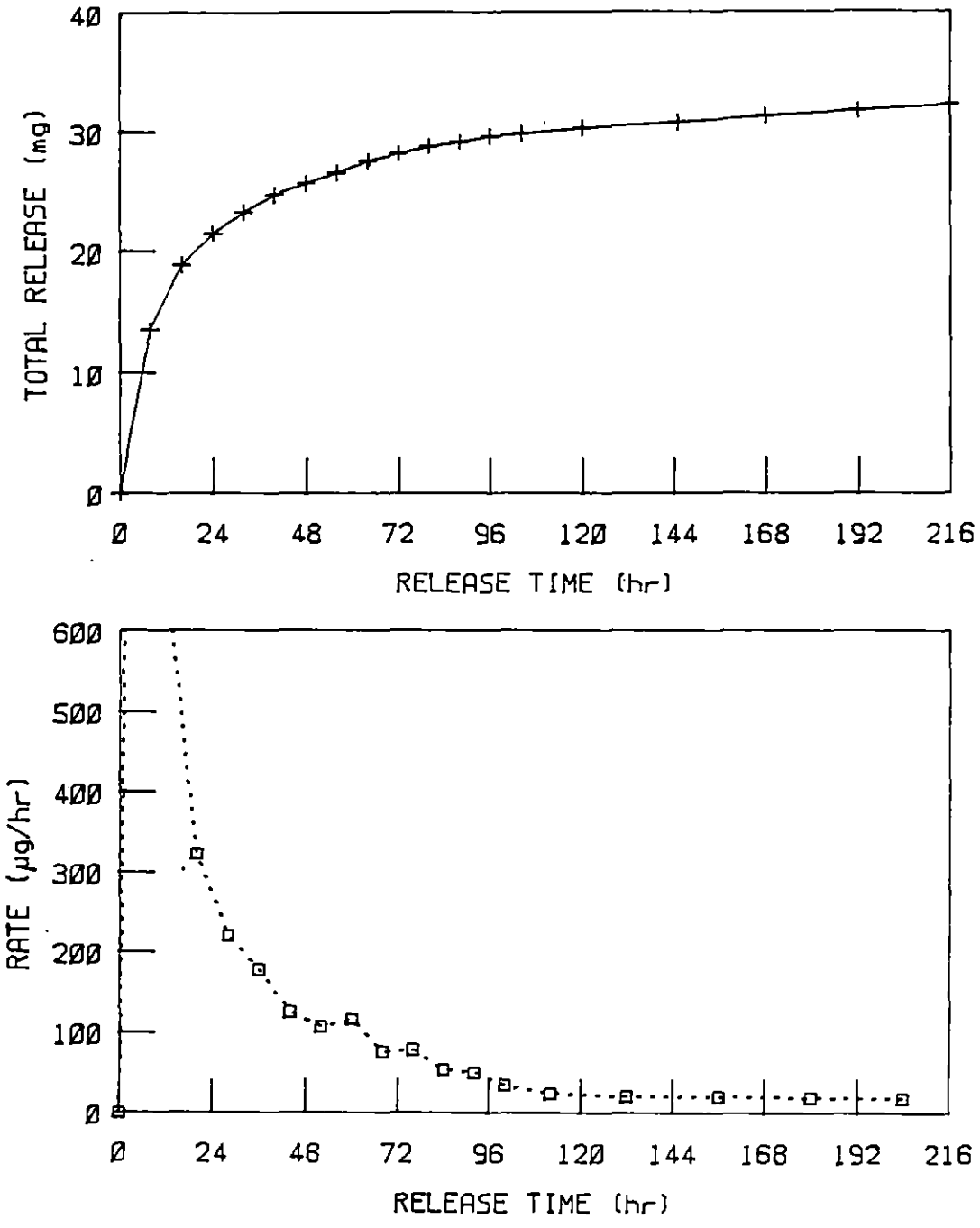

Figure 45. Release characteristics for device 6B. Top: total release vs. time; bottom: rate vs. time

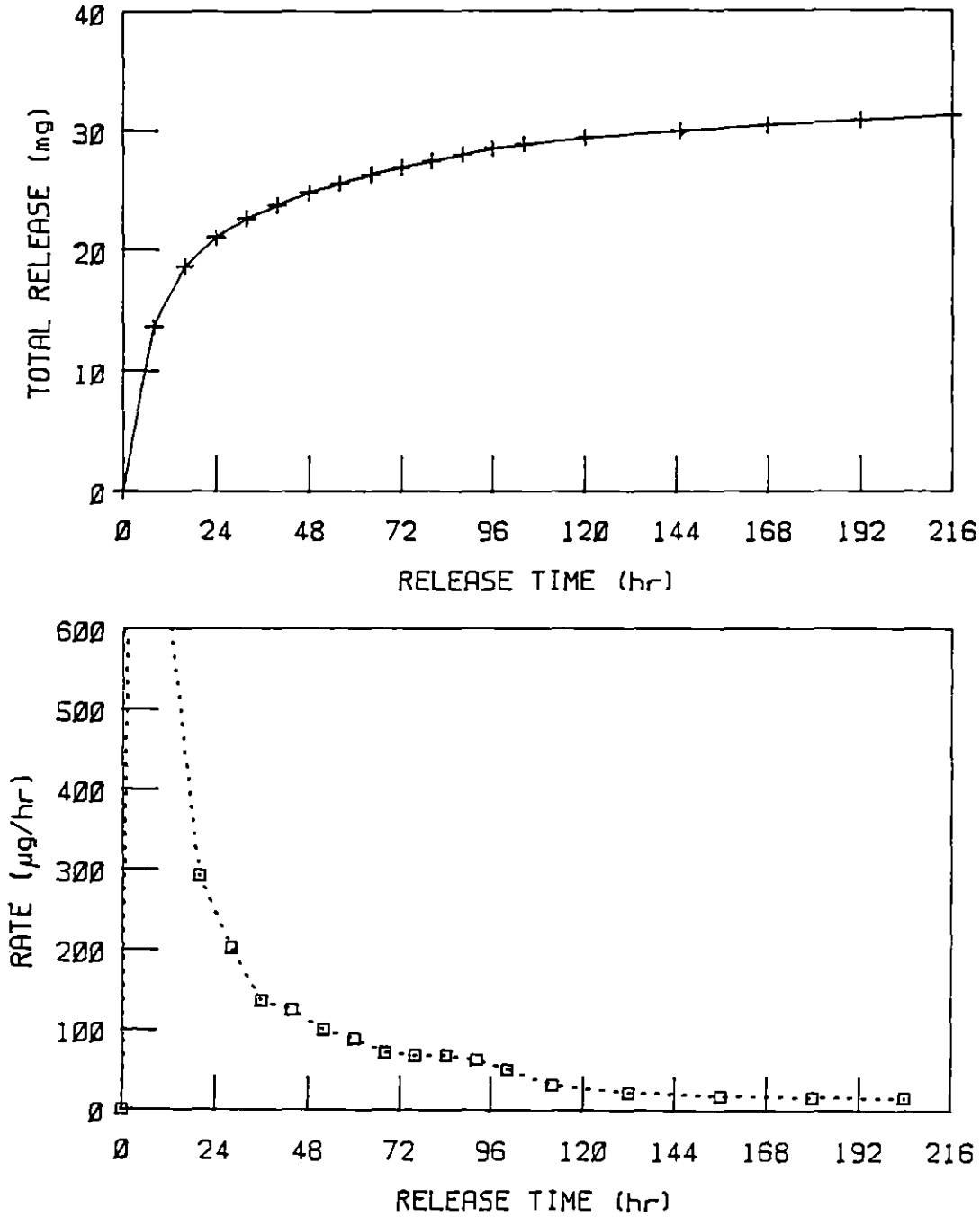

Figure 46. Release characteristics for device 7B. Top: total release vs. time; bottom: rate vs. time

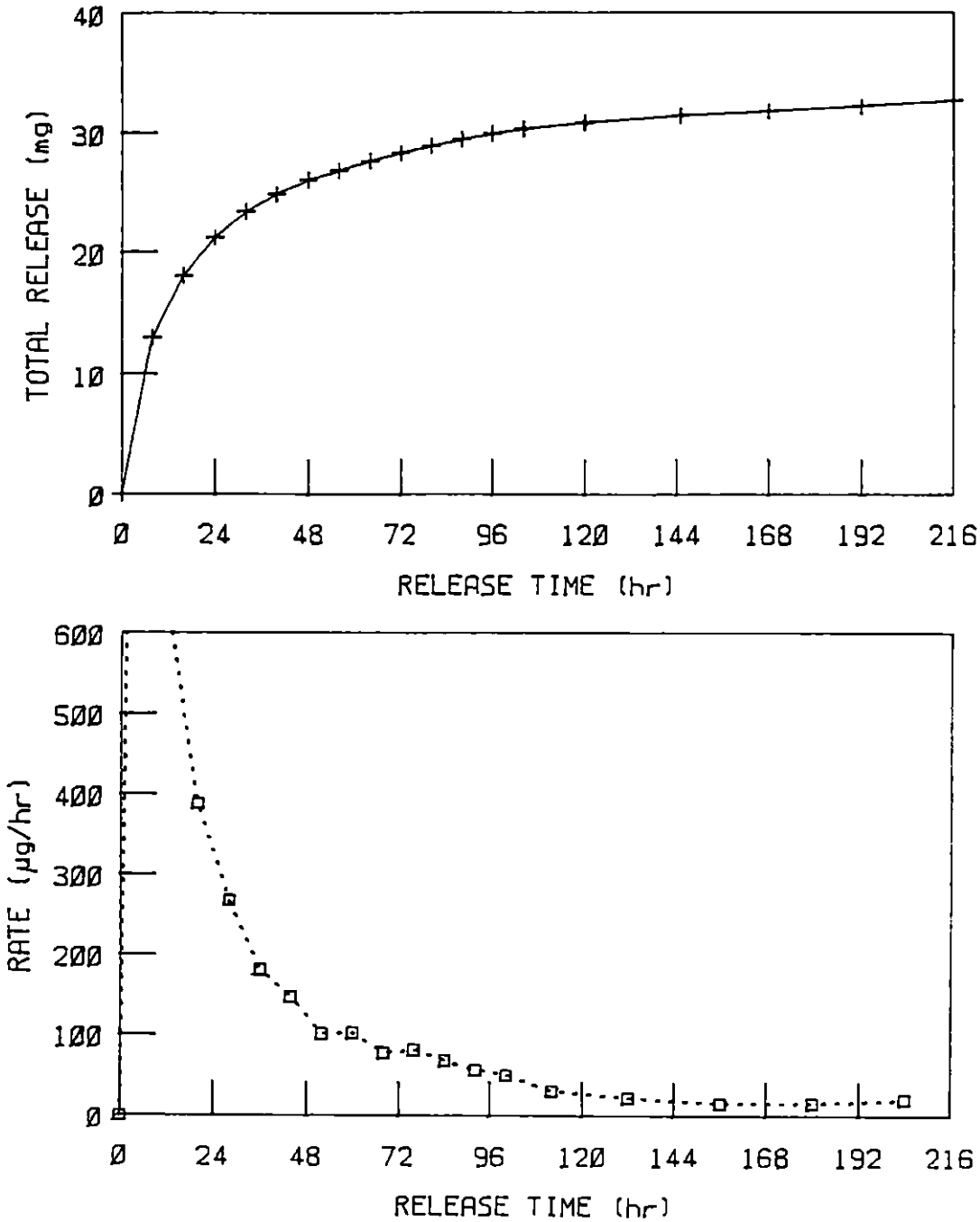

Figure 47. Release characteristics for device 8B. Top: total release vs. time; bottom: rate vs. time

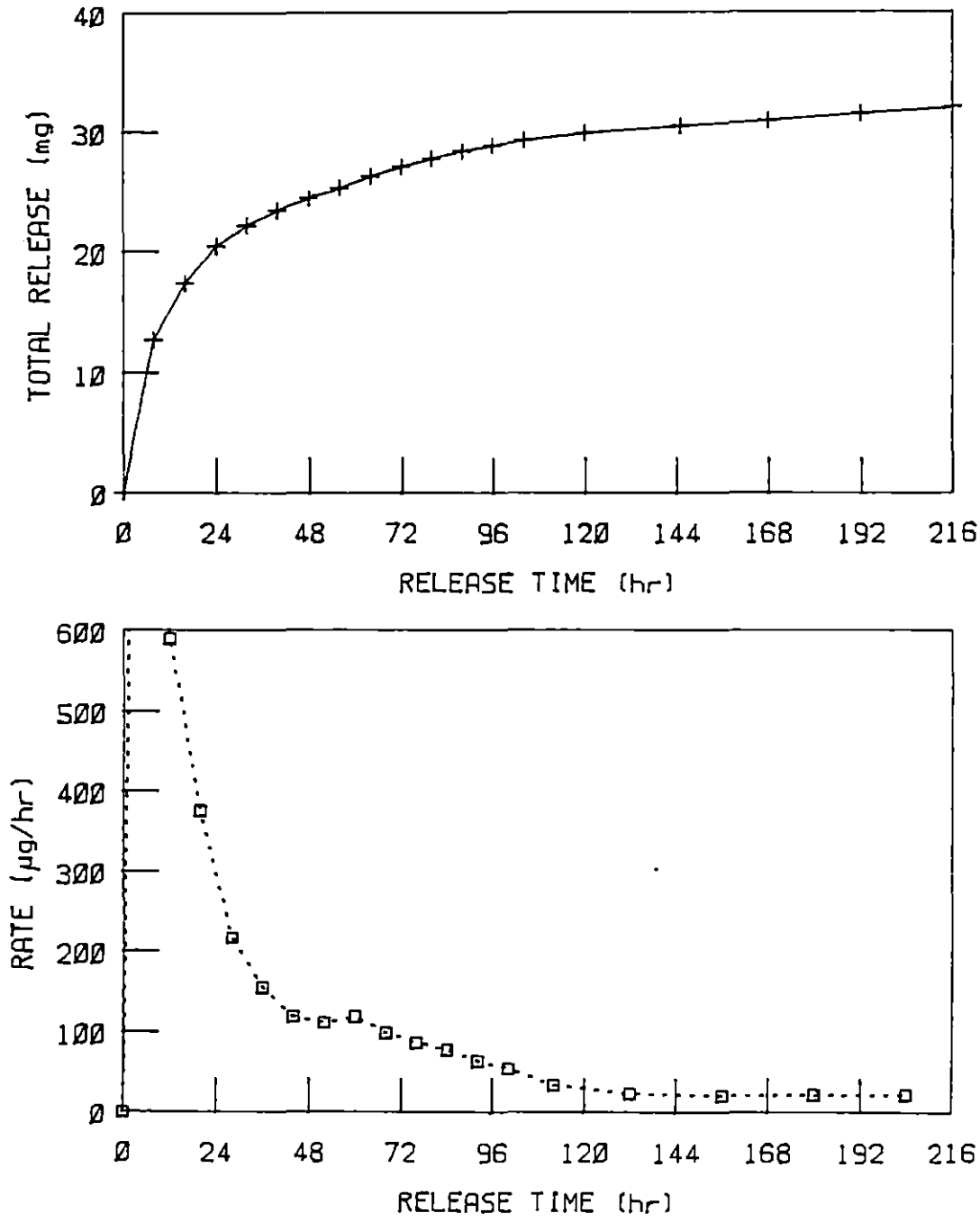

Figure 48. Release characteristics for device 9B. Top: total release vs. time; bottom: rate vs. time

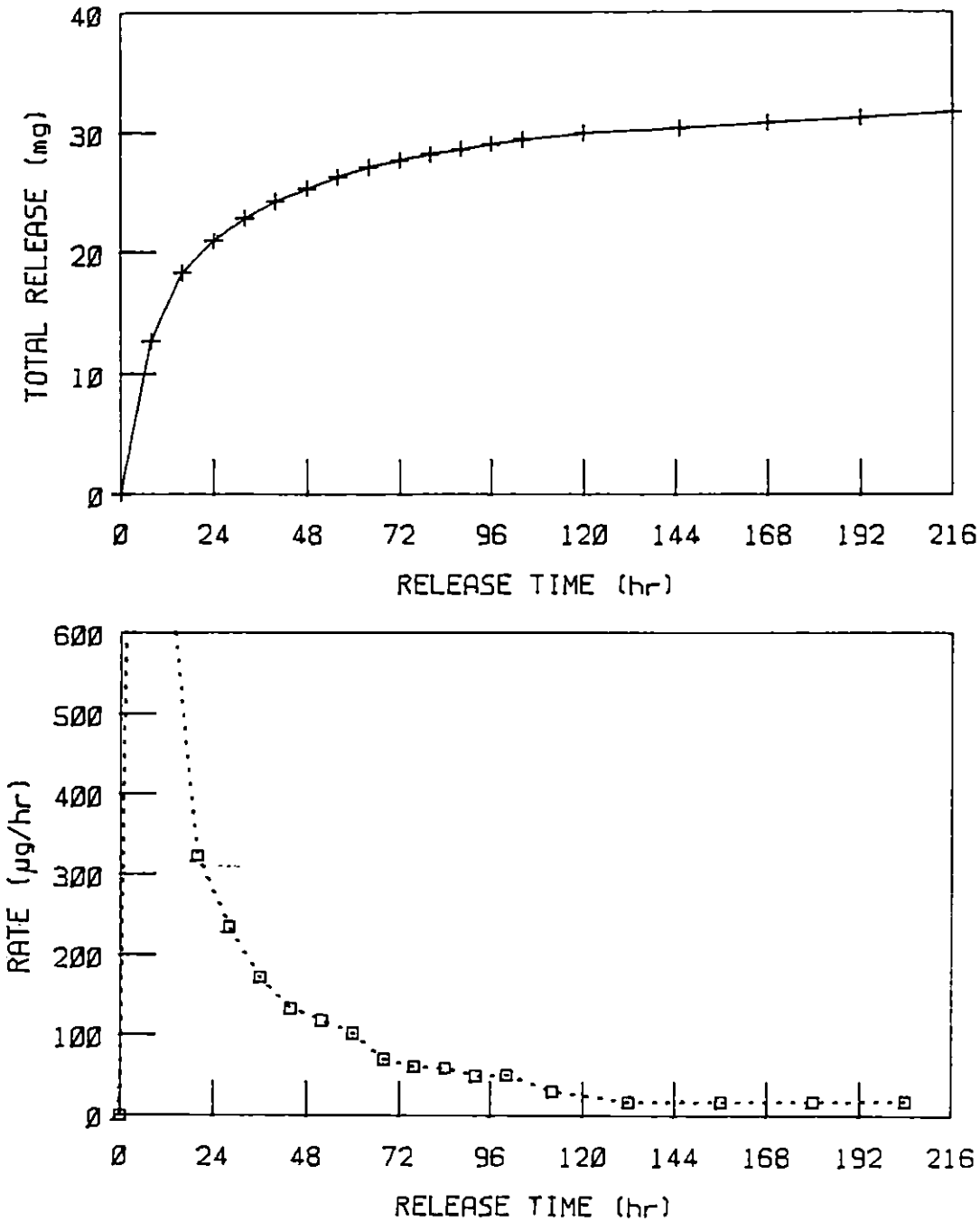

Figure 49. Release characteristics for device 10B. Top: total release vs. time; bottom: rate vs. time

released drug for the full nine-day experiment, and were releasing the tylosin tartrate at a rate of at least 6 ug/hr in the ninth day.

Table 9. Calculated values for release rate curves

Device number	release = a(time)^b	
	a	b
1B	8576	-1.19
2B	16078	-1.34
3B	21390	-1.45
4B	10291	-1.18
5B	13739	-1.32
6B	13380	-1.25
7B	11845	-1.22
8B	14167	-1.25
9B	10543	-1.16
0B	13690	-1.25

DISCUSSION

Production of the Controlled Release Systems

The release systems studied in this research were all fabricated from a solution of MMA/HEMA copolymer in dimethyl formamide. Olanoff et al. (1979) utilized acetone and dioxane solvents in their study, and Leytem (1984) used acetone and dimethyl formamide to dissolve the copolymer. The presence of acetone was found to increase the effect of humidity on the formation of the copolymer layer; when acetone was used, the copolymer frequently formed a brittle white layer, even when prepared over a desiccant. When only dimethyl formamide was used the copolymer consistently formed a firm, transparent layer when the procedure was carried out over a desiccant.

Reservoir devices

The first 19 reservoir devices, 1A-19A, were fabricated and tested in an attempt to determine the effectiveness of the methods for producing the copolymer tubes, and to determine if the desired release rate could be obtained from these reservoir systems. Four fabrication methods were tested based on the mandrel material and the coating method used: rotation coating on Silastic® mandrels, rotation coating on Teflon® mandrels, dip coating on Silastic® mandrels, and dip coating on Teflon® mandrels. With the exception of device 5A, which was observed to have holes in the wall of one of the copolymer tubes, these 19 devices

appeared to free of obvious physical defects prior to the release experiments. All four fabrication techniques worked for producing copolymer tubes, but as noted in the previous section, the rotation method produced copolymer tubes with less uniform wall thickness than the dip coating method. In addition, the dip coating method required less equipment than the rotation method, and less setup time. The Silastic® mandrels were coated more uniformly by the initial layer of copolymer solution, but the Teflon® tubes were easier to separate from the copolymer tubes. A more effective wetting agent for the surface of the Teflon® mandrels could be explored, as this would aid the uniformity of the initial coat of solution on the Teflon® mandrels. Devices 1A-19A were assembled using Silastic® medical grade adhesive, as this adhesive would not require any pretreatment before being introduced into the eye. No intermixing of the adhesive with the drug was noticed. The adhesive appeared to form a tight seal around the polyethylene tube, but the devices could be pulled apart with only a moderate force. Examination with the scanning electron microscope (SEM) revealed incomplete bonding of the adhesive to the polyethylene, and that gaps and holes resulted in some of the joints assembled in this manner. The Silastic® adhesive was also suspected of providing a permeable seal, and in order to eliminate this possibility devices 20A-29A were assembled using the copolymer solution as the adhesive, with PVC connecting segments. This approach was avoided, as it was desirable to wash the formed copolymer to remove any residual dimethyl formamide, and the finished devices could not be washed without affecting the subsequent release experiments. However,

this method does form a superior seal, and is recommended instead of the Silastic® adhesive. The dimethyl formamide in the copolymer solution effectively wets the surface of the PVC, and the SEM examination of these joints revealed a complete seal.

Monolithic devices

The monolithic devices were easy to produce, and required no assembly steps beyond the copolymer coating process. The tylosin tartrate dissolved completely in the copolymer solution, and no drug was seen to precipitate from the solution at any time during the experiment. The release from a monolithic device depends on the amount of drug initially present in the matrix (Schacht, 1984), so the solubility of the drug in the solution will determine, in part, the total amount of drug which can be incorporated into the matrix. As mentioned above, it is desirable to wash the copolymer layers to remove residual solvent; in the monolithic systems, this would also remove a portion of the drug. If enough extra drug could be incorporated into the matrix, the devices could be subjected to a pretreatment phase designed to remove the residual solvent while still leaving sufficient drug to obtain the necessary release.

Tylosin Tartrate Release Determination

Reservoir devices

As stated above, the first 19 reservoir devices, 1A-19A, were constructed in an attempt to evaluate the effectiveness of the copolymer coating techniques by comparing the release characteristics obtained from each set of devices. No consistent release characteristics were observed, so no conclusions could be drawn regarding the effectiveness of the fabrication methods. No correlations exist with respect to the wall thickness, mandrel material, or coating method. Devices 1A-6A, which were all prepared from copolymer segment produce on Teflon® mandrels using the rotation coating method, ranged in total release from 14,376 ug to 30,822 ug. Devices 12A-15A, which had nearly identical wall thicknesses, gave total drug release amounts ranging from 280 ug to 1142 ug. Devices 16A-19A, which had wall thicknesses similar to devices 1A-6A, gave total drug release amounts from 840-5429 ug, well below the amounts for devices 1A-6A.

Since such a wide range of release rates was found for devices 1A-19A, the possibility of drug release from structural imperfections was suspected. No obvious defects in the walls of the devices could be found under the stereomicroscopic examination, or during the subsequent SEM examination. The joints were seen to have holes and incomplete seals when assembled with Silastic® adhesive, and this could explain the uneven release characteristics. To eliminate the possibility of leakage through the PE/Silastic® adhesive seal, a different sealing method was employed

for devices 20A-29A. The dimethyl formamide in the copolymer solution readily wetted the PVC connecting tube, and a strong seal was obtained. In addition, the PVC segments in devices 25A-29A were encased in copolymer to eliminate the possibility of any release contribution through the PVC segment. Of these ten devices, only 20A and 23A gave any detectable drug release. Device 23A, which released a total of 1755 ug, was found to be incorrectly assembled, as it could be pulled apart as easily as the devices assembled with Silastic® adhesive. Device 23A was suspected of having insufficient bonding at the PVC/copolymer joint; a gap in the copolymer joining solution may have left a channel through which drug solution from inside the device could escape. However, the stereomicroscopic examination could not affirm this suspicion.

In general, the release rates found for the reservoir devices in these experiments are believed to be the result primarily of fabrication defects in the devices. The presence of such a wide range of detectable releases in 21 of the devices shows the need for better quality control in the production of the devices. The absence of release from devices 25A-29A indicates that the drug, tylosin tartrate, will not diffuse through the film of the copolymer used in these experiments at a rate sufficiently large to be of use. Although a thinner wall may increase the diffusion through the copolymer, the strength of the devices would be compromised. A different copolymer formulation, for instance one with a higher percentage of HEMA, may be more permeable to tylosin tartrate, and is suggested for future experimental release systems.

Monolithic systems

Baker and Lonsdale (1974) reported that the predicted release from a monolithic system should be proportional to $t^{-\frac{1}{2}}$. The release from the monolithic devices tested in these experiments did not conform to this prediction. The release rates from these devices were inversely proportional to time, but not to the one-half power. The reason for this release may be due to the physical state of the drug in the copolymer. The equations used to predict the drug release are for heterogeneous systems with dissolved drug within the matrix, or a combination of dissolved and dispersed drug. The systems studied in these experiments appeared heterogeneous due to their yellow color and the lack of any crystallized drug on the surface of the copolymer layer, but the actual distribution of the drug within the layer remains unknown. The presence of a greater concentration of drug near the surface of the copolymer layer would cause large initial release, and result in early depletion of the drug and, therefore, lower release rates later on. Similarly, the swelling of the copolymer could cause an unexpectedly high initial release. The presence of the tylosin tartrate, which is highly soluble, in the matrix may affect the equilibrium swelling of the matrix and the permeability of the swelled matrix. The permeability of the matrix may also change with time, as the drug within the matrix is depleted. All of these factors could contribute to varying degrees, resulting in the release characteristics which were observed.

Despite the lack of agreement with theory, the release rates from similarly prepared devices were consistent with each other, and all ten

of the devices provided detectable release after nine days. The release rate after nine days varied from 5-22 ug/hr. The release rate can be changed by adjusting the total amount of drug initially present in a matrix; the release rate from a monolithic device is proportional to the amount of drug initially present (Schacht, 1984). Tylosin tartrate comprised one third of the mass of the copolymer/drug matrix of the devices tested; the drug dissolved easily, and no precipitation of the drug was noticed at any time during the preparation of the devices.

CONCLUSIONS

The reservoir systems which were studied in this experiment did not provide consistent release, and the results indicated that the copolymer which was used was not sufficiently permeable to tylosin tartrate to allow adequate release from the ring-shaped devices so that they could be considered as a method for treating bovine ocular infections. Although a thinner control membrane may provide greater release, the physical strength of a thinner-walled copolymer tube would be insufficient for use in an *in vivo* situation. A more permeable polymer membrane would be required for these reservoir systems to be successful.

The ten monolithic devices all provided similar release characteristics, and gave a minimum release rate of 5 ug/hr after nine days. A release rate of tylosin tartrate of 2.5 ug/hr was calculated as being necessary for treating bovine ocular infections; the rate seen from the monolithic devices after nine days was above this value. Therefore, the monolithic devices which were studied in these experiments release drug at the rate necessary to treat bovine ocular infections, and might be considered as a possible treatment method for these infections.

RECOMMENDATIONS FOR FURTHER RESEARCH

Although the monolithic systems in this research were determined to give release rates of tylosin tartrate in the range needed for treating bovine ocular infections, many questions regarding the use of these devices still require study. The devices constructed by Hughes and Pugh (1975) were made from poly (vinyl chloride); the rings in this study were fabricated from a copolymer of methyl methacrylate and 2-hydroxyethyl methacrylate. The stiffness of the copolymer compared to PVC may prevent it from being used in the eye. In addition, the rigidity may cause it to be ejected from the eye more easily than the PVC rings constructed by Hughes and Pugh (1975). If these copolymer rings are selected as candidates for further studies, it is recommended that their ability to stay in the eyes of cattle without causing irritation be evaluated.

Tylosin tartrate was the only drug studied in this research, but it is not the only drug which could be considered for treating ocular infections in cattle. As pointed out earlier, the safe limits of tylosin tartrate within the eye have not been determined. Although instantaneous concentrations of tylosin tartrate as high as 10 mg/ml have been introduced into the eyes of cattle in the form of sprays (Ellis and Barnes, 1961), the effect of long-term concentrations this high have not been determined. The monolithic systems released tylosin tartrate at a rate greater than 1200 ug/hr for the first eight hours; with a lachrymal flow rate of 2 ml/hr, this gives a sustained concentration of 600 ug/ml

within the eye. It is recommended that a study be carried out, perhaps using a perfusion system, to determine the affects of these elevated concentrations over a long period of time.

The reservoir systems provide the only system of constant (zero-order) release available for the ring configuration. If further research is to be carried out on reservoir systems, it is recommended that a more permeable polymer formulation be found. The copolymer in this study provided insufficient release of tylosin tartrate, but a more permeable poloymer formulation may be found which could provide adequate release and the necessary physical strength for the ring systems.

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APPENDIX A

RELEASE DATA FOR THE RESERVOIR DEVICES

Table A.1. Results of the release experiment for device 1A

Time of Release (hr)	Volume[a] of Sample (ul)	Peak Weight (mg)	Std.[b] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	4000	NS[c]	1	0.00	0	0	0
8-16	4000	117.4	1	3.18	5088	636	5088
16-24	4000	131.3	1	3.44	5504	688	10592
24-32	4000	96.9	1	2.41	3856	482	14448
32-40	4000	25.8	1	0.31	496	62	14944
40-48	2000	33.2	2	0.61	488	61	15432
48-56	2000	35.4	3	1.11	888	111	16320
56-64	2000	30.2	3	0.96	768	96	17088
64-72	2000	31.4	3	1.00	800	100	17888
72-80	2000	33.9	3	1.09	872	109	18760
80-88	2000	23.2	4	0.77	616	77	19376
88-96	2000	36.6	4	1.26	1008	126	20384
96-104	2000	20.2	4	0.69	552	69	20936
104-112	2000	29.5	4	1.00	800	100	21736
112-120	2000	33.5	2	0.67	536	67	22272

[a]Amount of water added to dry sample to provide correct dilution so that a 2.5 ul spot size deposited an amount of drug within the 0.5-4.0 ug range onto the TLC plate.

[b]See Appendix C.

[c]No spot developed on the TLC plate.

Table A.2. Results of the release experiment for device 2A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std. Curve[a]	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	2000	14.8	5	0.73	584	73	584
32-40	2000	28.0	5	1.52	1216	152	1800
40-48	2000	41.6	5	2.52	2116	252	3816
48-56	2000	38.2	5	2.04	1632	204	5448
56-64	2000	57.1	6	1.73	1384	173	6832
64-72	2000	55.2	6	1.68	1344	168	8176
72-80	2000	43.0	6	1.37	1096	137	9272
80-88	2000	37.7	7	1.31	1048	131	10320
88-96	2000	35.7	7	1.25	1000	125	11320
96-104	2000	33.4	7	1.25	1000	125	12320
104-112	2000	34.9	8	1.35	1080	135	13400
112-120	2000	30.4	8	1.22	976	122	14376

[a]See Appendix C.

Table A.3. Results of the release experiment for device 3A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-4	0	NS	-	0.00	0	0	0
4-8	0	NS	-	0.00	0	0	0
8-12	0	NS	-	0.00	0	0	0
12-16	0	NS	-	0.00	0	0	0
16-20	0	NS	-	0.00	0	0	0
20-24	0	NS	-	0.00	0	0	0
24-28	0	NS	-	0.00	0	0	0
28-32	0	NS	-	0.00	0	0	0
32-36	1000	21.2	9	1.22	122	122	488
36-40	1000	98.9	9	6.90	690	690	3248
40-44	1000	60.5	9	3.80	380	380	4768
44-48	1000	53.8	9	3.25	325	325	6068
48-58	2000	81.5	10	2.73	218	218	8248
58-64	2000	108.3	10	4.30	573	573	11686
64-72	2000	57.4	10	1.80	180	180	13126
72-80	2000	54.8	10	1.72	172	172	14502
80-88	2000	33.4	11	1.04	104	104	15334
88-104	2000	53.5	11	1.52	76	76	16550
104-112	2000	22.7	11	0.70	70	70	17110
112-120	2000	22.5	12	0.78	78	78	17734
120-144	4000	18.7	12	0.68	45	45	18814
144-168	4000	20.5	12	0.73	49	49	19990

[a] See Appendix C.

Table A.4. Results of the release experiment for device 4A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	4000	109.6	13	3.06	4896	612	4896
8-16	4000	84.3	13	2.04	3264	408	8160
16-20	1000	136.5	13	4.15	1660	415	9820
20-24	1000	65.9	13	2.32	928	232	10748
24-28	1000	79.0	14	2.14	856	214	11604
28-32	1000	33.8	14	0.77	308	77	11912
32-36	1000	44.8	14	1.16	464	116	12376
36-40	1000	9.7	15	0.20	80	20	12456
40-44	1000	68.1	15	1.51	604	151	13060
44-48	1000	37.7	15	0.79	316	79	13376
48-58	1000	85.3	16	2.23	890	89	14266
58-64	1000	89.5	16	2.37	948	158	15214
64-72	1000	47.8	16	0.98	392	49	15606
72-80	2000	63.4	17	1.21	968	121	16574
80-88	2000	111.3	17	2.57	2056	257	18630
88-104	2000	77.6	17	1.61	1296	81	19926
104-112	8000	110.7	18	2.62	8384	1048	28310
112-120	2000	44.4	18	0.74	592	74	28902
120-144	6000	25.6	18	0.43	1032	43	29934
144-168	6000	21.8	18	0.37	888	37	30822

[a]See Appendix C.

Table A.5. Results of the release experiment for device 5A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	8000	93.6	19	3.08	9856	1232	9856
8-16	1000	50.5	19	1.46	584	73	10440
16-20	1000	54.2	19	1.58	632	158	11072
20-24	2000	57.4	19	1.72	1376	344	12448
24-28	1000	4.1	20	0.13	52	13	12500
28-32	250	NS	20	0.00	0	0	12500
32-36	250	NS	20	0.00	0	0	12500
36-40	1000	2.3	21	0.30	30	30	12620
40-44	1000	20.0	21	1.51	604	151	13224
44-48	1000	41.4	21	2.87	1148	287	14372
48-58	250	NS	22	0.00	0	0	14372
58-64	250	NS	22	0.00	0	0	14372
64-72	2000	18.7	22	1.26	1006	126	15380
72-80	2000	46.3	22	2.79	2232	279	17612
80-88	500	4.9	23	0.35	72	9	17684
88-104	500	NS	23	0.00	0	0	17684
104-112	500	NS	23	0.00	0	0	17684
112-120	500	NS	24	0.00	0	0	17684
120-144	2000	NS	24	0.00	0	0	17684
144-168	2000	48.3	24	1.52	2400	100	20084

[a] See Appendix C.

Table A.6. Results of the release experiment for device 6A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-20	100	NS	-	0.00	0	0	0
20-24	100	NS	-	0.00	0	0	0
24-28	4000	111.3	25	5.18	8288	2072	8288
28-32	4000	78.3	25	2.78	4448	1112	12736
32-36	2000	64.4	25	1.77	1416	354	14152
36-40	1000	47.8	26	1.39	556	139	14708
40-44	1000	49.7	26	1.43	572	143	15280
44-48	1000	45.2	26	1.34	536	134	15816
48-58	1000	55.4	27	1.65	660	66	16476
58-64	2000	35.1	27	1.02	816	136	17292
64-72	2000	18.1	27	0.56	448	56	17740
72-80	2000	20.6	27	0.64	512	64	18252
80-88	2000	20.3	28	0.66	528	66	18780
88-104	4000	73.5	29	2.50	4000	250	22780
104-112	2000	25.3	28	0.85	680	85	23460
112-120	2000	7.3	29	0.28	224	28	23684
120-144	6000	31.4	29	1.08	2592	108	26276
144-168	6000	24.9	29	0.88	2112	88	28388

[a] See Appendix C.

Table A.7. Results of the release experiment for device 7A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	600	59.6	30	1.80	1552	194	1552
8-16	1100	34.6	30	1.11	496	62	2048
16-24	600	104.2	30	3.38	2912	364	4960
24-32	1100	114.4	30	3.75	3232	404	8192
32-40	200	26.5	30	0.89	72	9	8264
40-48	500	11.8	31	0.54	112	14	8376
48-56	200	54.8	31	1.66	136	17	8512
56-64	500	67.6	32	1.87	376	47	8888
64-72	700	116.9	32	3.63	1024	128	9912
72-80	700	110.7	32	3.40	960	120	10872
80-88	1000	68.2	32	1.88	752	94	11624
88-96	1000	83.8	33	2.39	960	120	12784
96-104	2000	87.2	34	3.73	2984	373	15768
104-112	100	58.6	34	2.16	68	9	16312
112-120	100	18.5	34	0.74	30	4	16342
120-144	500	14.5	34	0.65	384	16	16726
144-168	500	24.3	34	0.88	528	22	17254

[a]See Appendix C.

Table A.8. Results of the release experiment for device 8A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	1100	47.9	35	1.81	816	102	816
16-24	1100	71.1	35	2.70	1216	152	2032
24-32	1100	46.4	35	2.55	1152	144	3184
32-40	1100	66.9	35	1.75	792	99	3976
40-48	1100	88.2	36	3.01	1352	169	5328
48-56	1100	78.4	36	2.65	1192	149	6528
56-64	1000	64.7	36	2.16	864	108	7384
64-72	1000	35.9	36	1.12	448	56	7832
72-80	1000	23.2	36	1.02	408	51	8240
80-88	500	35.1	37	1.02	208	26	8448
88-96	500	37.7	37	1.08	216	27	8664
96-104	500	44.6	37	1.25	248	31	8912
104-112	500	95.1	37	2.67	536	67	9448
112-120	500	130.7	37	3.96	792	99	10240
120-144	1000	103.6	38	3.00	1200	50	11440
144-168	1000	90.3	38	2.58	1032	43	12472

[a] See Appendix C.

Table A.9. Results of the release experiment for device 9A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	1100	9.2	39	0.44	200	25	200
16-24	1100	7.5	39	0.52	232	29	432
24-32	1100	12.0	39	0.60	272	34	704
32-40	1100	43.6	39	1.48	664	83	1368
40-48	1100	25.4	39	1.00	448	56	1816
48-56	600	27.5	40	0.91	408	51	2224
56-64	500	69.0	40	1.92	384	48	2608
64-72	500	90.7	40	2.58	520	65	3128
72-80	500	98.0	40	2.82	568	71	3696
80-88	1000	67.7	41	1.96	784	98	4480
88-96	1000	90.9	41	2.92	1168	146	5648
96-104	1000	57.3	41	1.70	464	85	6328
104-112	1000	50.0	41	1.52	608	76	6936
112-120	1000	40.8	41	1.29	520	65	7456
120-144	1000	75.5	38	2.11	840	35	8296
144-168	1000	75.9	38	2.12	840	35	9136

[a]See Appendix C.

Table A.10. Results of the release experiment for device 10A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	300	53.9	42	1.69	208	26	208
40-48	300	60.5	42	1.86	224	28	432
48-56	500	33.6	42	1.17	232	29	664
56-64	500	27.7	42	1.02	200	25	864
64-72	300	101.0	42	3.39	408	51	1272
72-80	500	34.7	46	1.16	232	29	1504
80-88	300	64.1	43	1.86	224	28	1728
88-96	300	81.8	43	2.80	336	42	2064
96-104	300	58.1	43	1.70	200	25	2264
104-112	300	22.3	43	0.74	88	11	2352
112-120	300	15.6	43	0.55	64	8	2416
120-144	1000	70.4	44	2.06	816	34	3232
144-168	1000	82.2	44	2.42	816	34	4048

[a]See Appendix C.

Table A.11. Results of the release experiment for device 11A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	1100	47.2	45	1.57	702	88	704
16-24	200	73.5	45	2.28	184	23	888
24-32	600	26.0	45	1.07	304	38	1194
32-40	1100	62.5	45	1.93	872	109	2066
40-48	1100	55.4	45	1.76	792	99	2858
48-56	1100	33.9	46	1.17	528	66	3386
56-64	600	26.7	46	0.95	272	34	3658
64-72	500	19.2	46	0.71	144	18	3802
72-80	100	8.9	46	0.44	9	1	3811
80-88	200	NS	47	0.00	0	0	3811
88-96	200	NS	47	0.00	0	0	3811
96-104	200	NS	47	0.00	0	0	3811
104-112	200	8.0	47	0.39	8	1	3819
112-120	200	NS	47	0.00	0	0	3819
120-144	1200	68.3	44	1.99	960	40	4779
144-168	1200	86.1	44	2.54	1224	51	5187

[a]See Appendix C.

Table A.12. Results of the release experiment for device 12A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total[b] Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	200	77.6	48	3.50	280	12	280

[a] See Appendix C.

[b] The release experiments for devices 12A, 13A, 16A, and 17A were conducted using a different sample collection technique than the other experiments. At each collection time, only half the volume of the release medium was collected. The calculations for the hourly and cumulative release from these devices were more complicated, and had to take into account the amount of drug in the sample jar at the beginning of each sampling time.

Table A.13. Results of the release experiment for device 13A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total[b] Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	200	14.7	49	0.73	117	15	117
72-80	200	NS	49	0.00	0	0	117
80-88	200	4.3	49	0.23	37	5	153
88-96	200	6.7	49	0.35	38	5	191
96-104	200	NS	49	0.00	0	0	191
104-112	200	3.8	49	0.20	32	4	223
112-120	200	NS	49	0.00	0	0	223
120-144	200	69.6	49	3.54	566	24	789
144-168	200	125.2	49	6.46	234	10	1023

[a]See Appendix C.

[b]See footnote (b), page 114.

Table A.14. Results of the release experiment for device 14A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	500	30.8	50	0.75	150	19	150
8-16	500	7.1	50	0.19	38	5	188
16-24	500	NS	50	0.00	0	0	188
24-32	500	NS	50	0.00	0	0	188
32-40	500	NS	50	0.00	0	0	188
40-48	500	5.9	50	0.16	32	4	220
48-56	500	10.0	50	0.27	54	7	274
56-64	500	14.3	50	0.38	76	10	350
64-72	500	21.8	51	0.30	60	8	410
72-80	500	22.5	51	0.31	62	8	472
80-88	500	10.5	51	0.15	30	4	502
88-96	500	8.7	51	0.12	19	2	521
96-104	500	7.6	51	0.11	30	4	551
104-112	500	4.1	52	0.07	14	2	565
112-120	500	8.0	52	0.14	28	4	593
120-144	500	6.8	52	0.12	24	1	617
144-168	500	11.0	52	0.20	41	2	658

[a] See Appendix C.

Table A.15. Results of the release experiment for device 15A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	500	6.1	53	0.24	48	6	48
88-96	500	25.3	53	0.78	125	16	204
96-104	500	10.5	53	0.42	112	14	288
104-112	500	13.6	53	0.52	104	13	392
112-120	500	5.0	53	0.20	40	5	432
120-144	500	90.6	53	2.20	146	18	871
144-168	500	52.0	53	1.36	90	11	1142

[a]See Appendix C.

Table A.16. Results of the release experiment for device 16A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total[b] Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	44.1	54	1.92	154	19	154
32-40	600	19.4	54	1.17	485	61	638
40-48	600	21.2	54	1.22	305	38	943
48-56	600	20.1	54	1.19	278	35	1222
56-64	600	24.1	54	1.31	343	43	1565
64-72	600	36.8	55	1.15	238	30	1802
72-80	200	73.4	55	2.02	48	6	1850
80-88	200	49.7	55	1.46	72	9	1922
88-96	600	9.4	55	0.31	32	4	1954
96-104	700	8.1	55	0.27	77	10	2031
104-112	200	16.8	56	0.53	8	1	2040
112-120	200	10.5	56	0.33	10	1	2050
120-144	700	23.1	56	0.77	405	17	2455
144-168	700	41.8	56	1.29	146	6	2600

[a] See Appendix C.

[b] See footnote (b), page 114.

Table A.17. Results of the release experiment for device 17A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total[b] Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	200	9.1	57	0.50	80	10	80
8-16	200	35.7	57	1.58	213	27	293
16-24	200	57.1	57	2.48	270	34	563
24-32	200	48.1	57	2.09	136	17	699
32-40	200	14.5	57	0.72	0	0	699
40-48	200	72.6	57	3.15	446	56	1146
48-56	200	66.2	58	3.82	359	45	1505
56-64	200	60.0	58	3.40	238	30	1743
64-72	200	33.5	58	1.58	0	0	1743
72-80	200	54.6	58	3.02	357	45	2100
80-88	200	72.0	58	4.21	432	54	2532
88-96	700	61.7	59	1.81	677	85	3208
96-104	600	58.4	59	1.71	314	39	3523
104-112	600	42.3	59	1.19	161	20	3684
112-120	600	17.6	59	0.50	0	0	3684
120-144	1100	19.2	59	0.54	355	15	4039
144-168	1100	32.7	59	0.90	158	7	4198

[a]See Appendix C.

[b]See footnote (b), page 114.

Table A.18. Results of the release experiment for device 18A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	200	39.1	60	1.56	125	16	125
8-16	1200	19.7	60	0.84	403	50	528
16-24	1200	21.1	60	0.90	432	54	960
24-32	1200	27.9	60	0.99	475	59	1435
32-40	400	23.3	60	1.14	182	23	1618
40-48	1200	28.8	60	1.19	571	71	2189
48-56	1200	32.3	61	1.19	571	71	2760
56-64	1200	19.7	61	0.79	397	47	3139
64-72	1200	31.3	61	1.15	552	69	3691
72-80	200	65.0	61	2.78	222	28	3914
80-88	500	7.0	61	0.39	78	10	3992
88-96	700	36.7	61	1.38	386	39	4378
96-104	700	18.5	62	0.49	137	23	4515
104-112	700	19.1	62	0.50	140	18	4655
112-120	700	14.6	62	0.38	106	13	4762
120-144	1200	25.4	62	0.64	307	13	5069
144-168	1200	30.5	62	0.75	360	15	5429

[a]See Appendix C.

Table A.19. Results of the release experiment for device 19A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	200	106.6	63	3.50	840	35	840

[a]See Appendix C.

Table A.20. Results of the release experiment for device 20A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	500	47.1	64	0.99	198	25	198
40-48	500	58.2	64	1.30	260	33	458
48-56	200	19.6	64	0.40	32	4	490
56-64	700	79.2	64	1.87	523	66	1014
64-72	500	75.2	64	1.76	352	44	1366
72-80	500	63.3	64	1.43	286	36	1652
80-88	200	48.5	65	1.02	82	10	1733
88-96	200	NS	65	0.00	0	0	1733
96-104	100	NS	-	0.00	0	0	1733
104-112	100	NS	-	0.00	0	0	1733
112-120	200	11.8	65	0.27	22	3	1755
120-144	100	NS	-	0.00	0	0	1755
144-168	100	NS	-	0.00	0	0	1755

[a]See Appendix C.

Table A.21. Results of the release experiment for device 21A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a] See Appendix C.

Table A.22. Results of the release experiment for device 22A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a]See Appendix C.

Table A.23. Results of the release experiment for device 23A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	500	111.7	65	3.15	630	79	630
16-24	400	91.6	65	2.33	373	47	1003
24-32	400	77.6	66	1.65	264	33	1267
32-40	200	21.4	66	0.41	33	4	1300
40-48	200	NS	66	0.00	0	0	1300
48-56	100	NS	66	0.00	0	0	1300
56-64	700	97.6	66	2.12	594	74	1894
64-72	200	40.3	66	0.82	66	8	1960
72-80	100	NS	-	0.00	0	0	1960
80-88	1200	69.8	66	1.48	710	89	2670
88-96	1200	100.7	67	2.14	1027	128	3697
96-104	200	88.8	67	1.91	153	19	3850
104-112	200	28.2	67	0.45	36	5	3886
112-120	200	52.8	67	1.16	93	12	3979
120-144	2200	90.5	67	1.94	5122	213	9101
144-168	2200	79.3	67	1.71	4514	188	13615

[a]See Appendix C.

Table A.24. Results of the release experiment for device 24A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a] See Appendix C.

Table A.25. Results of the release experiment for device 25A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a] See Appendix C.

Table A.26. Results of the release experiment for device 26A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a]See Appendix C.

Table A.27. Results of the release experiment for device 27A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a]See Appendix C.

Table A.28. Results of the release experiment for device 28A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a]See Appendix C.

Table A.29. Results of the release experiment for device 29A

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	100	NS	-	0.00	0	0	0
8-16	100	NS	-	0.00	0	0	0
16-24	100	NS	-	0.00	0	0	0
24-32	100	NS	-	0.00	0	0	0
32-40	100	NS	-	0.00	0	0	0
40-48	100	NS	-	0.00	0	0	0
48-56	100	NS	-	0.00	0	0	0
56-64	100	NS	-	0.00	0	0	0
64-72	100	NS	-	0.00	0	0	0
72-80	100	NS	-	0.00	0	0	0
80-88	100	NS	-	0.00	0	0	0
88-96	100	NS	-	0.00	0	0	0
96-104	100	NS	-	0.00	0	0	0
104-112	100	NS	-	0.00	0	0	0
112-120	100	NS	-	0.00	0	0	0
120-144	100	NS	-	0.00	0	0	0
144-168	100	NS	-	0.00	0	0	0

[a]See Appendix C.

APPENDIX B

RELEASE DATA FOR THE MONOLITHIC DEVICES

Table B.1. Results of the release experiment for device 1B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	68.9	68	2.35	11280	1410	11280
8-16	4000	57.4	68	1.96	3136	392	14416
16-24	2000	59.0	68	2.01	1608	210	16024
24-32	1700	51.1	68	1.78	1208	151	17232
32-40	1700	38.7	68	1.43	976	122	18208
40-56	2000	26.7	76	1.80	1440	90	19648
56-65	1200	21.4	76	1.50	720	80	20368
65-73	1200	17.8	76	1.30	624	78	20992
73-80	700	17.4	76	1.76	490	70	21482
80-88	700	25.9	76	1.28	496	62	21978
88-96	500	34.0	76	2.21	440	55	22418
96-104	500	28.1	73	1.54	312	39	22666
104-112	400	74.3	82	1.75	280	35	22946
112-120	400	60.0	82	1.32	208	26	23154
120-145	400	95.5	82	2.52	400	16	23554
145-168	400	85.0	82	2.10	345	15	23899
168-192	400	80.8	82	1.95	312	13	24211
192-216	400	81.2	82	1.96	312	13	24523

[a] See Appendix C.

Table B.2. Results of the release experiment for device 2B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	71.7	69	2.48	11904	1488	11904
8-16	4000	82.5	69	2.90	4640	580	16544
16-24	2000	91.6	69	3.25	2600	325	19144
24-32	1700	77.5	69	2.70	1840	230	20984
32-40	1700	54.2	69	1.80	1224	153	22208
40-56	2000	55.6	69	1.85	1448	93	23696
56-65	1200	28.8	73	1.57	756	84	24452
65-73	1200	20.0	73	1.15	552	69	25004
73-80	700	27.8	73	1.52	427	61	25431
80-88	700	26.0	73	1.44	400	50	25831
88-96	500	35.4	73	1.88	376	47	26207
96-104	500	29.1	75	1.57	312	39	26519
104-112	400	38.8	75	2.03	328	41	26847
112-120	400	34.9	75	1.85	296	37	27143
120-145	400	34.6	81	1.75	275	11	27418
145-168	400	36.5	81	1.87	299	13	27717
168-192	400	31.0	81	1.54	240	10	27957
192-216	400	29.7	81	1.46	240	10	28197

[a]See Appendix C.

Table B.3. Results of the release experiment for device 3B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	56.7	70	2.42	11616	1452	11616
8-16	4000	60.8	70	2.59	4144	518	15760
16-24	2000	65.6	70	2.80	2240	280	18000
24-32	1700	49.0	70	2.09	1424	178	19424
32-40	1700	35.4	70	1.53	1040	130	20464
40-56	2000	50.2	70	2.14	1712	107	22176
56-65	1200	25.8	75	1.42	765	85	22941
65-73	1200	22.9	75	1.29	616	77	23557
73-80	700	42.0	75	2.23	546	78	24103
80-88	700	68.0	77	1.87	520	65	24623
88-96	500	75.9	77	2.08	416	52	25039
96-104	500	55.7	77	1.54	312	39	25351
104-112	400	58.0	77	1.61	256	32	25607
112-120	400	46.6	77	1.31	208	26	25815
120-145	400	21.0	81	0.96	150	6	25959
145-168	400	16.6	81	0.79	115	5	26079
168-192	400	23.6	80	1.06	168	7	26247
192-216	400	21.7	80	0.97	144	6	26391

[a]See Appendix C.

Table B.4. Results of the release experiment for device 4B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std. Curve[a]	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	56.2	71	2.44	11712	1464	11712
8-16	4000	67.1	71	2.86	4576	572	16288
16-24	2000	71.0	71	3.01	2408	301	18696
24-32	1700	52.5	71	2.29	1560	195	20256
32-40	1700	46.1	71	2.04	1384	173	21640
40-56	2000	26.9	71	1.88	1504	94	23144
56-65	1200	52.1	77	1.45	693	77	23837
65-73	1200	57.5	74	1.56	752	94	24589
73-80	700	79.2	74	2.21	616	88	25205
80-88	700	76.1	74	2.11	592	74	25797
88-96	500	85.9	74	2.43	488	61	26285
96-104	500	52.5	74	1.41	280	35	26565
104-112	400	82.7	74	2.33	376	47	26941
112-120	400	75.5	78	2.49	400	50	27341
120-145	400	66.8	80	3.44	550	22	27891
145-168	400	71.0	80	3.70	598	26	28489
168-192	400	60.3	80	3.04	480	20	28469
192-216	400	31.4	80	1.43	240	10	29209

[a]See Appendix C.

Table B.5. Results of the release experiment for device 5B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	51.1	72	2.06	9888	1236	9888
8-16	4000	66.3	72	2.57	4112	514	14000
16-24	2000	65.3	72	2.54	2032	254	16032
24-32	1700	47.4	72	1.94	1320	165	17352
32-40	1700	49.7	72	1.99	1352	169	18704
40-56	2000	58.3	72	2.30	1840	115	20544
56-65	1200	59.1	78	1.95	936	104	21480
65-73	1200	39.0	78	1.35	648	81	22128
73-80	700	49.0	78	1.65	462	66	22590
80-88	700	38.8	78	1.34	376	47	22966
88-96	500	51.2	78	1.71	344	43	23310
96-104	500	28.5	79	1.49	296	37	23606
104-112	400	34.4	79	1.80	288	36	23894
112-120	400	28.2	79	1.48	240	30	24134
120-145	400	30.6	79	1.60	250	10	24384
145-168	400	27.1	79	1.42	230	10	24614
168-192	400	29.9	79	1.57	240	10	24854
192-216	400	24.4	79	1.28	216	9	25070

[a]See Appendix C.

Table B.6. Results of the release experiment for device 6B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	67.9	83	2.83	13584	1698	13584
8-16	4000	79.9	83	3.32	5312	664	18896
16-24	2000	77.7	83	3.23	2584	323	21480
24-32	2000	52.2	83	2.20	1760	220	23240
32-40	1700	49.6	83	2.09	1421	178	24661
40-48	1700	35.9	83	1.48	1006	126	25667
48-56	1200	43.2	83	1.81	869	108	26536
56-64	1200	50.2	88	1.95	936	117	27472
64-72	700	57.1	88	2.21	619	77	28091
72-80	700	59.0	88	2.27	636	80	28727
80-88	500	56.9	88	2.20	440	55	29167
88-96	500	52.6	88	2.05	410	51	29577
96-104	400	27.5	88	0.87	278	35	29855
104-120	400	66.6	88	2.54	406	25	30261
120-144	400	66.7	93	3.10	496	21	30757
144-168	400	67.2	93	3.12	499	21	31256
168-192	400	64.2	93	2.97	475	20	31731
192-216	400	61.8	93	2.85	456	19	32187

[a]See Appendix C.

Table B.7. Results of the release experiment for device 7B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	76.0	89	2.86	13728	1716	13728
8-16	4000	84.8	84	3.10	4960	620	18688
16-24	2000	80.4	84	2.92	2336	292	21024
24-32	2000	57.7	84	2.02	1616	202	22640
32-40	1700	45.6	84	1.61	1095	137	23735
40-48	1700	41.5	84	1.48	1006	126	24741
48-56	1200	48.0	84	1.69	811	101	25552
56-64	1200	40.8	89	1.50	720	90	26272
64-72	700	59.5	84	2.09	585	73	26857
72-80	700	54.1	89	1.98	554	69	27411
80-88	500	73.0	89	2.74	548	69	27959
88-96	500	67.9	89	2.53	506	63	28465
96-104	400	68.1	89	2.54	406	51	28871
104-120	400	85.3	89	3.23	517	32	29388
120-144	400	72.9	93	3.42	547	23	29935
144-168	400	61.8	93	2.85	456	19	30391
168-192	400	50.5	94	2.59	414	17	30805
192-216	400	47.4	94	2.40	384	16	31189

[a] See Appendix C.

Table B.8. Results of the release experiment for device 8B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	78.5	85	2.71	13008	1626	13008
8-16	4000	91.7	85	3.19	5104	638	18112
16-24	2000	110.3	85	3.88	3104	388	21216
24-32	2000	77.8	85	2.68	2144	268	23360
32-40	1700	63.0	85	2.14	1455	182	24815
40-48	1700	50.9	85	1.73	1176	147	25991
48-56	1200	50.0	85	1.70	816	102	26807
56-64	1200	37.7	90	1.71	821	103	27628
64-72	700	49.1	90	2.25	630	79	28258
72-80	700	50.9	90	2.23	652	82	28910
80-88	500	56.6	90	2.61	548	69	29458
88-96	500	49.9	90	2.29	458	57	29916
96-104	400	58.1	90	2.68	406	51	30322
104-120	400	62.0	90	2.87	517	32	30839
120-144	400	65.9	94	3.51	562	23	31401
144-168	400	47.2	94	2.39	382	16	31783
168-192	400	46.8	94	2.37	379	16	32168
192-216	400	60.9	94	3.21	514	21	32676

[a]See Appendix C.

Table B.9. Results of the release experiment for device 9B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std. Curve[a]	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	52.2	86	2.65	12720	1590	12720
8-16	4000	57.7	86	2.95	4720	590	17440
16-24	2000	72.6	86	3.75	3000	375	20440
24-32	2000	43.3	86	2.51	1736	217	22176
32-40	1700	36.6	86	1.82	1238	155	23414
40-48	1700	28.4	86	1.41	959	120	24373
48-56	1200	37.6	86	1.87	898	112	25271
56-64	1200	41.3	91	2.00	960	120	26231
64-72	700	58.2	91	2.82	790	99	27021
72-80	700	51.3	91	2.49	697	87	27718
80-88	500	65.4	91	3.17	634	79	28352
88-96	500	53.6	91	2.60	520	65	28872
96-104	400	56.8	91	2.75	440	55	29312
104-120	400	70.8	91	3.43	549	34	29861
120-144	400	66.6	94	3.56	570	24	30431
144-168	400	74.1	95	3.19	510	21	30941
168-192	400	77.6	95	3.35	536	22	31477
192-216	400	77.8	95	3.36	538	22	32015

[a]See Appendix C.

Table B.10. Results of the release experiment for device 10B

Time of Release (hr)	Volume of Sample (ul)	Peak Weight (mg)	Std.[a] Curve	Amount of Tylosin in spot (ug)	Total Drug in Sample (ug)	Average Release Rate (ug/hr)	Cumulative Release (ug)
0-8	12000	55.8	87	2.65	12720	1590	12720
8-16	4000	75.5	87	3.55	5680	710	18400
16-24	2000	68.4	87	3.23	2584	323	20984
24-32	2000	49.1	87	2.35	1880	235	22864
32-40	1700	41.9	87	2.02	1374	172	24238
40-48	1700	34.4	87	1.58	1074	134	25312
48-56	1200	41.2	87	1.98	950	119	26262
56-64	1200	32.8	92	1.71	821	103	27083
64-72	700	39.3	92	2.02	566	71	27649
72-80	700	34.2	92	1.78	498	62	28147
80-88	500	44.9	92	2.39	478	60	28625
88-96	500	38.7	92	1.99	398	50	29023
96-104	400	47.1	92	2.53	405	51	29428
104-120	400	56.1	92	3.12	499	31	29927
120-144	400	62.9	95	2.69	530	18	30357
144-168	400	62.1	95	2.65	424	18	30781
168-192	400	63.0	95	2.69	430	18	31211
192-216	400	66.9	95	2.87	459	19	31670

[a]See Appendix C.

APPENDIX C

DENSITOMETER DATA: STANDARD CURVES

Densitometer Settings: B-A Mode
Scan Rate 2 cm/min
Normal Output
Plotter Settings: 10 mv full scale

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
1	64	10	0.5	26.0
			1.0	45.4
			2.0	90.4
			4.0	141.6
2	64	10	0.5	24.6
			1.0	46.9
			2.0	78.7
			4.0	122.1
3	64	10	0.5	17.2
			1.0	32.3
			2.0	56.4
			4.0	114.5
4	64	10	0.5	14.7
			1.0	32.3
			2.0	59.7
			4.0	122.0
5	128	10	1.0	19.3
			2.0	35.9
			3.0	46.8
			4.0	57.5
6	64	10	1.0	28.6
			1.5	48.2
			2.0	67.1
			2.5	81.9
7	64	10	0.5	14.0
			1.5	43.1
			2.0	59.6

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
8	64	10	0.5	14.7
			1.0	22.8
			1.5	40.0
			2.0	52.8
9	128	12	0.5	5.8
			1.0	16.4
			2.0	38.1
			4.0	63.0
10	64	12	1.0	30.0
			2.0	64.8
			3.0	87.7
			4.0	103.6
11	64	12	0.5	16.8
			1.0	31.8
			2.0	73.6
12	64	12	0.5	12.0
			1.0	30.5
13	64	12	0.5	24.3
			1.0	44.1
			2.0	83.3
			4.0	132.9
14	64	12	0.5	21.7
			1.0	44.1
			2.0	60.7
			4.0	75.0
15	64	12	0.5	24.2
			1.0	47.8
			2.0	87.7
16	64	12	0.5	21.7
			1.0	48.8
			1.5	63.6
			2.5	93.4
17	64	12	0.5	24.5
			1.0	47.1
			2.0	96.4
			4.0	148.2

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
18	64	12	0.5	29.9
			1.0	60.0
			2.0	93.6
			4.0	121.3
19	64	12	0.5	16.5
			1.0	36.4
			2.0	66.9
			4.0	116.2
20	64	12	0.5	15.6
			1.0	29.3
			2.0	69.0
21	128	12	0.5	3.8
			1.0	10.8
			2.0	28.9
			4.0	57.8
22	128	12	0.5	6.9
			1.0	13.6
			2.0	33.0
			4.0	59.3
23	128	12	0.5	7.0
			1.0	13.5
			2.0	35.4
			4.0	58.8
24	64	12	1.0	31.9
			2.0	63.5
			4.0	115.0
25	64	12	1.0	28.2
			2.0	67.6
			4.0	95.1
26	64	12	1.0	28.6
			1.5	53.0
27	64	12	0.5	15.9
			1.0	34.5
			2.0	66.7

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
28	64	12	0.5	16.3
			1.0	29.2
29	64	12	0.5	13.0
			1.0	28.8
			2.0	61.6
			4.0	108.7
30	64	12	0.5	12.0
			1.0	30.8
			2.0	66.8
			4.0	121.2
31	64	12	0.5	10.3
			1.0	28.8
			2.0	67.9
32	64	12	0.5	15.2
			1.0	24.0
			2.0	74.1
			4.0	126.5
33	64	12	0.5	17.0
			1.0	33.3
			2.0	74.5
			4.0	121.7
34	64	12	0.5	8.1
			1.0	29.4
			2.0	55.6
			4.0	92.2
35	64	12	0.5	10.0
			1.0	24.3
			2.0	52.5
			4.0	105.2
36	64	12	0.5	7.8
			1.0	22.3
			2.0	60.3
			4.0	115.6

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
37	64	12	0.5	16.0
			1.0	34.2
			2.0	76.6
			4.0	131.7
38	64	12	0.5	14.6
			1.0	37.2
			2.0	72.1
			4.0	135.0
39	64	12	0.5	8.5
			1.0	25.3
			2.0	63.8
40	64	12	0.5	12.5
			1.0	30.9
			2.0	72.4
			4.0	134.7
41	64	12	0.5	13.0
			1.0	28.9
			2.0	69.3
			4.0	116.5
42	64	12	0.5	9.3
			1.0	26.8
			2.0	66.1
			4.0	116.3
43	64	12	0.5	13.9
			1.0	31.6
			2.0	69.2
			4.0	100.8
44	64	12	0.5	11.7
			1.0	30.4
			2.0	68.6
			4.0	133.5
45	64	12	0.5	10.1
			1.0	22.9
			2.0	65.6
			4.0	121.1

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
46	64	12	0.5	12.3
			1.0	28.4
			2.0	68.5
47	64	12	0.5	10.2
			1.0	32.9
48	64	12	1.0	23.2
			2.0	41.1
			4.0	85.5
49	64	12	0.5	9.5
			1.0	21.0
			2.0	78.3
50	64	12	0.5	18.7
			1.0	43.0
51	64	12	0.5	35.9
			1.0	61.7
52	64	12	0.5	27.8
53	64	12	0.5	12.6
			1.0	35.5
54	128	12	1.0	13.8
			2.0	46.6
55	64	12	0.5	15.0
			1.0	30.4
			2.0	72.5
			4.0	125.7
56	64	12	0.5	15.9
			1.0	29.3
			2.0	72.3
57	64	12	0.5	9.0
			1.0	21.7
			2.0	46.0
			4.0	92.6

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
58	64	12	0.5	5.6
			1.0	18.0
			2.0	39.7
			4.0	68.9
59	64	12	0.5	17.5
			1.0	36.5
			2.0	67.5
60	64	12	0.5	11.5
			1.0	23.6
			2.0	51.2
			4.0	91.2
61	64	12	0.5	8.9
			1.0	27.6
			2.0	51.8
			4.0	85.8
62	64	12	0.5	19.0
			1.0	42.1
			2.0	74.4
			4.0	122.9
63	64	12	2.0	67.3
			4.0	119.8
64	64	12	0.5	24.6
			1.0	47.7
			2.0	84.0
65	64	12	0.5	21.7
			1.0	47.8
			2.0	83.4
			4.0	132.7
66	64	12	0.5	25.9
			1.0	48.2
			2.0	93.6
			4.0	158.8
67	64	12	0.5	31.2
			1.0	45.2
			2.0	93.3
			4.0	166.6

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
68	64	12	1.0	23.7
			2.0	58.7
			4.0	117.7
69	64	12	1.0	20.1
			2.0	46.9
			4.0	93.8
70	64	12	1.0	22.5
			2.0	46.9
			4.0	93.8
71	64	12	1.0	28.0
			2.0	45.0
			4.0	96.2
72	64	12	1.0	23.1
			2.0	49.3
			4.0	108.9
73	128	12	1.0	16.7
			2.0	38.0
			4.0	72.9
74	64	12	1.0	38.4
			2.0	72.8
			4.0	133.1
75	128	12	1.0	16.8
			2.0	38.2
			4.0	71.8
76	128	12	1.0	12.5
			2.0	30.2
			4.0	66.9
77	64	12	1.0	35.0
			2.0	73.0
			4.0	138.8
78	64	12	1.0	27.3
			2.0	60.8
			4.0	120.8

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
79	128	12	1.0	18.9
			2.0	38.3
			4.0	65.8
80	128	12	1.0	22.4
			2.0	43.5
			4.0	75.9
81	128	12	1.0	22.1
			2.0	38.7
			4.0	70.9
82	64	12	1.0	49.6
			2.0	82.5
			4.0	132.2
83	64	10	1.0	25.1
			2.0	47.4
			4.0	96.6
84	64	10	1.0	27.2
			2.0	57.2
			4.0	107.5
85	64	10	1.0	28.1
			2.0	59.2
			4.0	113.6
86	64	10	1.0	20.1
			2.0	40.2
			4.0	77.2
87	64	10	1.0	24.6
			2.0	41.5
			4.0	85.3
88	64	10	1.0	30.2
			2.0	51.3
			4.0	107.5
89	64	10	1.0	27.2
			2.0	54.6
			4.0	104.6

Standard Curve Number	Atten. Setting	Paper Speed (cm/min)	Amount In Standard (ug)	Peak Weight (mg)
90	64	10	1.0	22.2
			2.0	44.0
			4.0	85.3
91	64	10	1.0	17.7
			2.0	41.2
			4.0	82.7
92	64	10	1.0	17.5
			2.0	39.0
			4.0	69.6
93	64	10	1.0	25.4
			2.0	45.3
			4.0	84.3
94	64	10	1.0	21.2
			2.0	40.7
			4.0	74.0
95	64	10	1.0	24.3
			2.0	47.7
			4.0	91.9