In vitro studies of the release of tylosin tartrate from hydrogel ocular inserts

by

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INTRODUCTION

IBK (Infectious Bovine Keratoconjunctivitis) is a bovine eye disease which is rarely fatal, but it may result in a major economic loss. The principal treatment method consists of topical applications of antibiotics and sulfonamide eye drops, powders, or ointments for a period of five to seven days. Because lacrimal fluid rapidly washes the drug from the eye, it is desirable to develop a more efficient method for the administration of the drug.

To address this problem, a controlled drug release system has been developed and characterized for its suitability for this application. Hydrogels have been selected as the polymer for the ocular controlled release system, and tylosin tartrate, an agricultural antibiotic, has been chosen as the release system drug which is capable of treating IBK. The drug release rates for ring devices have been evaluated in a continuous flow apparatus. Also, batch-to-batch reproducibility of release characteristics for the copolymer formulations chosen has been checked. Two different hydrogel copolymer formulations have been studied: 90:10 mole percent and 25:75 mole percent of methyl methacrylate (MMA) and hydroxyethyl methacrylate (HEMA).

LITERATURE REVIEW

Nature of Problem

Infectious Bovine Keratoconjunctivitis (IBK) is a disease occurring perennially in all areas where cattle are raised. This disease is commonly called "pinkeye". It is caused by a bacteria, Moraxella bovis, which is found in discharges from the eyes and noses of infected animals.

Signs of the disease may be divided into four stages: the first stage is exemplified by moist eyes or slight tearing; the second stage is the ulcerative stage associated with drooping of the eyelids; the third stage (or the period of vascularization) occurs when the corneal repair process predominates; the fourth stage is the time when the scar tissue is resolved. Occasionally, weakening of the cornea leads to rupture and subsequent loss of the eye. The disease may occur any time during the year, but it usually manifests itself most often during summer when disease-enhancing factors such as ultraviolet radiation and fly population are increased (Hughes, 1981).

The average outbreak lasts about three weeks, with the largest economic damage associated with weight loss in beef herds and with a reduction in milk production (may be cut in half for infected dairy herds). These economic losses result from various causes. For example, cattle with severely diseased eyes tend to stay away from other cattle in order to protect their infected eyes and to reduce the pain caused by direct sunlight. This inactivity can result in a reduction of feeding and a decrease of growth rate and milk production.

Present Treatment Methods

IBK is a contagious disease, so management of diseased cattle is effective and practical. Affected animals are placed in dark quarters, to avoid fly irritation and direct sunlight, and are separated from the herd. It is recommended that cattle should be treated with antibiotics or other chemo-therapeutic agents before they are returned to a herd. Fly control measures, sanitation, sprays, dusts, and systemic insecticides are also recommended.

Once an infected animal is found in a herd, all animals should be treated. Various kinds of eye medications may be useful in preventing the development of secondary infections. Suggested treatment for IBK ranges from topical application of eye drops, ointments, or sprays to continuous injection of a variety of antibiotic solutions and compounds containing chloramphenicol, oxytetracycline, penicillin-streptomycin (Jensen and Mackey, 1979; Blood et al., 1979), or tylosin (Burger, 1970; Rossoff, 1974; Hughes, 1981). The use of eye drops and sprays represents direct treatment techniques for infected eyes, but it is difficult to maintain therapeutic levels of drug in the tears and in the conjuntival sacs, and therefore repeated applications are necessary.

> Antiseptic eye drops such as boric acid, mercurials, argyrol, silver nitrate, and copper sulfate were used during the first half of the century.

Zinc sulfate in a 1:40 solution and ethidium bromide were found useful (Hughes, 1981). The eye drop type dosage form is easy to use, but suffers from inherent drawbacks. A great part of the medication is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac, and is rapidly drained away from the

ocular cavity by constant tear flow. In order to maintain a continuous, sustained level of drug medication, frequent instillation of eye drops or an application of higher concentration of drug is necessary, but the loss of drug through lacrimal-nasal drainage system may result in some considerable side effects (Chien, 1982a). Eighty percent of an eye drop is lost immediately after instillation (information for humans; Gelatt et al., 1979). When sprays with a drug such as tylosin tartrate were applied twice daily, the clinical signs of IBK were eliminated within 5 days (Ellis and Barnes, 1961). Even though the sprays have similar retention times as seen for eye drops, a spray results in less irritation than an eye drop. A treatment must be applied in the beginning of infection to achieve best results.

For achieving the therapeutic efficiency of an ophthalmic drug, viscosity-enhancing agents such as methyl cellulose are added into the eye drop preparation, or the ophthalmic drug is provided as an ointment to sustain intimate drug/eye contact. Unfortunately, these methods also do not yield a constant drug bioavailability, and they give only marginally more sustained drug/eye contact than the eye drop solution. Repeated medications are still required through a period of a few days.

Recently, delivery systems (for humans) have been developed to prolong the retention time of a drug in the eye through the use of soft contact lenses or ocular inserts. Soft contact lenses are presoaked in eyedrop solutions. The effects of drug last 24 hours after application, and the drug concentration declines rapidly after that (Gelatt et al., 1979). Ocular inserts, which were fabricated from insoluble or soluble polymers, were placed in the upper or lower conjunctival sac. This

appeared to overcome the difficulty of maintaining therapeutic drug concentration at the site of the infection. A controlled release device must be able to be inserted easily in the eye, and must be able to remain there during the course of the therapy. Also, the device must not cause tissue reaction.

In cattle, several forms of prolonged release devices have been tested. Hawley (1954) reported that an eye pellet can provide therapeutic levels of drug, such as terramycin in tears for up to 31 hours. Certain polymers and copolymers such as polylactic acid, ethylene/vinyl acetate, polyglycolic acid, polylactic/polyglycolic acid composite and others have been used as controlled drug delivery system. Theodorakis et al. (1983) prepared an ocular insert which was made of composite of chloramphenicol sodium succinate (CASS) with polylactic acid (PLA). This was attached onto the third eyelid of cattle and rabbits. The inserts were retained in the eye at least 4 days, and achieved a therapeutic level of drug in ocular fluids of healthy cattle. On this basis, these inserts may be useful in treating IBK. The ocular inserts released CASS at a rate that diminished with time. The release kinetics did not follow the square root of time law or a first-order equation.

Graham and Hibbs (1981) have developed a composite material for the prophylactic treatment of pinkeye. They applied attenuated Moraxella bovis directly to the eyes of cattle to provide cellular immunity to prevent bacterial infection. Attenuation of Moraxella bovis was accomplished by a heat treatment of pure cultures of Moraxella bovis which were isolated from active cases of pinkeye. The attenuated cultures may be formulated as a time release medication in the form of an

ocular insert. The ocular insert contained attenuated Moraxella bovis and an antibiotic.

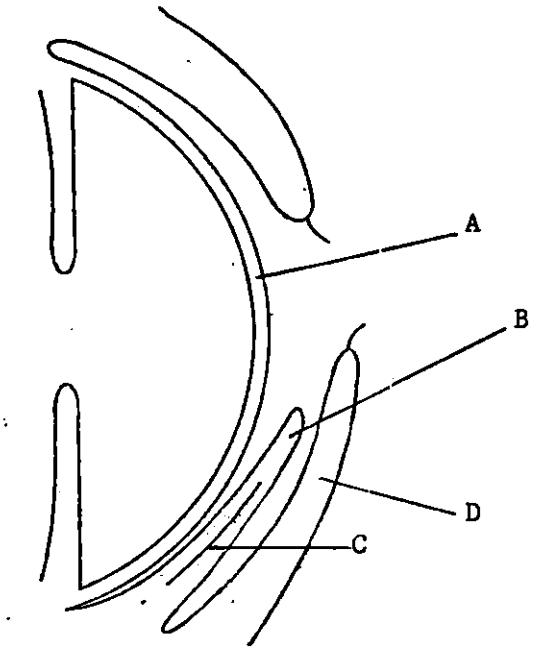
Structure and Lacrimal System of a Bovine Eye

Structure of bovine eye

The eye is the sense organ for vision. Within its protective casing, each eye has a large number of receptors, a lens system for focusing light on these receptors, and a system of nerves for conducting impulses from the receptors to the brain.

The ocular layer of each eye is tough, fibrous and (except at the front) opaque to light. This is the sclera, or "white" of eye. At the front, and bulging forward, the transparent cornea replaces the sclera, and outside the cornea is the thin protective membrane called the conjunctiva. The inner surface of the upper and lower eyelids, both sides of the 3rd eyelid, and the anterior surface of the eye itself are covered with this layer. The conjunctiva is constantly cleaned and moistened by a salty, bactericidal fluid called tears. The tears are secreted by several lacrimal glands (Prince et al., 1960; Severin et al., 1980). The lacrimal glands are located caudodorsally to the globe.

Also, located in the medial canthus or corner of a bovine eye is a transverse sheet of thin translucent membrane, the "third eyelid" (nictitating membrane). The third eyelid of cow is leaf or shovel-shaped and thicker than in the horse. The schematic drawing of a bovine eye is shown in Figure 1.



A: Cornea

B: Nictitating membrane

C: Cartilage of nictitating membrane

D: Lower eyelid

FIGURE 1. Schematic drawing of a bovine eye (from Theodorakis et al., 1983)

Lacrimal apparatus

The eye is protected by two mechanisms; blinking and the secretion of tears. Irritating particles and fumes are washed away from the sensitive cornea by the secretion of tears, and the surface of the globe is maintained in a normally moist condition. Blinking, besides its protective function, prevents dazzle by a blinding light and keeps the exposed surface of the globe moist by spreading the lacrimal secretions and by preventing evaporation during sleep. Also, the act of blinking assists in the drainage of tears.

The lacrimal apparatus consists of four structures (Figure 2); (1) lacrimal glands, (2) lacrimal canals, (3) lacrmal sac, and (4) nasolacrimal duct. Fluid secreted by the lacrimal glands washes over the eyeball and is swept up by the blinking. Tears drain through the

lacrimal canals from the punta to the lacrimal sac, which drains into the nasal cavity via the nasolacrimal duct (Pasquini, 1982; Severin et al., 1980). The eyeball is continuously irrigated by a gentle stream of fluid which prevents it from becoming dry and inflamed. Normally, the amount of lacrimal fluid renewed by frequent involuntary blinking movements is just sufficient to keep pace with its disappearance from the conjunctiva. However, an excessive formation and secretion of lacrimal fluid, or lacrimation, can occur when foreign bodies or other irritants get into the eye, when a bright light is shined into the eye, or when the cow is in emotional stress.

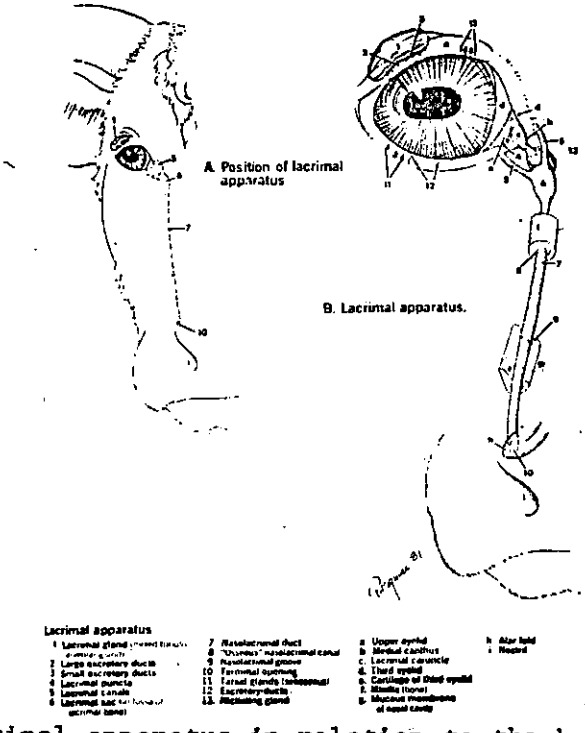


FIGURE 2. Lacrimal apparatus in relation to the bovine eye (from Pasquini, 1982)

One of the most essential requirements for the anatomical and functional integrity of the cornea is a moist environment. The moisture within the conjunctival sac, the serous component of tear fluid, is

largely produced by the lacrimal gland. The nictitans glands also contribute a serous component of tear fluid. Through a number of ducts, tears reach the conjunctival sac at the upper fornix, mixed with the mucous products of the Harder's gland. The tear fluid maintains a well organized fluid film over the entire anterior surface of the eyeball. A good deal of this fluid is lost through evaporation while the eye is open. In the normal tear film, the evaporation rate is low because of the protective oily surface. No more than 20 to 25 percent of the total tears secreted (human) are lost by evaporation (Milder, 1970).

Characterisitics of tear solution

Tears form a clear, salty, slightly alkaline, watery fluid. The tear fluid contains lysozyme, whose bactericidal activity reduces the bacterial count in the conjunctival sac. Water constitutes 98-99% of the tear fluid, and thus the specific gravity is only slightly above unity. The viscosity is 1.053 to 1.405 centipoise. The osmolarity and the pH are of clinical interest in the preparation of drops for instillation into the conjunctival sac in order to minimize discomfort and irritation. Modern studies have suggested that tear fluid is approximately isotonic with plasma. As discussed by Duke-Elder (1968), the osmotic pressure of human tears is equivalent to that of solutions of sodium chloride ranging in concentration from 0.903 to 1.014 gr NaCl/100g H_2O; the range was considerable, but close to that of "physiological saline". The pH of tears is usually in the range of 7.4 - 7.5 (approximately as alkaline as plasma).

Chemical constituents of tears can be divided into (1) nondiffusible substances of large molecular size and (2)

diffusible substances, which in turn are comprised of ionized and nonionized compounds.

The chief nondiffusible constituent of tears is protein (concentration in the range from 0.2 to 0.6 wt. %). In electrophoretic studies, there are three main bands: serum albumin, serum globulin and lysozyme. Of these three, lysozyme has bactericidal properties.

In general, its lytic properties depend on the reaction, the salt concentration and temperature.

In some pathological conditions, such as conjunctival infections (kerato-conjunctivitis sicca and sustained epiphora), the lysozyme content of the tears falls.

Other nondiffusible substances are mucopolysaccharides and lipids. The ionic component of tears includes cations such as Na^+ and K^+, and anions such as Cl^-, phosphate and thiocyanate. The concentration of Na^+ is approximately equal to that of serum, or is above it. The concentrations of potasssium and chloride are greater in tears than in serum. Nonionized substances include glucose, urea, and amino acids.

Measurement of tear flow rate

As discussed in Duke-Elder (1968), there is a constant output rate of tears which continues throughout all waking hours. There are various tests for measuring the flow rate of tears: for example, (a) Schirmer's test, (b) newer techniques based on introducing a dye and (c) photometry.

Hoffmann and Spradbrow (1978) reported bovine tear flow rates obtained from two cattle using the catheterization method. Values between 0.18 to 1.86 ml/hr were measured. Another method is to cannulate the nasolachrymal duct and to use a suction pump. Slatter and Edwards (1982) reported that the mean tear flow rate obtained from 327 collection

periods of 30 minutes for 11 cattle was 1.96±1.84 ml/hr. They saw no evidence of a difference of tear flow rates between left and right eyes. Time of day of collection was not seen to have an effect on the tear flow rate.

Controlled Release Systems

The term controlled release (delivery) systems can be distinguished from sustained systems. The release mechanisms for sustained systems are sensitive to environmental conditions, while those of the controlled release systems are determined by the device (or system) characteristics. A controlled release system includes not only the notion of prolonged release characteristics, but more accurate, reproducible, and predictable release kinetics.

Controlled release systems may be classified according to physical mechanisms of release of the incorporated solute. Controlled release systems may be divided into porous and nonporous types according to the molecular structure of the polymer. Membrane-moderated controlled release systems can consist of a reservoir (membrane) system and a matrix (monolithic) system. In reservoir systems, the bioactive agent is usually put into a device which is enclosed by a polymeric membrane, and the device is placed in contact with a dissolution medium (water or other biological fluid). The rate of drug release is controlled by its permeability between the membranes. Transport through these membranes may be described by Fick's first law of diffusion,

$$J = -D \frac{dC_m}{dX} \qquad (1)$$

where J is the flux, C_m is the membrane concentration of permeant, X is the position of release concentration, and D is the permeant diffusion coefficient through the polymer. However, since solute concentrations in the membrane are difficult to measure experimentally, it is customary to determine solute concentration in the membrane by using the partition (distribution) coefficient, K, which is determined easily with a measure of solute solubility in the swollen polymer. The solute concentrations on either side of the membrane surface are in equilibrium with the solute concentration in the adjacent solution, C, as shown in Figure 3 (Baker and Lonsdale, 1974).

$$C_m(0) = KC(0) \text{ at the upstream surface } (X=0) \tag{2}$$

$$C_m(1) = KC(1) \text{ at the downstream surface } (X=1) \tag{3}$$

Here, K is the distribution coefficient, which is the ratio of the solubility of the solute in the polymeric membrane to that in the liquid medium.

In the steady state, equation (1) can be integrated to give

$$J = D \frac{C_m(0)-C_m(1)}{1} = D \frac{\Delta C_m}{1} \tag{4}$$

where 1 is the thickness of a slab membrane. From equations (2),(3), and (4),

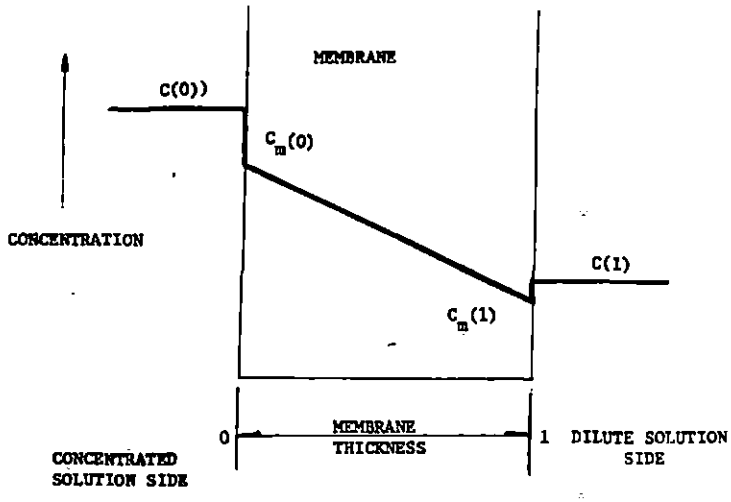


FIGURE 3. Schematic representation of the concentration gradient across a membrane (from Baker and Lonsdale, 1974)

$$J = \frac{DK \Delta C}{l} \tag{5}$$

where ΔC is the difference in concentrations on two sides of a membrane. If you have a constant activity sourse, the release rate of solute does not depend on time. This is commonly referred to in the literature as "zero order release". The release rate of this system will be constant, and for a slab, is represented using the equation (6).

$$\frac{dM_t}{dt} = \frac{ADK \Delta C}{l} \tag{6}$$

where A is the surface area of the slab. For a cylinder, the steady state release rate is given by

$$\frac{dM_t}{dt} = \frac{2\ hDK\Delta C}{\ln[\frac{r_o}{r_i}]} \tag{7}$$

where r_o and r_i are the outside and inside radius, respectively, of the

cylinder and h is the length of the cylinder. For the sphere,

$$\frac{dM_t}{dt} = \frac{4\pi DK\Delta C}{(r_o - r_i)/r_o r_i} \tag{8}$$

The above, for slabs and cylinders, assumes one-dimensional diffusion. This occurs under the assumptions of thin layer membranes for slabs and long cylinders for cylindrical systems.

On the other hand, for varying concentration experiments (i.e, nonconstant sources), the release rate falls exponentially. This is called "first order release".

Although constant activity source reservoir systems give constant release behavior in the steady state, they will initially exhibit release rates higher or lower than the steady state value. This is due to the burst effect and the time lag. The burst effect may cause the device to release at an initially high rate depending on the history of the device. The time lag means that a device which is used immediately requires time to establish the concentration gradient within the membrane.

Drug Release from a Matrix System

In matrix (monolithic) systems, the bioactive agent (such as a drug) is mixed with the membrane (either as dissolved molecules or as dispersed solid drug particles). The solubility of the solute in the polymer matrix becomes a controlling factor in the mathematical modeling of these systems.

Matix systems can be classified four different types:

1. Dissolved systems in which the initial solute loading is below or at the solubility limit (saturated solubility) in the polymer. Then, release is achieved by simple molecular diffusion through the polymer. Thus, the rate of release is dependent upon the initial drug load. The diffusion coefficient is dependent upon the properties of the drug and the polymer matrix.
2. Dispersed systems in which solute is dispersed as solid particles within polymer because the solute loading is above the solubility limit. In this case, dissolution of the solute in the polymer becomes the limiting factor in the releasing pattern.
3. Porous systems which are analogous to dispersed systems except that the initial drug loading is sufficient to produce continuous macroscopic pores of channels. The release rates are dependent upon transport within these solvent-filled pores or channels formed due to leaching of the solute.
4. Reservoir-dispersed matrix systems (or porous reservoir systems) which are analogous to the dispersed system except that a barrier layer is present at the surface of the device which is of much lower permeability to the drug than is the bulk polymer matrix.

Various derivatives of hydroxyalkyl methacrylates have been utilized for matrix devices because they have the following advantages: (1) nontoxicity, (2) high biocompatibility, (3) high permeability to both

hydrophobic and hydrophilic solutes, and (4) variable permeability, depending upon copolymer composition and crosslink density.

The major advantages of a matrix system are that the system can be made easily and that a drug will not be released suddenly upon rupture. The major disadvantage is that the solute release rate will decrease with time. The release kinetics from matrix systems are influenced by the initial drug loading and the geometry of devices. As the initial drug loading is increased, the matrix will become porous as drug is leached from the polymer. Thus, the free volume for diffusion increases as a result of the voids by the leached drug. This may bring changes in the effective diffusion coefficient of drug in the matrix phase. In addition to the initial drug loading effect, the geometry of these systems provides insight into the release characteristics of a polymer matrix.

An example of a typical release profile is shown in Figure 4, where the release rate for progesterone from a silastic cylinder is plotted versus time (Cardinal, 1984a).

The initial release rate in Figure 4 is very high relative to that seen at long times. In fact, this high release rate may not only reach a toxic level for a drug with a narrow therapeutic index, but also may result in a short life for the device with a drug which has to be maintained at a therapeutic level for a long time.

Next, the declining release rate has been explained by J. R. Cardinal (1984b). In a polymer containing a dispersed drug, a zone of depletion is formed as drug is released from the matrix system in Figure 5. A pictorial representation of the changes in this zone with time has been given by Roseman and Higuchi (1970). If the matrix is an

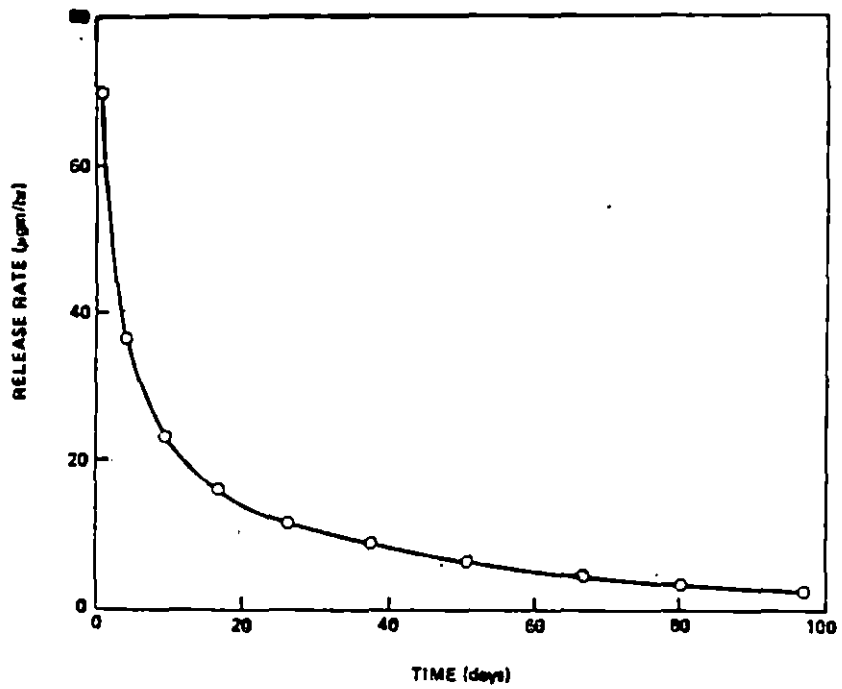


FIGURE 4. Typical profile for the variation in the release rate with time for a polymer matrix device in which the active agent is dispersed (from Cardinal, 1984a)

infinite slab, the surface area at the receding drug boundary is constant. The flux of drug away from this surface will follow a path which is perpendicular to the surface. Therefore, by Fick's first law, the concentration gradient in the zone of depletion will be linear. As the thickness of the zone increases, the diffusing distance will increase with time. Since flux is inversely proportional to the zone thickness, the release rate will decrease with time.

For other devices (e.g., cylinders or spheres), the flux of the drug will also follow the same pattern as seen for slabs. However, the volume of the depletion zone will increase rapidly from the surface, and the concentration gradient is nonlinear within the zone of depletion. Thus, the release rate will decrease more rapidly with time.

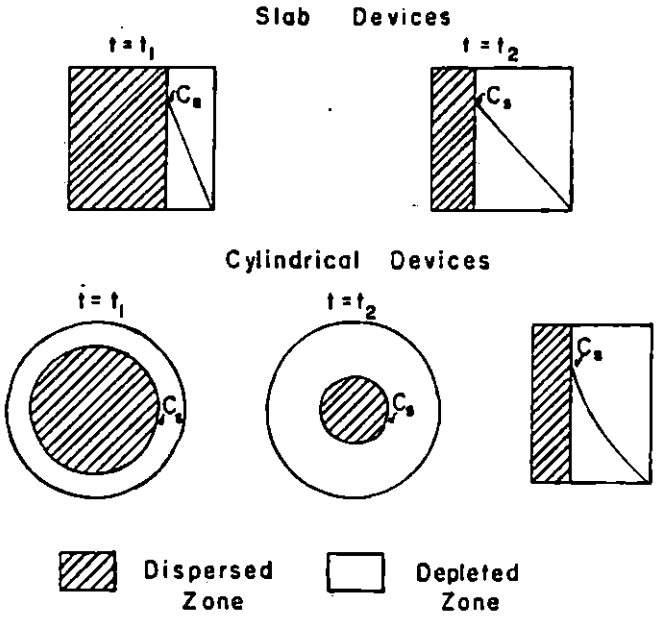


FIGURE 5. Schematic representation of the change in the dispersed and depleted zones of matrix devices with time ($t_2 > t_1$) (from Cardinal, 1984b)

In order to achieve a constant release rate, a number of methods have been developed. One approach is to design a device which has an increasing surface area of the zone of depletion with time. This method has been utilized by Brooke (1974), Lipper and Higuchi (1977), Hsieh et al. (1983) and Theeuwes (1980). A second approach is to incorporate a rate limiting barrier at the surface of the system, such as to include a crosslinked surface region, to alter the water content, or to dip coat and thus to establish a barrier layer. A third approach is to have an aqueous boundary layer which can provide a significant resistance to drug release by using an inherent property of the matrix and the drug. A fourth approach is to control the rate of swelling of the matrix in the fluid medium.

Most of the mathematical models for controlled release kinetics presented in the literature have been derived with the assumption of a boundary condition of zero (and constant) solute concentration at the interface of the device. This condition requires the complete elimination of boundary layer effect, such as by high agitation during the release experiment. Recent mathematical models and equations show the solution for certain boundary conditions.

Baker and Lonsdale (1974) have developed approximations for release equations (refer to Table 1). These equations are in the form of infinite series (infinite slab or infinite cylinder), and include the data obtained from the short and long time approximations. In the case of the slab, the early time approximation provides an excellent fit in the range of $M_t/M_\infty<0.6$ where M_t is the mass of drug released by time and M_∞ is the total mass of drug. The late time approximation is valid in the range of $0.4 <M_t/M_\infty<1.0$. For the cylinder, the early time approximation fits when $M_t/M_\infty<0.4$, whereas the late one is valid in the range of $M_t/M_\infty >0.6$. In the case of the sphere, the early time approximation fits when $M_t/M_\infty<0.4$, whereas the late time approximation is good in the range where $M_t/M_\infty>0.6$.

It is clear that when slabs of monolithic devices are kept at a constant external solute concentration, the fractional release (M_t/M_∞) is proportional to the square root of the release time over the first 60 % of release. After that, the fractional release decays in an exponential manner. However, in the dispersed drug system, the release rate falls according to the $t^{-0.5}$ law essentially throughout the lifetime. Solutions for other boundary conditions are available in the literature.

TABLE 1. Fractional release and release rate for a matrix device in which the active agent is dissolved

Device geometry	Early time	Late time
Slab	$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi l^2}\right)^{0.5}$	$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\exp\left(\frac{-\pi^2 Dt}{l^2}\right)$
	$\frac{dM_t}{dt} = 2M_\infty\left(\frac{D}{\pi l^2 t}\right)^{0.5}$	$\frac{dM_t}{dt} = \frac{8DM_\infty}{l^2}\exp\left(\frac{-\pi^2 Dt}{l^2}\right)$
Cylinder	$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi r^2}\right)^{0.5} - \frac{Dt}{r^2}$	$\frac{M_t}{M_\infty} = 1 - \frac{4}{(2.405)^2}\exp\left[\frac{-(2.405)^2 Dt}{r^2}\right]$
	$\frac{dM_t/M_\infty}{dt} = 2\left(\frac{D}{\pi r^2 t}\right)^{0.5} - \frac{D}{r^2}$	$\frac{dM_t/M_\infty}{dt} = \frac{4D}{r^2}\exp\left[\frac{-(2.405)^2 Dt}{r^2}\right]$
Sphere	$\frac{M_t}{M_\infty} = 6\left(\frac{Dt}{\pi r^2}\right)^{0.5} - \frac{3Dt}{r^2}$	$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\exp\left(\frac{-\pi^2 Dt}{r^2}\right)$
	$\frac{dM_t/M_\infty}{dt} = 3\left(\frac{D}{\pi r^2 t}\right)^{0.5} - \frac{3D}{r^2}$	$\frac{dM_t/M_\infty}{dt} = \frac{6Dt}{r^2}\exp\left(\frac{-\pi^2 Dt}{r^2}\right)$

Ocular Application of Controlled Release Systems

When drug delivery to the eye is used, the duration of drug activity must be adequate, and the drug levels attained must be uniform so that the frequency of drug administration can be minimized. Thus, the invention of suitable ocular inserts will be more valuable than other methods of drug delivery as the invention would provide drug availability in the eye at a constant level for long periods of time.

The eye has been considered to be a useful site for controlled release systems. Compared to other body sites, it is relatively easy for devices to be inserted into and to be removed from the eye. An insert is readily prepared by dissolving a drug and a polymer in a solvent. The solvent is then evaporated to form a thin film of the polymer which is made into the various shaped systems such as a square, rectangle, or doughnut, for example. Two ocular polymeric systems, Ocusert and Lacrisert, are already commercially available.

The Ocusert is a membrane controlled reservoir system used in the treatment of glaucoma. The active agent of this insert is pilocarpine. The polymer used in the Ocusert is a ethylene-vinyl acetate copolymer. Pilocarpine is surrounded on both sides with two polymer membranes. There are two different systems of Ocusert. One releases pilocarpine at 20 ug/hr (Pilo-20), and the other at 40 ug/hr (Pilo-40). Both systems display a "burst" effect. The major advantages of using Ocusert include: (1) longer duration of drug action (up to 7-8 days) (2) convenience of once a week application, and (3) better patient compliance with the therapy. However, there are problems of insertion, retention, leakage, and discomfort.

Lacrisert is valuable for the treatment of a dry eye. This device is a rod-shaped preparation (1.27 mm diameter, 3.5 mm in length) composed of 5 mg of hydroxypropyl cellulose, a water soluble polymer. The Lacrisert is inserted into the eye with a special reusable applicator. The system is placed in the conjunctival sac where it softens within 1 hour, and completely dissolves within 14 to 18 hours. This device acts to stabilize and thicken the precorneal tear film, and to prolong the tear film breakup time which is usually accelerated in patients with dry eye states. The difficulty of using Lacrisert is insertion (Conn and Langer, 1984).

Ozawa et al. (1983) have prepared ocular inserts impregnated with antibiotics for trachoma therapy. In in vitro experiments, they found that the release rate of a drug with relatively high solubility was influenced by the solubility of drug in water. A drug with low solubility in water had a constant release pattern when the water content of the hydrogel insert was more than 30%. In this case, the stirring of the solution increased the release rate. However, for a drug with high solubility, the rate determining step of the release was the diffusion of the drug within the gel. Consequently, a small change of water content of the hydrogel affected the release rate, although the stirring of the solution had little effect. The drug release rate was a little higher in vivo than in vitro for the low solubility drug case. In addition, they suggested that ocular inserts should meet the following three criteria:

1. Release kinetics of the effective drug from the insert should be zero or nearly zero order for a long time.

2. The insert should not be harmful when retained in the eye for a long time.
3. The insert must be retained easily in the eye and must cause no patient discomfort.

Several ocular inserts were suggested as the treatment system for providing pinkeye therapy for cattle. Hawley (1954) has used an eye pellet that provided therapeutic concentrations of terramycin in the eye for 31 hours. However, these eye tablets used tetracaine hydrochloride to reduce irritation from the tablet. Tetracaine has a severe inhibitory effect on the healing of injured cornea (Slatter et al., 1982). Theodorakis et al. (1983) have prepared an ocular insert which was made from a composite of CASS with PLA. The insert was retained in the eye at least 4 days and maintained therapeutic levels of drug in the eye of the rabbit in vivo. Hughes and Pugh (1975) tested the retention of plastic tubular rings. They did not report any drug incorporation or drug release experiments for bovine eyes. They emphasized that the retention of devices in the eye was dependent on the size (a range of 135 to 140 mm in circumference was satisfactory in their study) and the shape of device. They suggested that the use of the ring design and that a biodegradable matrix containing antibiotic could provide a good treatment method for the pinkeye of cattle. Punch et al. (1985) have used a soluble and an insoluble collagen film impregnated with gentamicin. For these collagen inserts, they found that soluble collagen films released significantly higher levels of antibiotic than the insoluble films, and that soluble collagen films maintained the therapeutic levels necessary to treat Moraxella bovis for only 24 hours. The inserts showed no

irritant properties when initially placed in the eye, and were well tolerated by the cow in contrast to their previous studies using hydrophilic contact lenses (Slatter et al., 1982) in which corneal edema was developed in some cattle right after insertion.

Hydrogels for Controlled Drug Delivery

Hydrogels may be prepared by different procedures of polymerization of hydrophilic monomers or of chemical modification of existing polymers. Hydrophilic polymers like HEMA are water-swollen gels with poor mechanical properties. Therefore, their application in a pure state is very limited. They must have an adequate number of hydrophilic groups to permit swelling (no dissolution) in water, but this decreases their mechanical stability. The hydrophilic monomers are usually copolymerized with hydrophobic monomers. Hence, the hydrophilicity of hydrogels can be modified by use of less water soluble monomers (e.g., MMA) for control of the mechanical strength and the degree of swelling. Hydrophilic monomers are mixed with hydrophobic ones in different ratios to yield hydrogels with a wide range of water contents (Piskin, 1984). Copolymerization of HEMA with MMA leads to poorly swollen hydrogels which have been reported to give an almost zero-order release of low molecular weight solutes for long periods of time (Cowsar et al., 1976).

Hydrophobic polymers are provided to make uncrosslinked release devices by compression of powdered polymer or by dissolution of the polymer, followed by evaporation of the nontoxic solvent. Polydimethyl siloxanes (PDMS) and ethylene-vinyl acetate (EVA) copolymers are available. On the other hand, drug delivery systems based on synthetic

biodegradable polymers have used mainly polylactic acid, polyglycolic acid and their copolymers.

Since hydrogels consist of hydrophilic macromolecules crosslinked to form a three-dimensional network, their permeability for low molecular weight solutes has been exploited in designing sustained release devices (Schacht, 1984). Also, this material is stable to varing pH, temperature and tonicity. Hydrogels in contact with aqueous solutions will swell to some equilibrium value. The equilibrium water content (EWC) is expressed by;

$$EWC(\%) = \frac{\text{weight of swollen gel - weight dry gel}}{\text{weight of swollen gel}} \times 100$$

This water content in a hydrogel influences the biocompatibility of polymer surfaces. The degree of hydration of hydrogels also affects the rate of release of drug. The equilibrium water content of a hydrogel is affected by the nature of the hydrophilic monomers used in preparing the polymer or copolymer, the nature and density of the crosslinks, and such factors as the temperature, tonicity and pH of the hydrating medium (Pedley et al., 1980), but poly(HEMA) is little affected by variations in these factors (equilibrium water content of 40 %). A copolymer of HEMA with hydrophobic monomers, such as styrene or methyl methacrylate, will have less than 40 % water content. More hydrophilic monomer combinations can provide hydrogels with water contents in excess of 90 %. Collett et al. (1980) observed that the hydration of HEMA was influenced by the method of preparation and by the use of different solutes for hydrogel. They used HEMA as a monomer, 0.875 w/o of ethylene glycol dimethacrylate as the crosslinking agent, 40 w/o water, and 0.2 w/v of ammonium

persulfate as a chemical initiator. Other were prepared using a dose of 300K rad of γ-radiation. Swelling properties and the degree of hydration of hydrogel were estimated from measurement of weight changes of gels immersed in water, and 0.5M aqueous solution of some substances including NaCl, urea, and sucrose. The results showed that NaCl and sucrose caused dehydration of the gels probably due to an osmotic effect, whereas urea increased the hydration.

> The presence of the persulfate initiator increased the hydration rates and equilibrium water contents, compared with those seen in gels produced by irradiation.

The magnitude of the permeability of a drug in a hydrogel matrix depends on the type of drug, the polymer composition, the water content in the hydrogel, and the amount and nature of the crosslinking agent (Schacht, 1984). The diffusion coefficient will decrease with increasing crosslinking density due to a reduced pore size and a decreased fraction of water. Therefore, the dissolution-diffusion process dominates the permeation mechanism. Schacht (1984) has obtained data for various crosslinker densities, and has indicated that the rate of release of procaineamide hydrochloride from hydrogels decreases as increasing amounts of crosslinking agents are added to the polymerization mixture. Water-swollen hydrogels with high degrees of swelling can provide rate-controlling barriers for diffusion of water-soluble drugs (Graham and McNeill, 1984).

In addition, the drug release rate from a trilaminate delivery system by Olanoff et al. (1979) can be controlled by the device geometry, coating membrane thickness, disk surface area, drug loading and membrane coating copolymer composition.

> Increasing disk surface area available for diffusion by 1.88 times (0.709 - 1.33 cm^2) produced a proportional increase in the release by 1.8 times (0.54 - 0.97 ug/day).

Correspondingly, increasing the coating membrane thickness by 1.73 times (0.081 - 0.14 mm), holding surface area constant, decreased the release rate by 1.43 times. Increasing the drug loading in the core material by an order of magnitude from 0.02 to 0.2 milligrams of drug per milligram of total core weight increased the release rate by 16 times when corrected for a difference in membrane thickness. The higher increase than predicted in the release rate is probably due to the osmotic effects that become significant for core materials with higher drug loading. There were effects of varying the coating membrane composition; a hydrophilic coating copolymer (22:78 mole of ratio of HEMA:MMA) had a higher release rate than that of a relatively hydrophobic coating copolymer (2:98 HEMA:MMA). Rhine et al. (1980) also reported that fabrication parameters such as drug particle size, drug loading and matrix coating significantly affected release kinetics in sustained macromolecule release. The release rate increases caused by increases in the particle size may result from the formation of larger channels or pores in the polymer matrix. Similarly, increased loading may provide simpler pathways and greater porosity for diffusion, both of which would facilitate the movement of water into the matrix and protein out of the matrix. Good and Mueller (1980) have shown the existence of a new diffusion mechanism which relies heavily on structure and thermodynamic interactions within a crosslinked polymer network. The networks described in their paper are composed of two component systems: the "macromer" component is a hydrophobic oligomer of butane oil while the

"main chain" is hydrophilic pHEMA (poly hydroxyethyl methacrylate). These networks were achieved by simultaneous copolymerization and crosslinking of pHEMA in the presence of the macromer, polytetramethylene oxide. The authors have shown that the usefulness of hydrogels can be expanded by using a structure based on 2-component systems in which the hydrophilic balance is adjusted to provide for the desired release rates for an enclosed solute.

In Vitro Evaluation of Controlled Release of Drugs

In order to develop a controlled release drug delivery system and to construct the controlled release rate profiles of drug from the device, the work requires the design of a proper in vitro drug elution system which will permit the accurate evaluation and analysis of the controlled release profiles prior to the initiation of more costly animal testing. Analytical sensitivity of the drug assay technique, long-term maintenance of a sink condition, hydrodynamic characteristics of solution diffusion, reproducibility of sampling, and the volume and temperature of the system are important design parameters in choosing an elution system.

Drug elution systems are divided into two types: a continuous flow apparatus or a constant rotation apparatus. Both of them are ideal for the measurement of in vitro drug release rate profiles. The continuous flow apparatus has been designed and used by Kalkwarf et al. (1972) and Roseman and Higuchi (1970). A typical setup is illustrated in Figure 6.

This apparatus is composed of the reservoir for the elution solution, a peristaltic pump, a drug elution column and a thermostatic bath. A prototype of a drug delivery device is positioned in a drug

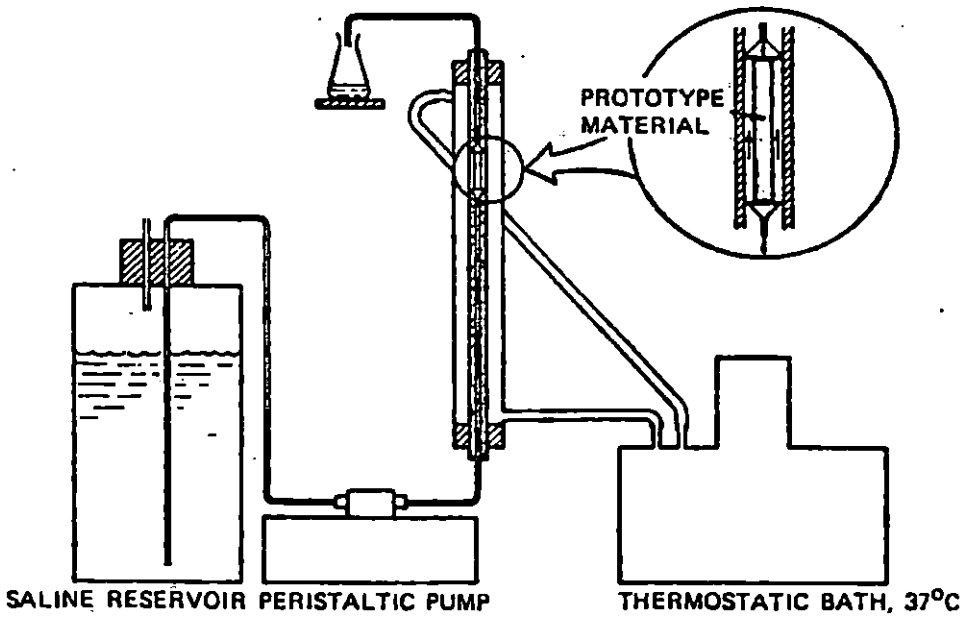


FIGURE 6. Schematic diagram of a continuous flow apparatus (from Chien, 1982b)

elution column which is thermostated at 37°C, and the device is exposed to a continuous flow of elution solution by using a peristaltic pump. Distilled water, isotonic saline and 3% isotonic solution of bovine serum albumin have been used as an elution solution. Samples from the effluent of the systems are then collected and assayed at various time intervals (Kalkwarf et al., 1972). As the flow rate of elution solution is increased, the rate of drug release also increases, and eventually reaches a plateau level. The in vitro release of progesterone from a matrix-type polyethylene drug delivery device using this apparatus was linear for a Q (rate of drug release) vs. $t^{0.5}$ (time) plot (i.e., was found to follow a Q versus $t^{0.5}$ relationship; Chien, 1982b). Kalkwarf and coworkers (1972) suggested that sink conditions were maintained during the test. Direct evaluation of drug concentration in the elution solution becomes extremely difficult and labor-intensive.

Another method for in vitro evaluation is the use of a constant rotation apparatus. Chien and his associates (1974) developed a constant rotation apparatus which was relatively simple in design and easy to build. The polymeric drug delivery device is mounted in a circular plexiglass holder (with a spin bar at the center of the holder), and then the spin bar is rotated in the elution solution at constant rotation rate to achieve the constant solution hydrodynamics required. The release rate has a similar pattern (linear Q vs. $t^{0.5}$ relationship) to that of a constant flow apparatus. In a drug elution system, the effect of temperature adjustment is one of the important factors in the thermodynamics of controlled release drug. The temperature is normally adjusted to 37°C to approximate body temperature. This temperature influences the in vivo evaluation. Y. W. Chien (1976) has reported that the Q vs. $t^{0.5}$ drug release profile is observed to be temperature-dependent and is linked to three energy-activated steps: dissolution and diffusion of drug molecules in the polymer structure, and dissolution of drug molecules from a crystal lattice.

Both drug elution systems are capable of maintaining a sink condition for better simulation of a biological in vitro/in vivo relationships. The in vitro/in vivo relationship established can then be applied to the design of a desirable drug release device.

PROPOSED TREATMENT METHOD

Design Factors

Selection of drug

A wide range of astringents, antiseptics, and antimicrobial agents has been used for the treatment of IBK. Among these treatment drugs, penicillin and streptomycin have been used widely to treat IBK, but they are not as effective as certain other drugs. Tetracycline, erythromycin, and tylosin are effective drugs against Moraxella bovis infection. Of these three, tylosin has an inhibitory concentration as low as 0.63 ug/ml (as cited in Leytem (1984)). Tylosin (molecular weight 916.14) is a marcrolide type antibiotic isolated from a strain of Streptomyceles fradiae found in soil from Thailand (Hamill et al., 1961). The structure of tylosin is shown in Figure 7.

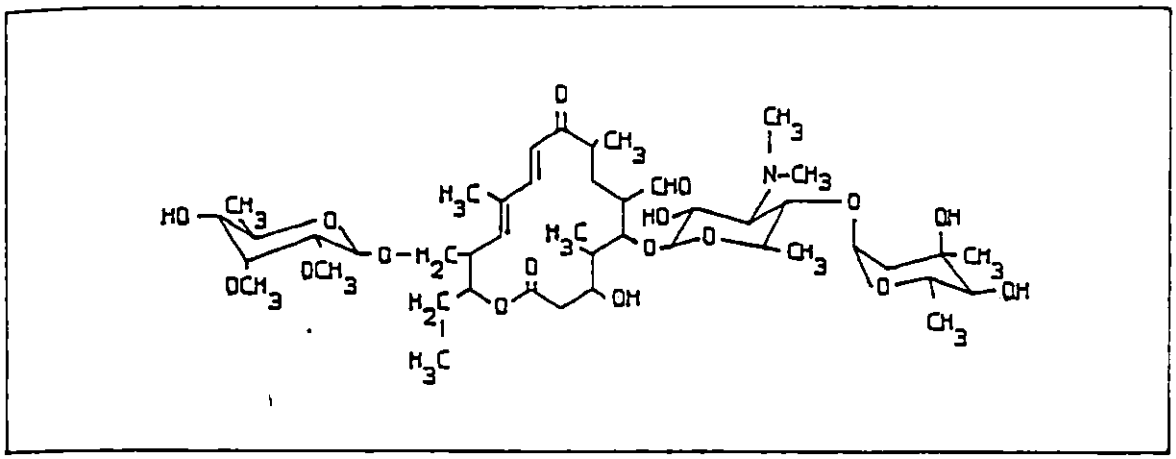


FIGURE 7. Molecular structure of tylosin (from Windholz et al., 1976)

This antibiotic is essentially nontoxic, nonirritating to the eye and conjunctival sac, stable and readily soluble in water (Ellis and Barnes, 1961). Tylosin tartrate (mol. wt. 1066.2) is commercially

available as an agricultural antibiotic (Burger, 1970), is soluble in water at concentrations greater than 300mg/ml, and is stable at room temperature in aqueous solutions (pH 4-9) for at least one month (Ose and Barnes, 1960).

Treatment device

The treatment device must be designed for prolonged retention in the eyes of cattle. In addition, the device should be easy to insert and should not induce tissue reaction. Hughes and Pugh (1975) have examined these factors to design an ocular insert for cattle. They have evaluated ring devices fabricated from intrademic polyethylene, medical grade polyvinylchloride, and nylon surgical tubing. Insertion of ring devices was no problem except that the ring had to be the proper size to fit into the conjunctival sac. Local eye reaction to the ring devices was minimal, and ranged from an increased tear pooling initially, to an increased mucous secretion after prolonged retention. These ring type devices were retained in the cattle eyes up to 19 days. The authors have reported that the long term retention of a ring device was made possible with rings having a circumference smaller than that of the conjunctival sac, but larger than that of the globe of the eye. If the ring is too large or too small, the ring tends to be forced out and is easy to distort. If these criteria are met, the ring devices will be uniquely suited for use in the bovine eye (which is anatomically different from that of human). Therefore, this ring-shape was chosen as the configuration of the proposed treatment devices.

Material for device

The material used for construction of the ring devices must be rigid enough to retain its ring shape, but should be flexible enough to conform to the shape of the conjunctival sac. Also, the smooth surface of the ring device is necessary to prevent eye irritation. The membrane coating material of the ring device should be biocompatible, and be capable of sustaining the drug release for the required period. As mentioned above, hydrogel is one of the materials which can satisfy all the above conditions. A copolymer of HEMA and MMA will be used as the fabrication material.

Design of the Apparatus for Release Experiments

The development of an in vitro method that can determine the release machanism of a controlled released release device is a very important step in evaluating potential drug delivery systems prior to in vivo testing. A continuous flow apparatus can be applied to study the drug elution system of our matrix device. The design is similar to the actual eye in which the drug delivery system will be used. The block diagram for modeling the drug release appartus is shown in Figure 8.
This continuous flow apparatus is composed of the reservoir for the elution solution, a constant flow pump, drug elution system and thermostatic bath. The drug elution system is designed to be close in function to the lacrimal apparatus in the eye as shown in a schematic comparison in Figure 9.

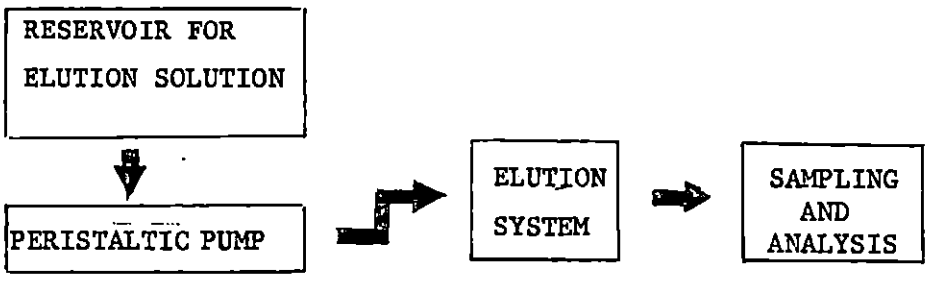


FIGURE 8. The block diagram of ocular drug release apparatus

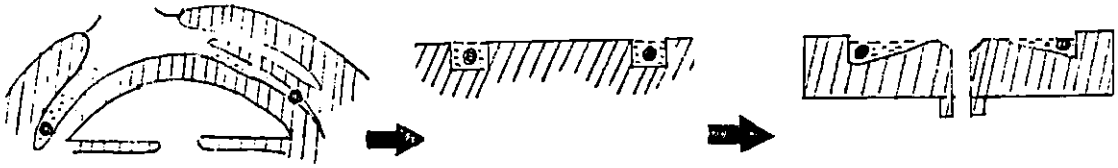


FIGURE 9. Drug elution system for ocular drug release device

Quantitative Design Parameters

Required drug release rate

In order to design an optimum release device for treating pinkeye, it is very important to determine the release characteristics of the device. The variables influencing release profile are the duration of the treatment of drug and mininmum and maximum concentrations required in treating the disease within a given period. Recovery times from pinkeye can not be clearly determined because of the difference in healing times depending on the severity of the disease (Blogg, 1980). To ensure a sufficient treatment duration, the prolonged device will be designed to release tylosin tartrate for at least 7 days. If a minimum inhibitory concentration of 0.63 ug/ml of tylosin tartrate is used, the required drug release rate can be calculate based on tear flow rate data for cattle. As mentioned before, Slatter and Edwards (1982) have measured bovine tear flow rates as being 1.96 ± 1.84 ml/hr. Using these data, a minimum release rate of the order of 1.2 to approximately 2.4 ug/hr of tylosin tartrate is required. These values are useful in design considerations for the system to be described.

Calculation of release rate for drug release system

Leytem (1984) has made disk-shaped laminates of 90:10 MMA-HEMA copolymer which contained tylosin tartrate. The disks were studied for release characteristics in five-day in vitro experiments. He obtained the release results of approximately 1 - 33.3 ug/hr (for up to 84 hrs) from the controlled release disks containing 50 mg of tylosin tartrate. Weiss (1985) has measured release rates from a reservoir system and a

monolithic system. The reservoir system did not provide consistent release characteristics, while ten monolithic devices provided similar release profiles and gave a minimum release rate of at least 5 ug/hr throughout nine days. He concluded that the monolithic system could be applied for the treatment of pinkeye in cattle, since the rate seen from the monolithic devices after nine days was above the calculated minimum release values (approximately 2.4 ug/hr) desired. In his system, the drug release rates were seen to be inversely proportional to time, but not to the one-half power.

In general, the determination of the release pattern of the delivery device is essential in anticipating release rates for similar systems. The release rate can be a function of device geometry and coating copolymer composition. The geometry of the device of the current study is that of a cylinder, the outer portion of which contains the active antibacterial agent. The cylinder is joined at both ends, and a ring shape results. As mentioned before, the solubility of the solute in the polymer matrix determines the type of system. Therefore, if the solubility of tylosin tartrate in the hydrogel is below or at the solubility limit (saturation solubility), the system will be a dissolved system. This system depends on the diffusivity of tylosin tartrate in a homogeneous matrix containing the dissolved drug. For a drug dissolved in a device having the cylinderical geometry, the fractional release (M_t/M_∞) and the release rate (dM_t/dt) at any time can be obtained easily by using the equations in Table 1. However, our system is not exactly a cylinder model or a slab model. Therefore, when those equations are applied to our system, any assumptions must be considered carefully.

METHODS AND MATERIALS

Production of MMA:HEMA Copolymers (90:10 and 25:75)

The MMA:HEMA copolymers were made in a batch process. The following materials were added in the order listed to a one-liter Erlenmeyer flask: 570 ml of absolute ethanol, 380 ml of type-one water, 6.1 ml of HEMA[1], 46.6 ml of MMA[2], 0.25 gm of reagent grade sodium persulfate and 0.125 gm of reagent grade potassium persulfate to provide a MMA:HEMA copolymer on a 90:10 molar basis. For a 25:75 (MMA:HEMA) copolymer, the added amount of the materials was the same as the above except that 37.2 ml of HEMA and 10.9 ml of MMA were added to the water, ethanol and initiator mixture. The flask was sealed with a rubber stopper and the contents were bubbled vigorously with nitrogen for 30 minutes. After thirty minutes, the nitrogen pressure was adjusted to a slow continuous bubbling throughout the ten day copolymerization period. The reaction was carried out at room temperature. On the tenth day, the 90:10 copolymer was present in the form of a white sticky precipitate. The solution containing the copolymer was added to three liters of water. However, the 25:75 solution did not exhibit a change in color until the copolymer solution was added to three liters of type-one water even though the reaction was complete. The liquid was filtered through Whatman 1-quantitative filter paper in a Buchner funnel by using a vacuum. Each time the funnel was full of precipitate, the precipitate was washed four

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

[2] Aldrich Chemical Co. Inc., Lot #041557, Milwaukee, Wis.

times with 25 ml of water, and was placed into a glass container for drying. This container was covered with filter paper which prevented contaminants from entering during the drying period. The copolymer was dried in an oven at 50°C for five days under a vacuum (25 in. Hg).

Fabrication of Controlled Release System

Preparation of copolymer/drug mixture

Monolithic devices used in this study were made by dip-coating a polymer tube. The tube was dipped into a drug/copolymer mixture and dried; this process was repeated several times to build up a surface layer of the drug and copolymer.

In order to reduce the time required for a complete dissolution of the copolymer in the solvent, the copolymer particles were crushed or ground. For the 90:10 copolymer, a ceramic crucible was used to grind the hard, brittle copolymer. However, the 25:75 copolymer was a transparent, glassy material after drying, and was not easy to grind or crush. Liquid nitrogen was used to break down this material into a small particles (by adding liquid nitrogen while crushing). One gram of the copolymer powder was added to 20 ml of dimethyl formamide, mixed, and the mixture was heated at 50°C for six hours to dissolve the polymer. Then, the copolymer/drug mixture was made by first cooling the solution at room temperature and then adding 500 mg (or 1000 mg) of tylosin tartrate to the solution and mixing. One half gram of tylosin tartrate was easily dissolved in the copolymer/solvent mixture. The 25:75 copolymer required more time for dissolution in the solvent than for the 90:10 copolymer. The finished copolymer/drug mixture became a yellow, viscous solution.

Preparation of mandrel

All the devices were fablicated by dip-coating ring shaped plastic tubes. These mandrels were made by inserting a copper wire through a piece of tubing such as Silastic tubing[3], Teflon[4], or polyvinylchloride tubing[5] and wrapping the tubing (containing the wire) around a cylinder form of the correct diameter (35 - 40 mm). If the mandrel material was Teflon, the mandrel was soaked overnight in a soap solution[6] and dried in air without rinsing to improve subsequent adherence of the copolymer. Any particular mandrel was weighed before and after coating to establish the weight of the copolymer-drug coating, and the drug loading of a ring device was calculated on the basis of the original drug:copolymer ratio.

Dip-coating process

The mandrel was dipped into the copolymer/drug mixture and was hung in a drying chamber containing nitrogen and Drierite ($CaSO_4$) to eliminate moisture. The moisture in the air can otherwise cause the copolymer layer to swell. In this study, two different compositions of copolymer, 90:10 and 25:75, were prepared to evaluate the effects of the drug loading and the copolymer composition on drug release rates. Each coat

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[3] Dow Corning, Silastic medical grade tubing, Size: 0.025"(I.D.)x0.047"(O.D.), Midland, Mich.

[4] Cole-Parmer, Cat.#P/N 6417-21. Size: 0.022"(I.D.)x0.042"(O.D.), Chicago, Ill.

[5] Becton, Dickinson and Co., Transparent/radiopaque type, Sizes: 0.034"(I.D.)x0.046"(O.D.) and 0.020"(I.D.) x 0.036"(O.D.), Rutherford, N.J.

[6] Proctor and Gamble, Ivory Snow Flakes, Cincinnati, O.

deposited 10-15 mg of copolymer/drug mixture on the mandrel, so nine to fifteen coats of solution were required to deposit the desired amounts of drug and copolymer on the devices. In the case of working with the 25:75 copolymer, precoating with 90:10 copolymer improved the ease of applying (subsequent) coatings with the 25:75 copolymer. The 100 mg drug loading in a copolymer was more yellowish and more brittle than the that of 50 mg drug loading. The 50 mg loading for a 90:10 copolymer exhibited a smooth, transparent surface. The 25:75 copolymer ring device had a less smooth surface with a straw tint compared with the 90:10 ring devices. As the solvent (dimethyl formamide) in the copolymer/drug mixture was capable of dissolving certain plastics such as PVC, the physical properties of the PVC tubing were changed somewhat after dip-coating; the inner PVC tube of a ring device became softer and more flexible, while the diameter of a ring device was reduced to 85-90% of the original diameter. However, the Silastic or the Teflon tubing did not exhibit a reduction in diameter.

Production of finished ring device

After drying dip coated ring devices in the desiccator for 3 days, the wire was removed, and both sides of a polymer ring device were then joined using a short piece of a PVC connecting tube (0.020" I.D. x 0.036" O.D.). A small bead of an adhesive such as silicone rubber or a mixture of copolymer solution and tetrahydrofuran (THF)/PVC mixture was used to connect the tube sides. The tips of a PVC ring device widened slightly compared to the rest of the tubing, which made the smaller diameter joint easy to insert into a dried ring device. Twenty-four

monolithic type devices were fabricated for the drug release experiments; the coating materials and drug loadings for producing these devices are shown in Table 2.

TABLE 2. Characteristics of the fabricated ring devices

Device number	Mandrel material	Coating material (copolymer)	Ring device diameter (mm)	Drug loading amount (mg)	Joint material
011	Silastic	90:10	40	51.8	Copper wire
021	Silastic	90:10	40	49.9	Copper wire
022	Silastic	90:10	40	49.6	Copper wire
031	Silastic	90:10	40	52.2	Copper wire
032	Silastic	90:10	40	109.7	Copper wire
033	Silastic	25:75	40	44.6	Copper wire
041	Silastic	90:10	40	48.5	Copper wire
042	Silastic	90:10	40	101.8	Copper wire
043	Silastic	25:75	40	61.5	Copper wire
044	Silastic	90:10	40	111.2	Copper wire
051	Silastic	90:10	40	116.5	Copper wire
052	Silastic	90:10	40	99.9	Copper wire
053	Silastic	25:75	40	53.8	Copper wire
054	Silastic	25:75	40	56.9	Copper wire
061	PVC	90:10	35	50.8	PVC
062	PVC	90:10	34.5	48.0	PVC
063	PVC	90:10	35.5	52.8	PVC
064	PVC	90:10	37	49.4	PVC
065	PVC	90:10	35	55.2	PVC
071	PVC	25:75	35	48.7	PVC
072	PVC	25:75	36	54.9	PVC
073	PVC	25:75	36.5	57.3	PVC
074	PVC	25:75	36.5	50.5	PVC
075	PVC	25:75	36	56.1	PVC

Drug Release Process

A continuous flow apparatus was used in the tylosin tartrate release experiment. This apparatus was comprised of a 500ml flask, a peristaltic pump, a drug elution system, and a water bath as shown in Figure 10.

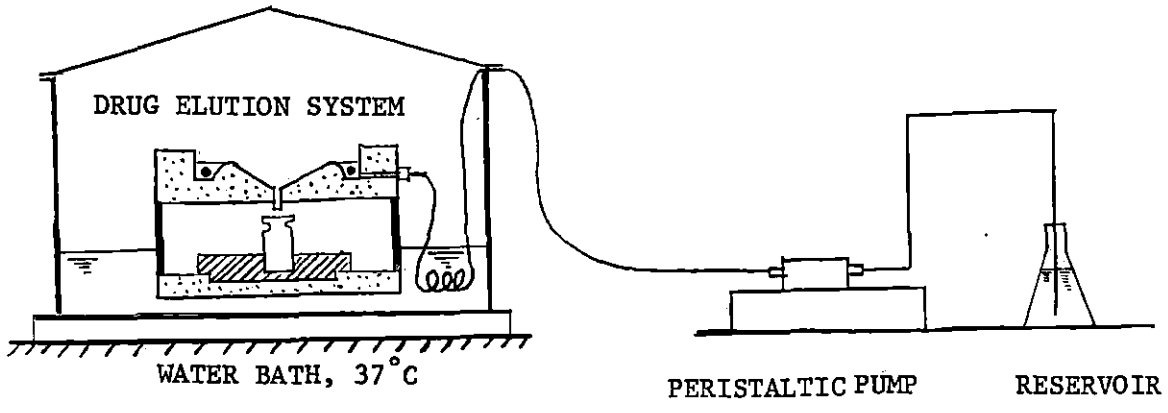

FIGURE 10. Apparatus for drug release experiment

Mammalian Ringer's solution[7] was used for the elution solution in the flask. The drug elution system was placed in the water bath. Each device was put into the inner ring-shaped hole of drug elution system to permit subsequent measurement of the amount of drug released at a specific time. The temperature of elution solution in the elution system was maintained at 37°C during the entire experiment period. In order to adjust the flow rate and the operating temperature of the elution solution during the release experiment, three methods were studied: use

[7] 8.60 g NaCl, 0.30 g KCl. 0.33 g $CaCl_2$ in 1.0 liter type-one water.

of a cassette pump[8], of a syringe pump[9], and of a flow meter. The comparison of the three methods is listed in Table 3.

TABLE 3. Comparison of three different methods for a continuous flow apparatus

Item \ Equipment	Flow meter	Syringe pump	Cassette pump
Maximum operating capacity	9 days/unit	1 day/unit	9 days/unit
Flow rate adjustment	poor	good	poor
Temperature adjustment	good	good	good
Sampling	good	excellent	excellent
Performance test before experiment	necessary	not necessary	necessary

The samples collected from the drug elution system were stored in small vials. The sampling time was an important factor in determining the release profiles seen for the continuous elution model. Since sequential samples obtained in the initial period (up to 2 days) typically contained rapidly increasing concentrations of drug (Weiss, 1985), a short sampling time period was necessary within the initial two day period. In addition, other sampling frequencies were used in this experiment.

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[8] Cassette pump, Monostat, New York, N.Y.

[9] Dual infusion/withdrawal pump, Model# 945 and 946, Harvard Apparatus Co., Millis, Mass.

Table 4 lists the conditions of the release process for each device.

TABLE 4. Conditions of drug release experiment for each device

Device number	Elution fluid name	Elution fluid flow rate (ml/hr)	Equipment (pump)	Sample collection frequency
011	Ringer's	2	syringe	1 hour and random[a]
021-022	Ringer's	2	syringe	1 hour and 7 hours
031-033	Ringer's	2	syringe	8 hours
041-043	Ringer's	2	syringe	8 hours
044	Ringer's	2	cassette	8 hours
051-075	Ringer's	2	cassette	8 hours

[a]Every hour as well as additional random sample.

Each device was examined using a stereomicroscope before and after the release experiment to check for poor seals around the connected parts or for surface cracks. Also, several were examined by scanning electron microscopy to check for surface structural defects.

Quantitative Analysis of Tylosin Tartrate

After the release experiment, the samples were dried in an oven at 50°C. Then, a volume of water was added to the dried samples in particular quantities related to the anticipated concentration of drug and associated thin layer chromatography (TLC) requirements. For example, if one sample was expected to release 160 ug/hr of drug, 100 ul - 800 ul of water was added to the dried sample to make a concentration in the range of 0.5 ug/2.5 ul - 4.0 ug/2.5 ul. TLC was used to determine

the amount of tylosin tartrate released from the ring device for in vitro tests. Whatman LKC18F 20x20 cm TLC plates[10] were fully developed in a standard developing chamber[11] containing reagent grade methanol. The developed plates were air-dried at room temperature for three days. The solvent wet no more than the bottom 3 mm of a plate once the plate was placed in the chamber. A volume of 2.5 ul of each redissolved sample was applied onto the 2 cm region below the reverse phase/preadsorbent interface of a TLC plate by using a Drummond 0-10 ul micropipette[12]. By touching the edge of the drop to the preadsorbent layer of the TLC plate, the drop was transferred to the plate. The standard samples were four known concentrations of tylosin tartrate (0.5, 1.0, 2.0, and 4.0 ug/2.5 ul) which were applied to the same plate. Since the salts in the sample solution have an effect on the spot size and R_f value, the standard solutions are made with a concentration of salts to match the ressolved samples closely (Leytem, 1984). For this reason, the standards were made with a salt concentration equal to ten times that in the original release medium. Four standards and six samples were applied onto a plate. The spots on the TLC plate were dried thoroughly before development. A developing solution of 85 v/o methanol and 15 v/o type-one water was used (prior to development, the developing chamber was equilibrated by lining it with Whatman 3 mm filter paper[13] on the side facing the plate

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[10] Whatman Chemical Separation Inc., Clifton, N.J.

[11] Whatman Chemical Separation Inc., Type CDC-12, Clifton, N.J.

[12] Drummond Scientific Co., Broomall, Pa.

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

surface). Each plate was developed a distance of 8 cm above the spotting point. A developed plate was removed and dried at room temperature. Spots were visualized with an ultraviolet light source[14] to give preliminary relative intensity information. If any samples spots were darker than all of the standard spots, additional subsamples were diluted, spotted onto another plate, and visualized in ultraviolet light. Direct visualization of the spots on the developed plates was accomplished by spraying 10 v/o of reagent grade sulfuric acid in methanol onto the developed area of the plate using a sprayer[15]. The spray flow rate was 15 ml/min for approximately 15 seconds. The sprayed plate was placed in an oven at 100°C to 105°C for 5 minutes, and was then allowed to cool to the room temperature. Some fading of the dry spots occurred. After the initial fading, the spot intensity stayed constant for several hours (Leytem, 1984). A quantitative analysis on the plate was carried out within two hours. Tylosin tartrate on a plate was evaluated by using a Kontes fiber optic scanner[16]. This densitometer was used to measure the intensity of spots by a method of cross-scanning the TLC plate (perpendicular to the direction of development). The densitometer scan rate was 2 cm/in. The output was recorded on a chart recorder[17] to provide peak tracings to be used for subsequent calculation

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[14] UVP, Inc., Model UVGL-25 Minerallight, San Gabriel, Cal.

[15] Kontes, Model K-422550, Vineland, N.J.

[16] Kontes scientific Instrument Group, Model 800, Vineland, N.J.

[17] Linear Instruments Group Corp., Model 255/MM, Irvine, Cal.

[18] Mettler Instrument Corp., Model H31AR, Princeton, N.J.

of peak weights. The peaks were cutout and weighed[18]. The amount of tylosin tartrate for a sample was calculated by comparing the weight of a sample peak to that of a series of standards. Linear interpolation was used. The amount of tylosin tartrate released from the ring device was then calculated on the base of the amount of drug found in the TLC spot for the 2.5 ul volume of sample. The initial dilution of samples prior to spotting was taken into account. The dilution limitation of our sample was 50 ul of water diluting for a 1 hour sample of 2 ml.

Preliminary In Vivo Testing

A preliminary test of retention time of a ring device, level of irritation and effect on tear flow rate was made. The detectability of drug in the collected tear sample was measured using the TLC method.

In addition, several ring devices for in vivo test were checked for their flexibility and joint strength under dry and wet conditions. A ring device was pushed down in two directions; one direction was parallel to the joint part of a ring device and another was vertical. In the case of the wet condition, a ring device was first immersed in a saline solution for 1-2 hours.

RESULTS

Physical Characteristics of Controlled Release System

In the copolymerization of 25:75 HEMA/MMA, it was difficult to confirm whether or not the reaction was completed, since there was no obvious physical change as seen for the 90:10 copolymer. However, after ten days, the addition of water to the viscous mixture resulted in forming a precipitate of a white-sticky copolymer. The dried 25:75 copolymer was a transparent, glassy material. The use of fresh initiators produced a good yield (85-90%). In the dipcoating work, multiple coats were required to deposit the desired amounts of drug and copolymer on the devices. The number of coats for a ring device depended on the composition of hydrogel, the amount of drug loading and the type of tubing. The average number of coats was summarized in Table 5.

TABLE 5. The average number of coats for the finished ring device

Polymer / Drug loading / Mandrel	90:10 Silastic	90:10 Teflon[a]	90:10 PVC	25:75 Silastic	25:75 Teflon	25:75 PVC
50 mg	13-15	12-13	9-10	15-18	-	10-12
100 mg	13-15	12-13	-	-	-	-

[a]Tubing was presoaked in soap solution.

Small differences in the amount of drug deposited on the ring devices were due to the physical characteristics of the tubings and the solubility of drug in the copolymers; the deviation of the average drug amount in the 100 mg loading had a greater value than that of 50 mg drug

loading because the 100 mg loading of drug compared with a 50 mg amount was less soluble in the same amount of copolymer.

PVC was the best tube variety of polymer of the three for dip-coating, and the PVC had a smooth copolymer surface after coating. The advantages of using PVC tubing were flexibility, ease of fabrication as a ring, good retention characteristics when used in vivo, and low cost. The coating procedure produced a 10-15% decrease in diameter for a PVC ring. Table 6 lists the diameter reductions seen for the various types of ring devices.

TABLE 6. Shrinkage of the diameter of ring device during fabrication

Device No.	Diameter (mm) original	Diameter (mm) after dip-coat	Type of adhesive[a]	Material joint	Material mandrel
011-054	40	40	none	copper wire	Silastic or Teflon
061	40	35	THF	PVC	PVC
062	40	34.5	90:10 polymer	"	"
063	40	35.5	"	"	"
064	40	35	"	"	"
065	40	37	"	"	"
071	40	35	"	"	"
072	40	36	"	"	"
073	40	36.5	"	"	"
074	40	36.5	"	"	"
075	40	36	"	"	"

[a]THF = tetrahydrofuran

Table 7 contains descriptions of the physical appearance of the ring devices. The devices are divided into five groups (according to the characteristics of each ring device).

TABLE 7. Physical appearance of ring devices

Group	Characteristics of ring devices	Device No.	Comments
I	90:10 copolymer 50 mg loading copper wire joint	011,021 022,031 041	All appeared uniform and transparent. No defect found under stereomicroscopic examination.
II	90:10 copolymer 100 mg loading copper wire joint	032,042 044,051 052	A slightly rough surface and yellowish. Device 041 had visible air bubbles and a crack in side.
III	25:75 copolymer 50 mg loading copper wire joint	033,043 053,054	A slightly rough surface,and a gray color. Had a crack of the side of ring.
IV	90:10 copolymer 50 mg loading PVC joint	061-065	Smooth surface and good connection. Transparent, no defects.
V	25:75 copolymer 50 mg loading PVC joint	071-075	The same as for group IV above.

Color differences were observed for devices prepared under conditions of different drug loadings and environmental conditions. The 90:10 copolymer (50 mg loading) devices were transparent while the 90:10 copolymer (100 mg loading) devices were straw yellow. The 25:75 copolymer (50 mg loading) devices were gray. Devices prepared in air, rather than under nitrogen, were white and had a more open microstructure on the micron size level; to insure uniformity, fabrication under a nitrogen environment was preferred. The 100 mg drug loading devices had rougher surfaces and were more brittle than the 50 mg loading examples. The composition of a copolymer also affected the surface condition of a device and the ease of coating. The 25:75 copolymer devices were more

difficult to dip-coat and rougher on the surface compared with the 90:10 copolymer devices.

The 90:10 copolymer mixture was a better adhesive for joining segments of a ring than any other adhesives such as Silicone rubber or tetrahydrofuran. A PVC/THF solution was also satisfactory.

Drug Release Characteristics of Ring Device

After the release experiment, all of the samples were dried, redissolved with water, and spotted on the TLC plates to evaluate the release rate for each sampling period. The TLC analysis data for a release experiment performed on a monolithic device include the collection time, the amount of drug in the spot, the average release rate over the time period, and the cumulative drug release.

Figures 11 - 34 show the drug release characteristic curves for devices 011-075. All of the ring devices had similar release profiles; the initial release is high; then the rate decreases (as an exponential function) until it reaches a nearly constant level throughout the rest of the test period. Table 8 summarizes the drug release characteristics of all of ring devices.

Table 9 summarizes release characteristics by device type. The devices are divided into five groups (Table 9) according to the drug loading amount, the geometry of device, and the type of the copolymer used for the ring device.

As shown in these results, all of ring devices released measurable amounts of drug for nine days at levels of at least 3 ug/hr of drug. The initial release rate (through the first 2 days) was high, and then the

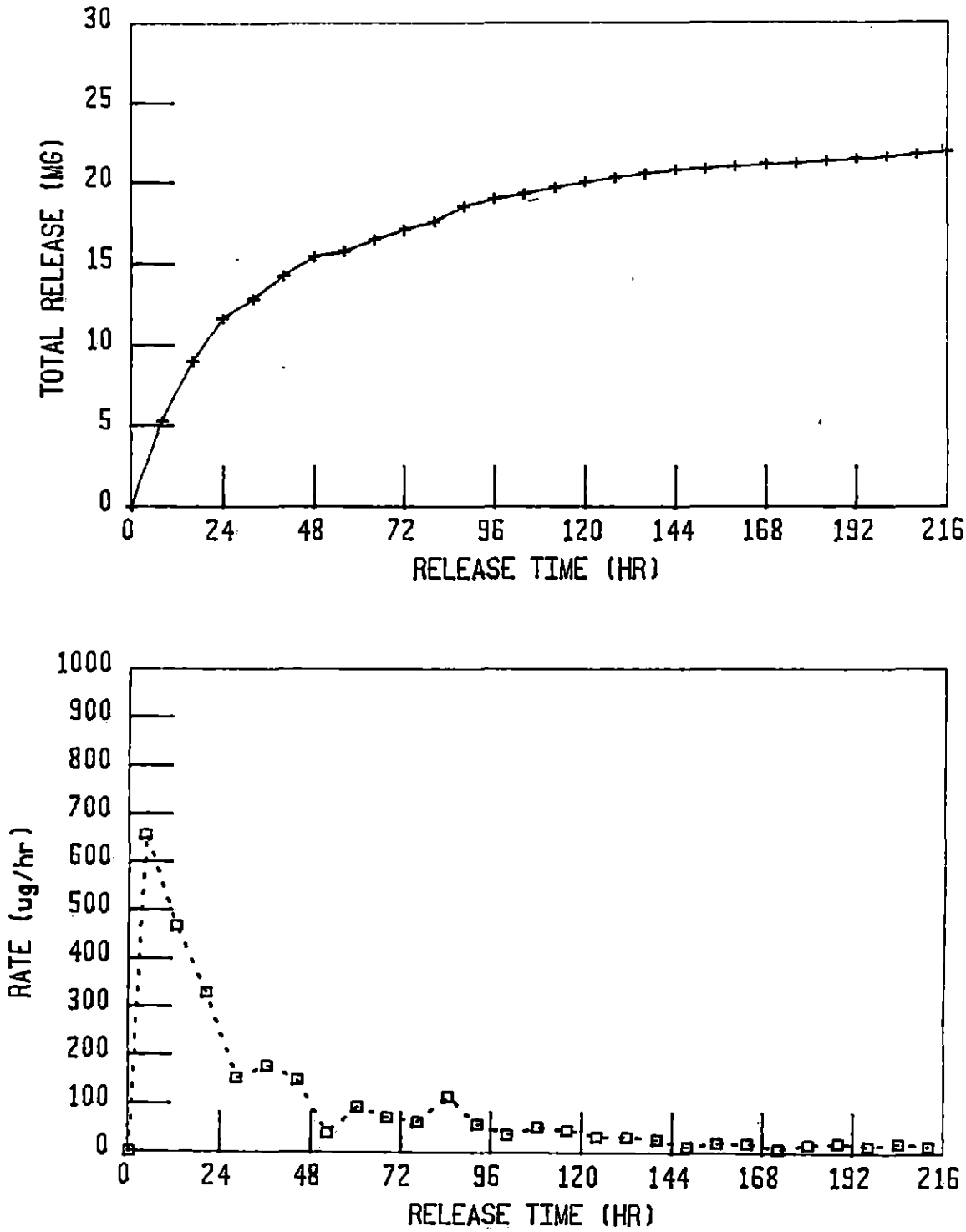

FIGURE 11. Drug release characteristics for ring device 011. Top: total release vs. time; bottom: rate vs. time

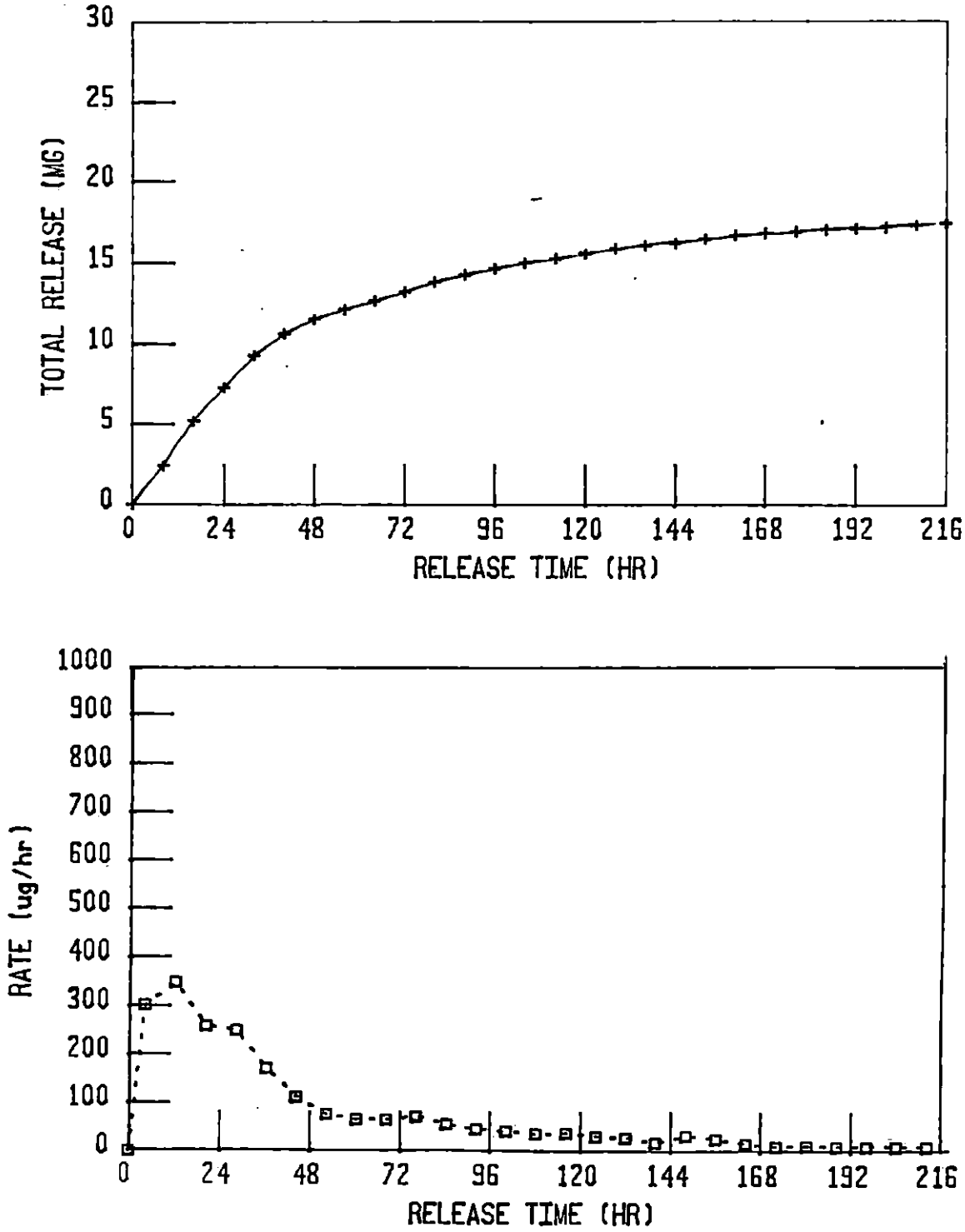

FIGURE 12. Drug release characteristics for ring device 021. Top: total release vs. time; bottom: rate vs. time

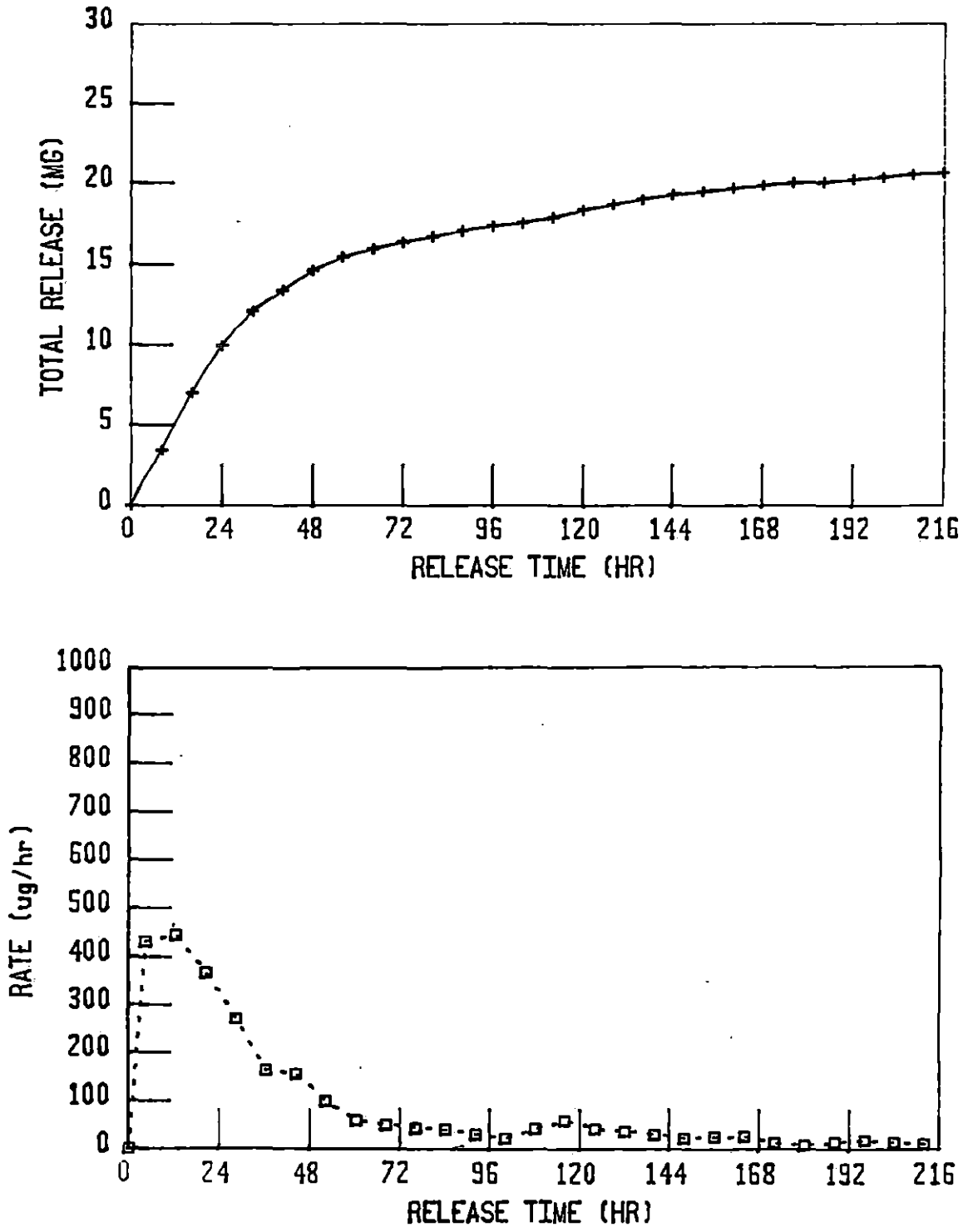

FIGURE 13. Drug release characteristics for ring device 022. Top: total release vs. time; bottom: rate vs. time

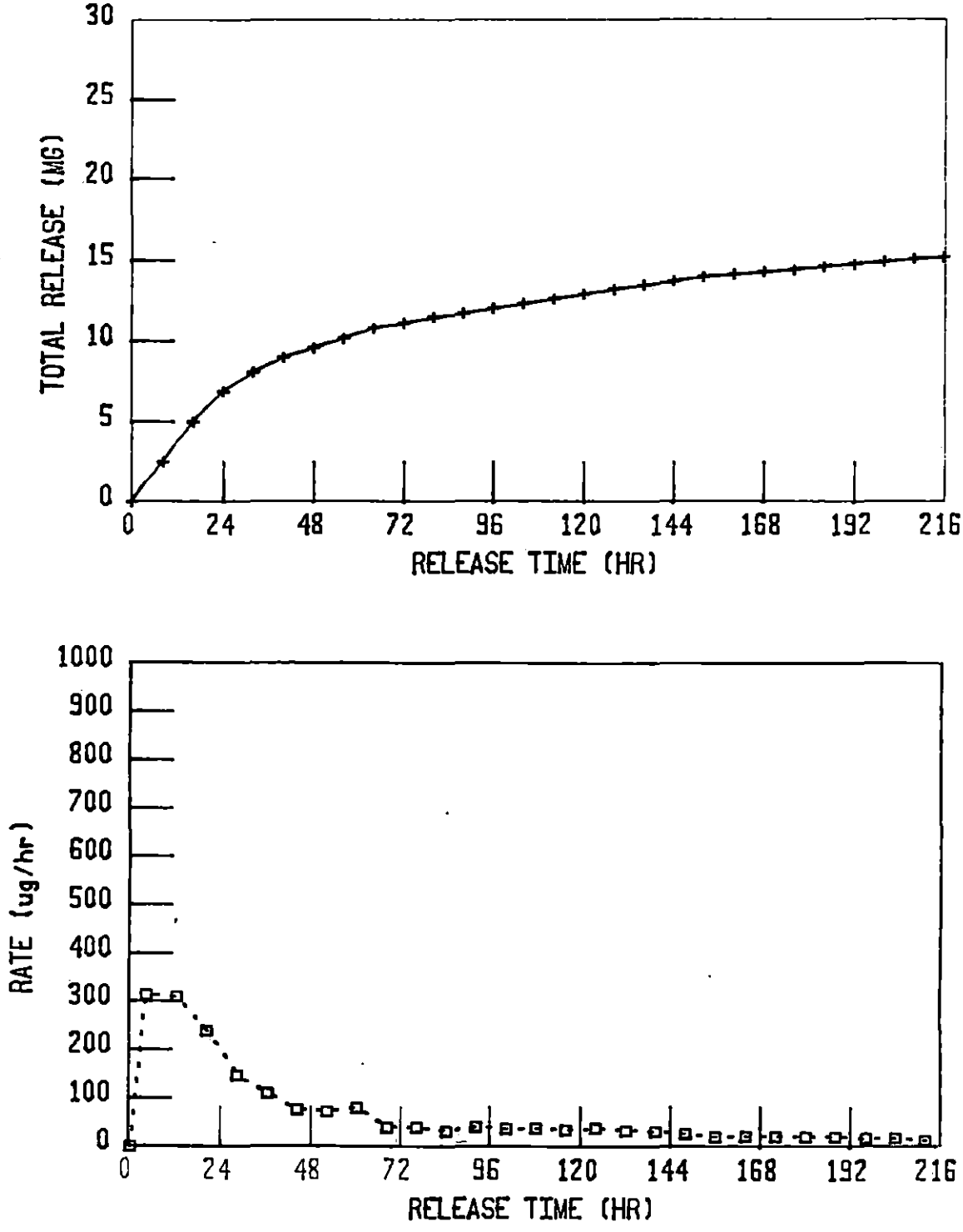

FIGURE 14. Drug release characteristics for ring device 031. Top: total release vs. time; bottom: rate vs. time

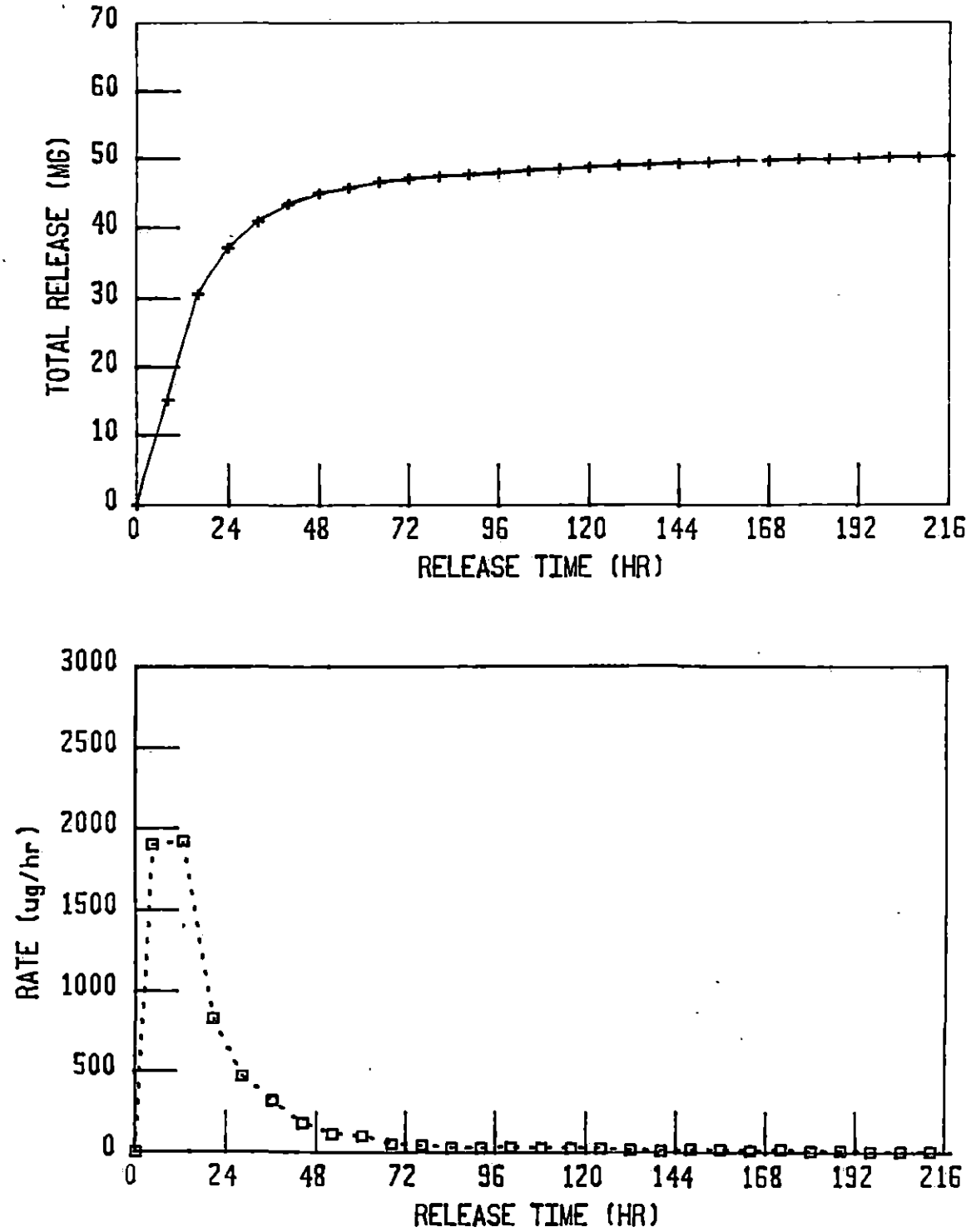

FIGURE 15. Drug release characteristics for ring device 032. Top: total release vs. time; bottom: rate vs. time

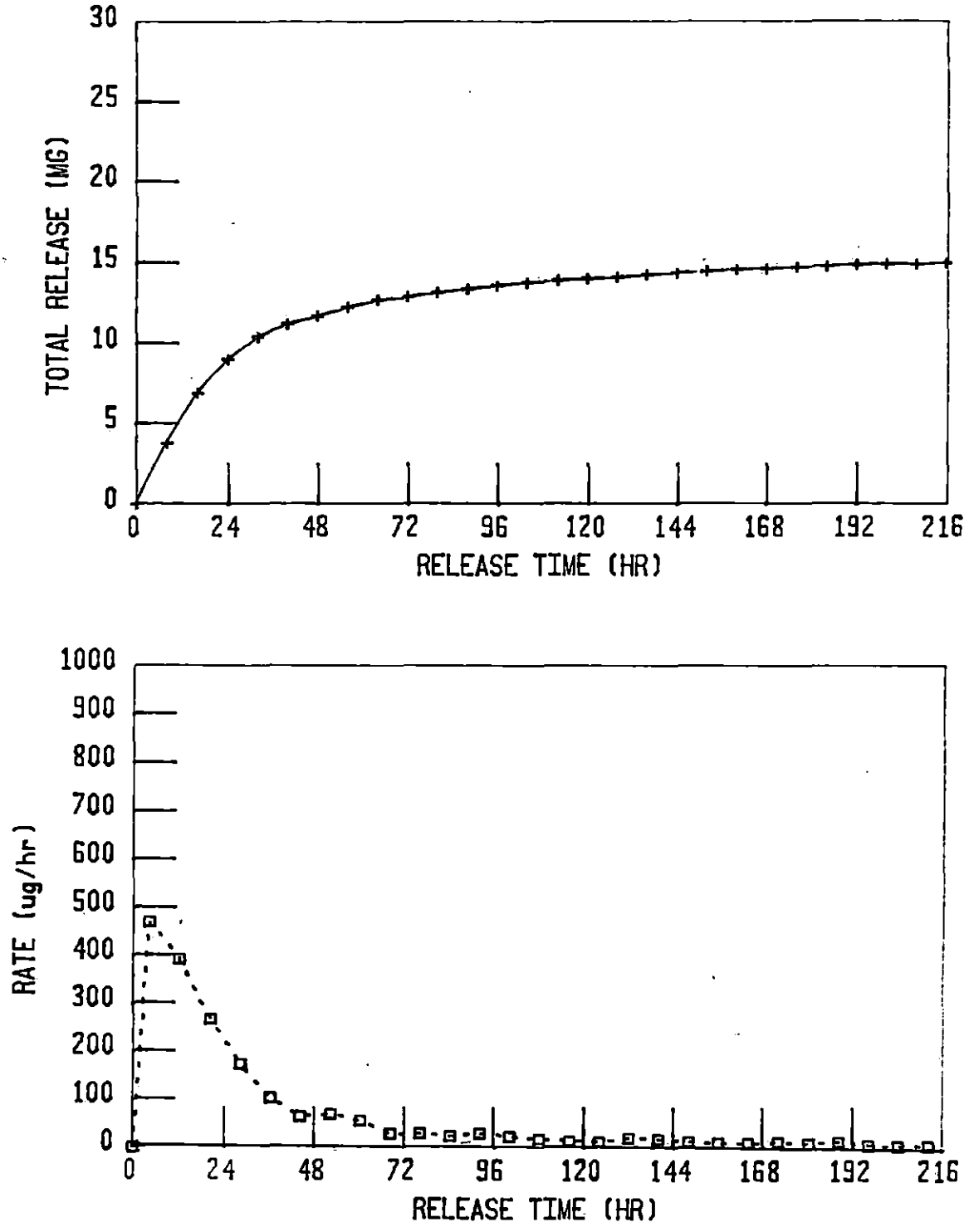

FIGURE 16. Drug release characteristics for ring device 033. Top: total release vs. time; bottom: rate vs. time

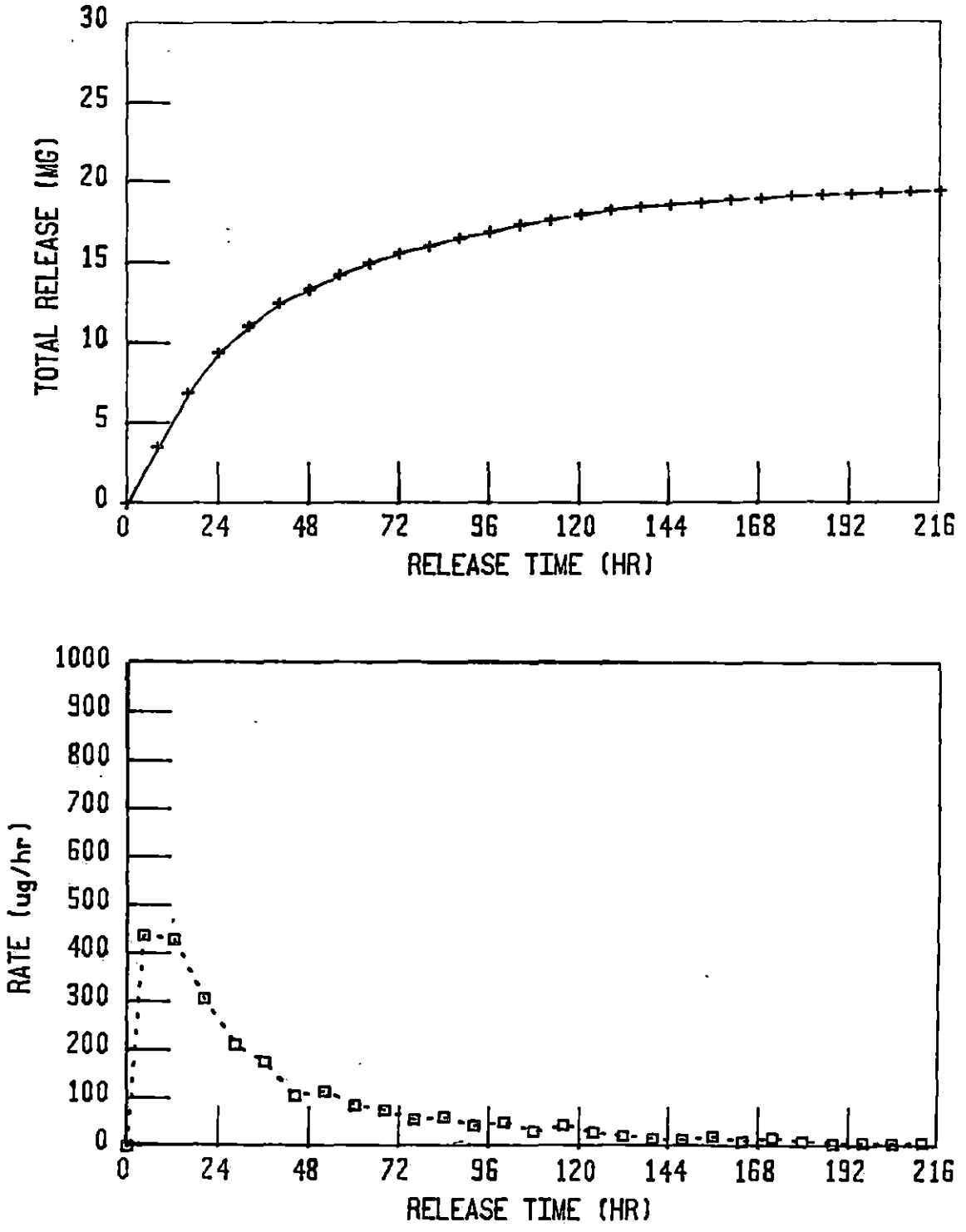

FIGURE 17. Drug release characteristics for ring device 041. Top: total release vs. time; bottom: rate vs. time

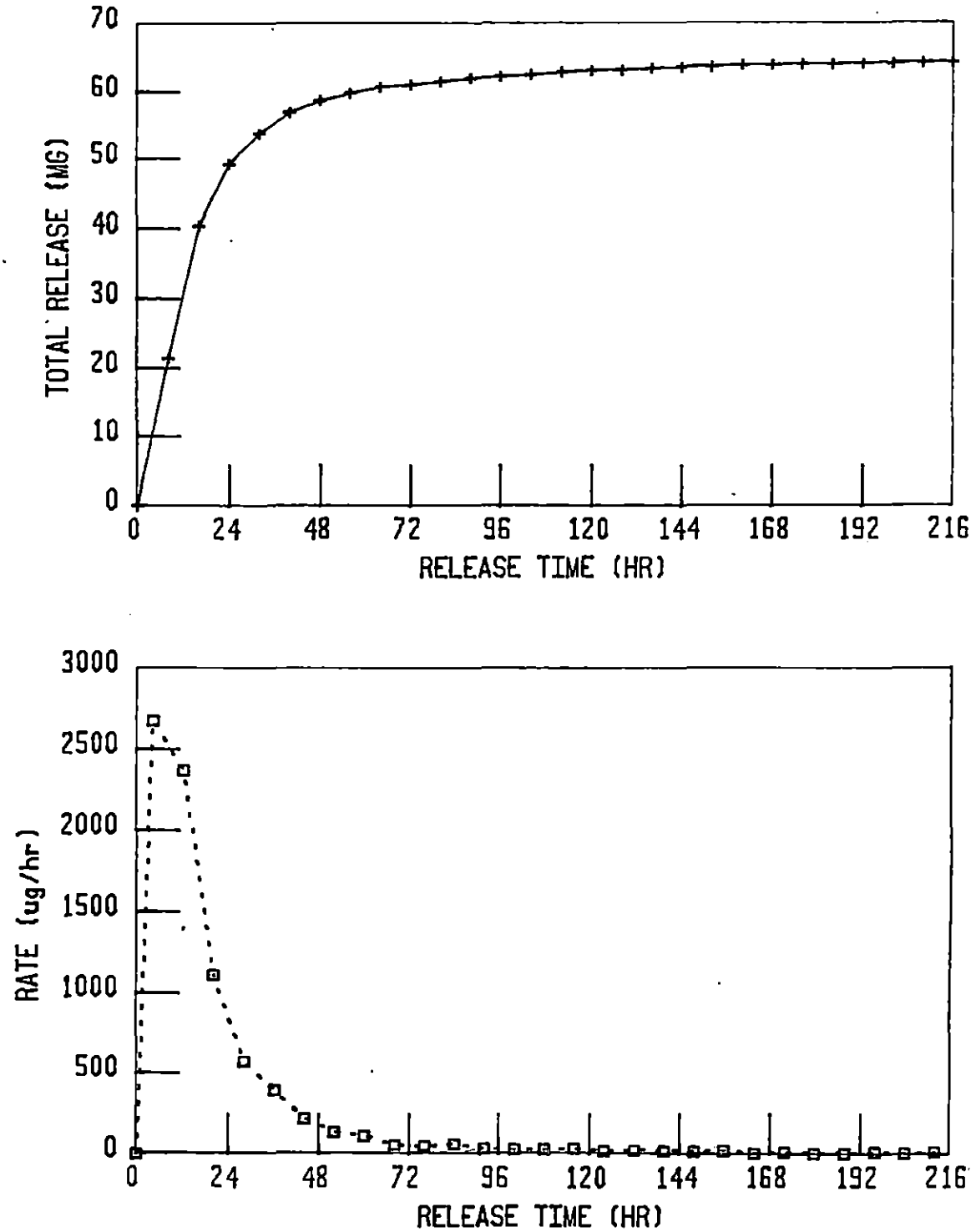

FIGURE 18. Drug release characteristics for ring device 042. Top: total release vs. time; bottom: rate vs. time

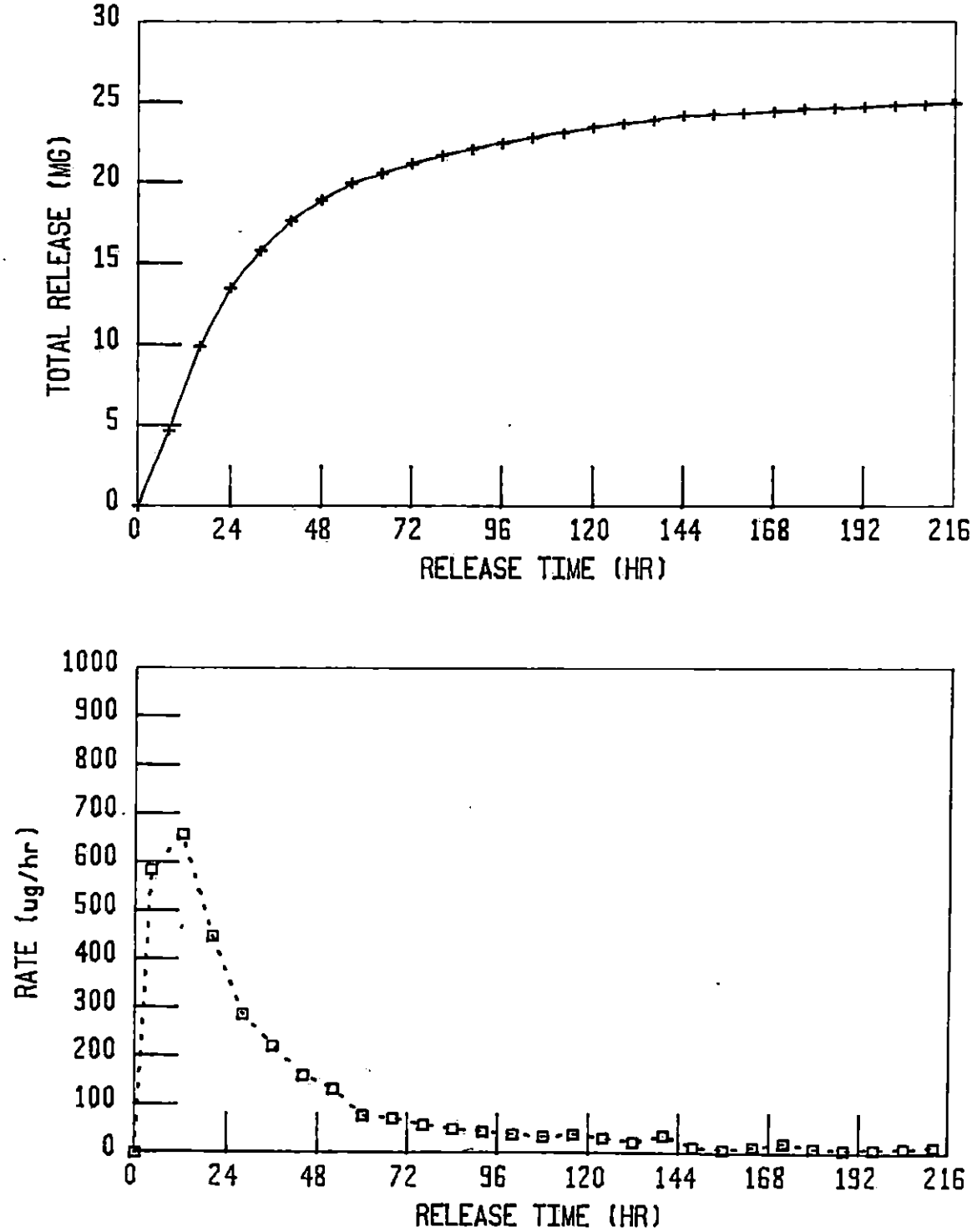

FIGURE 19. Drug release characteristics for ring device 043. Top: total release vs. time; bottom: rate vs. time

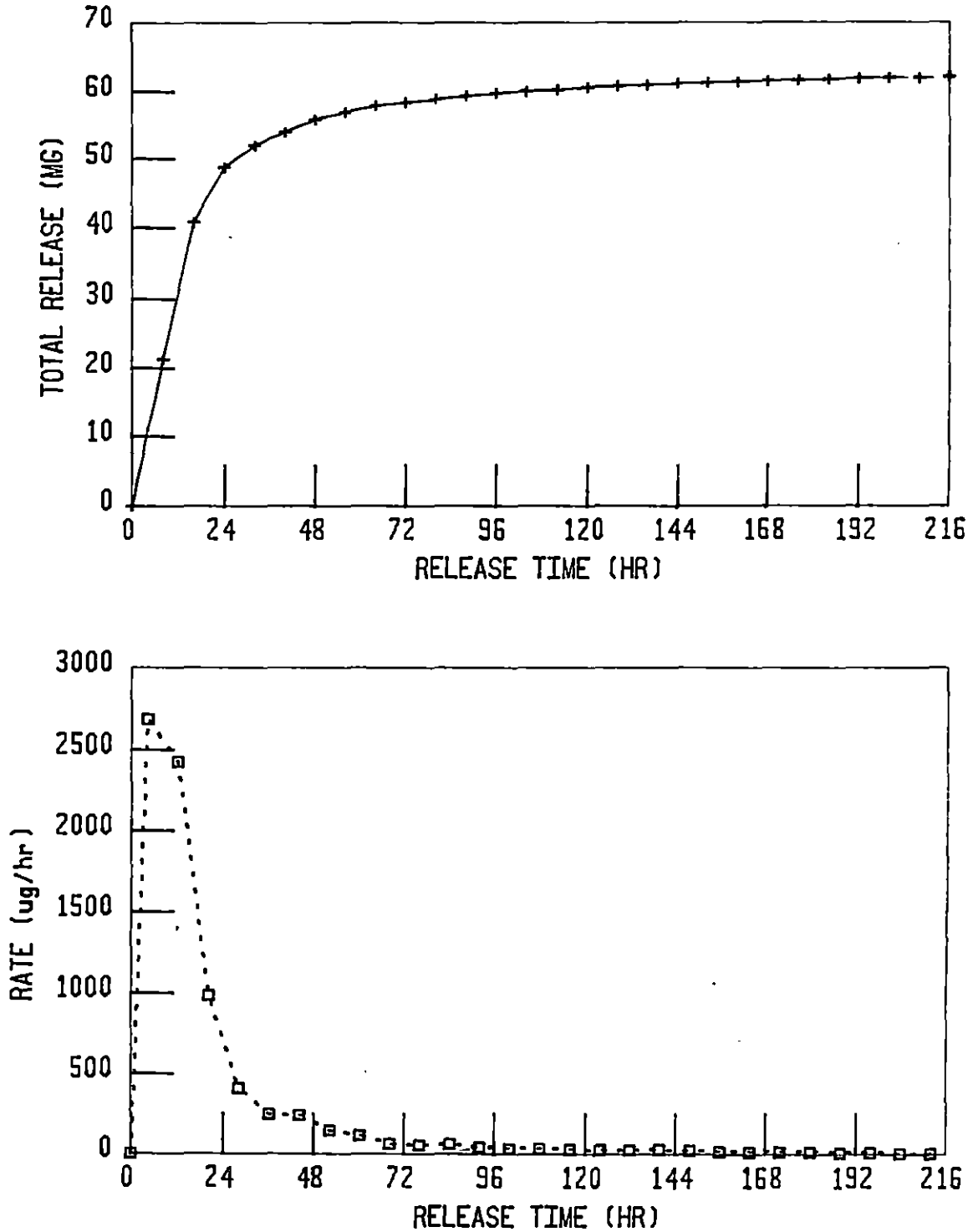

FIGURE 20. Drug release characteristics for ring device 044. Top: total release vs. time; bottom: rate vs. time

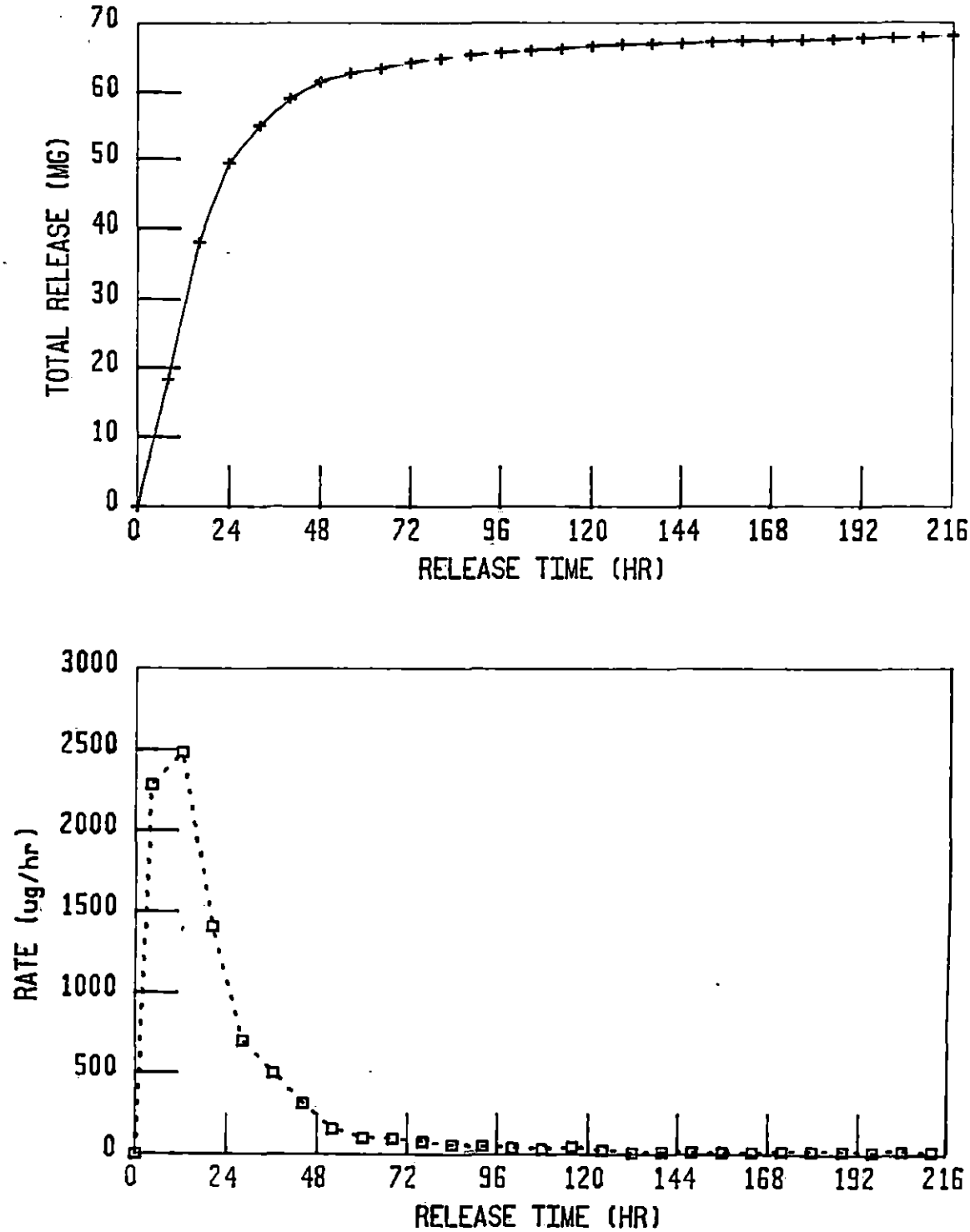

FIGURE 21. Drug release characteristics for ring device 051. Top: total release vs. time; bottom: rate vs. time

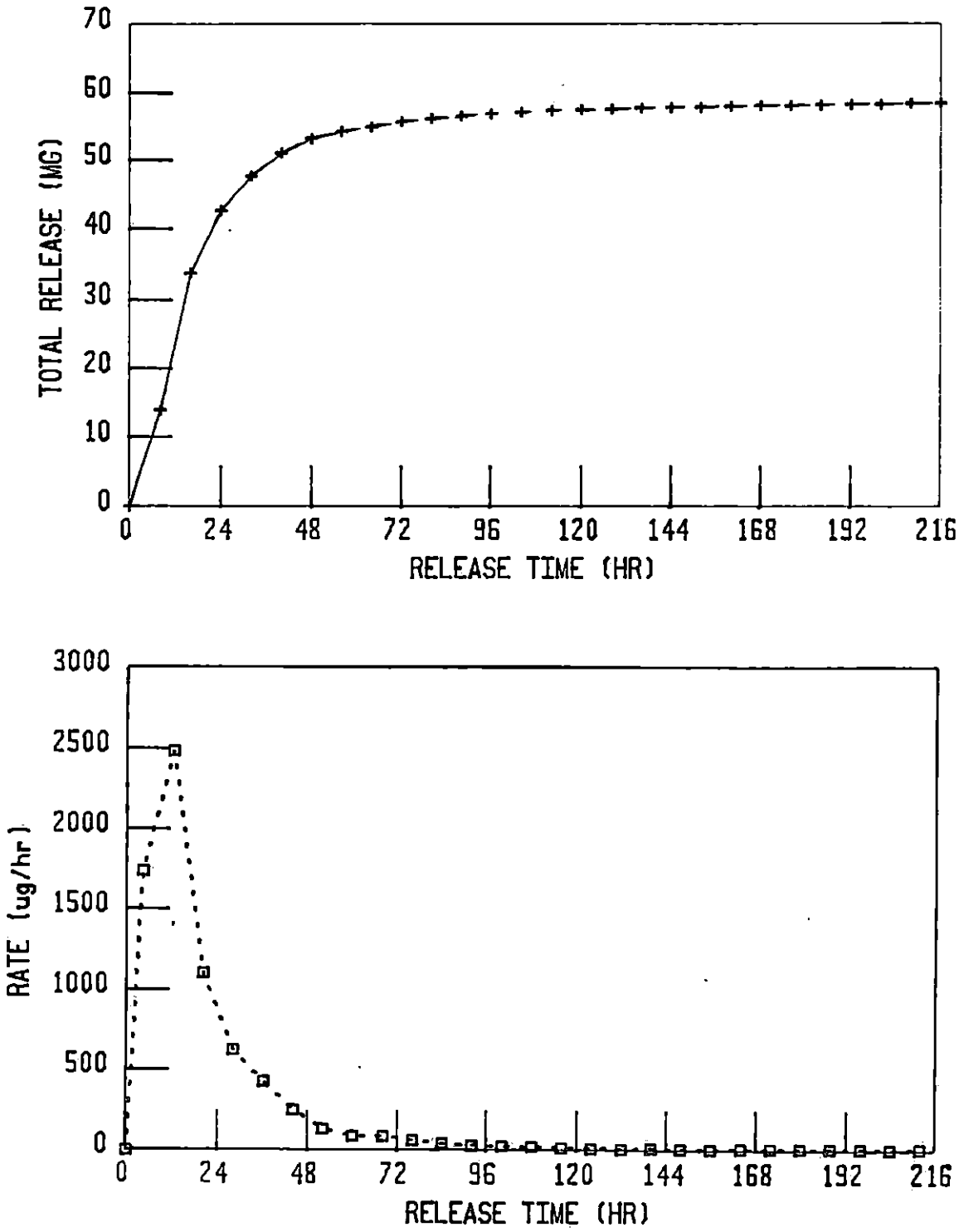

FIGURE 22. Drug release characteristics for ring device 052. Top: total release vs. time; bottom: rate vs. time

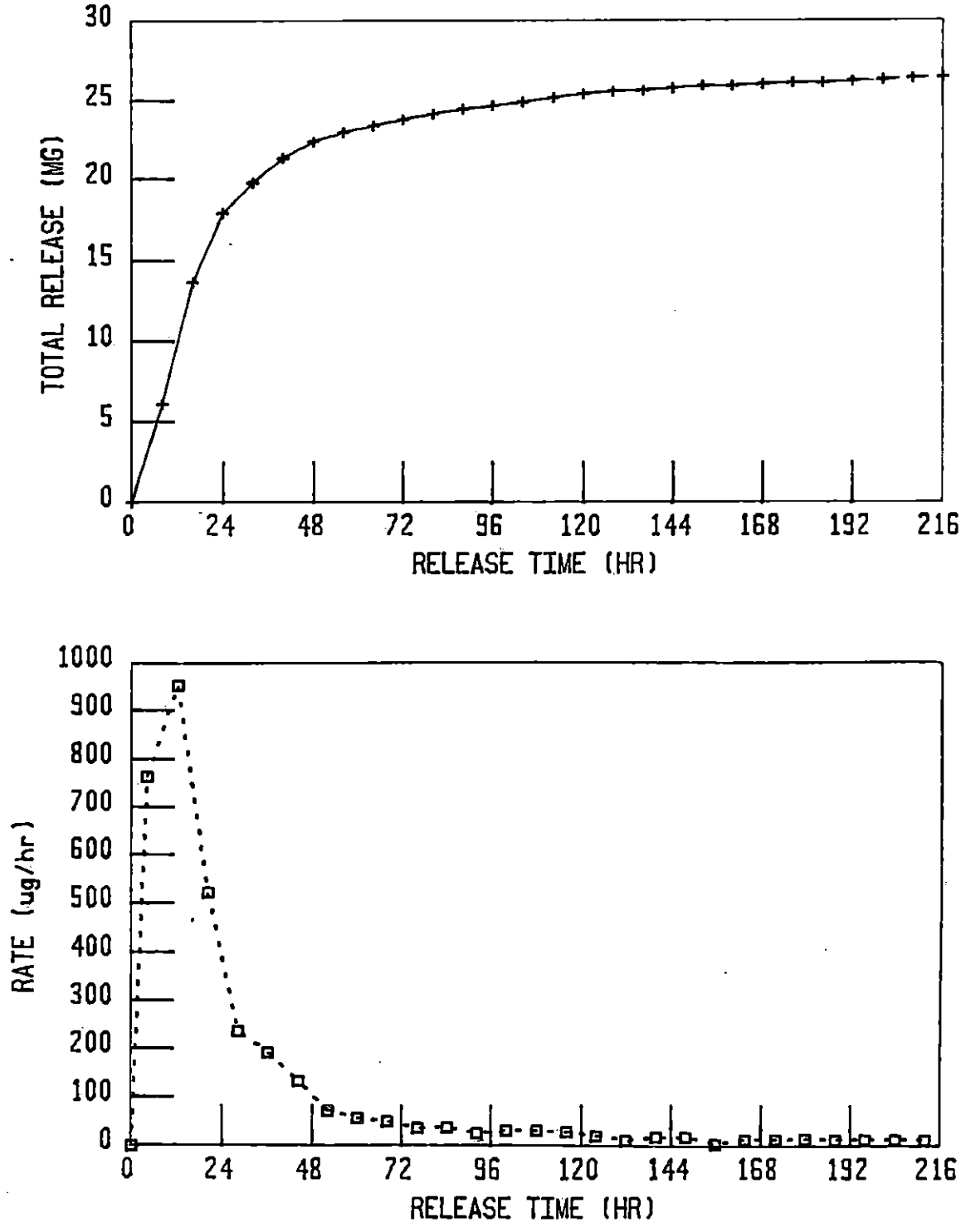

FIGURE 23. Drug release characteristics for ring device 053. Top: total release vs. time; bottom: rate vs. time

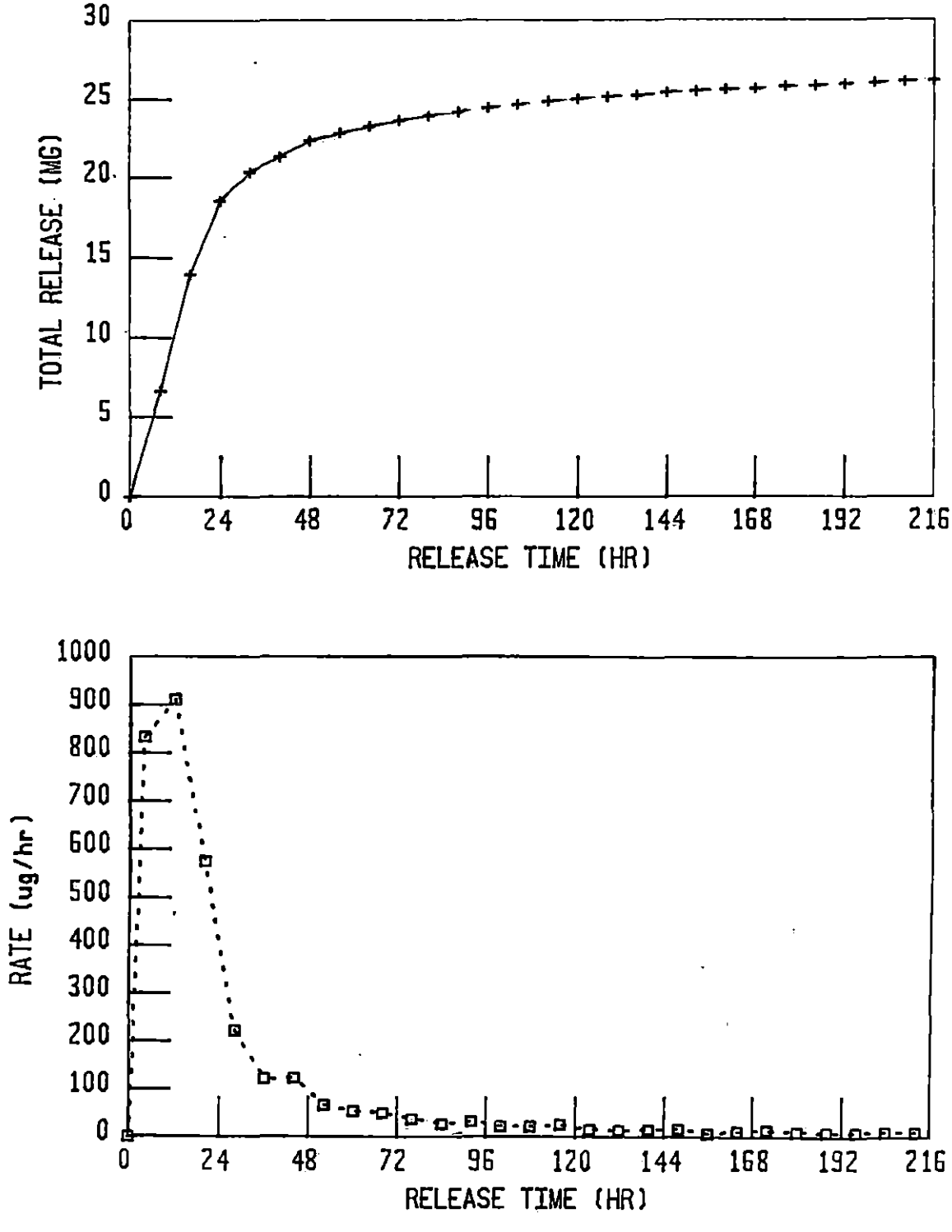

FIGURE 24. Drug release characteristics for ring device 054. Top: total release vs. time; bottom: rate vs. time

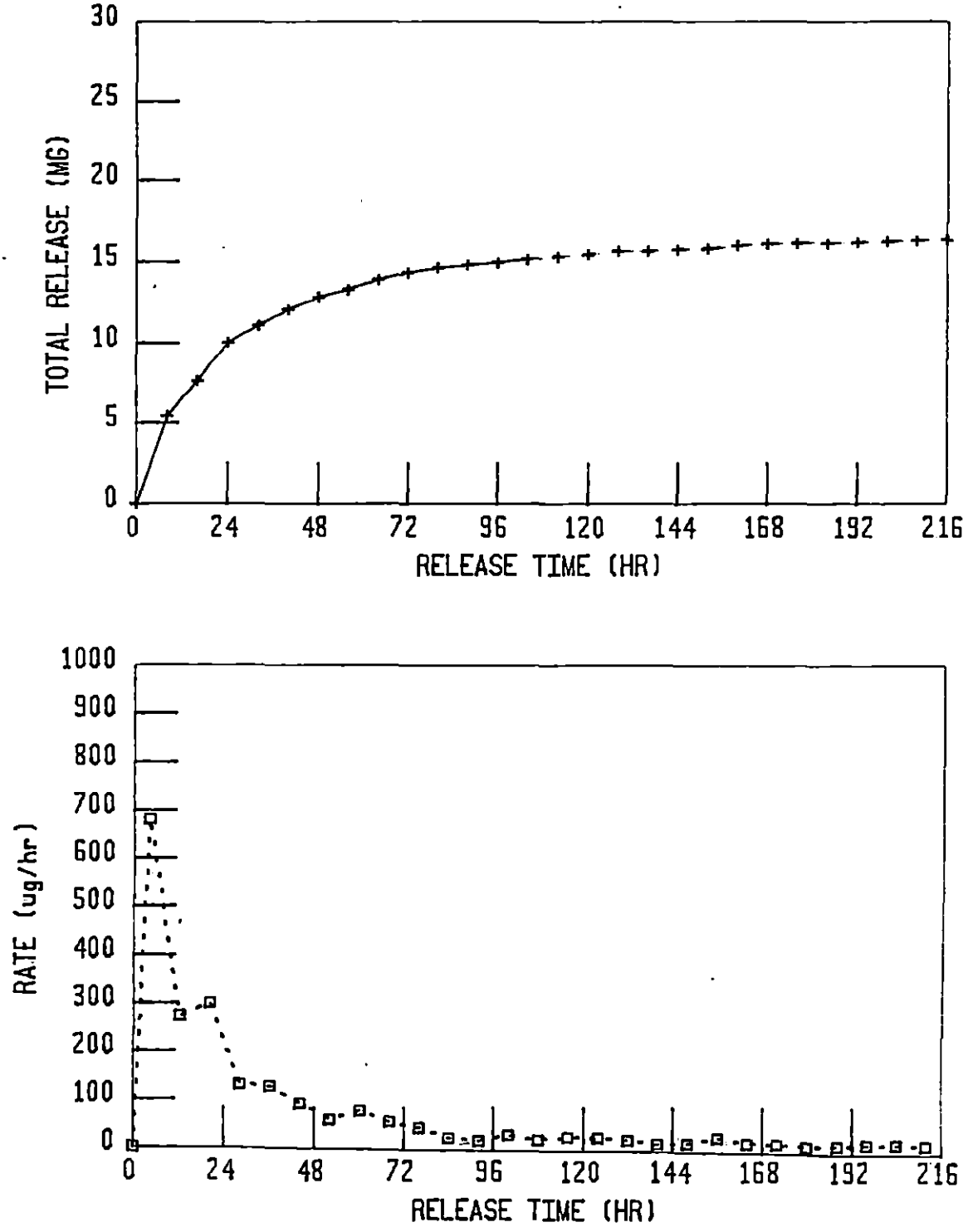

FIGURE 25. Drug release characteristics for ring device 061. Top: total release vs. time; bottom: rate vs. time

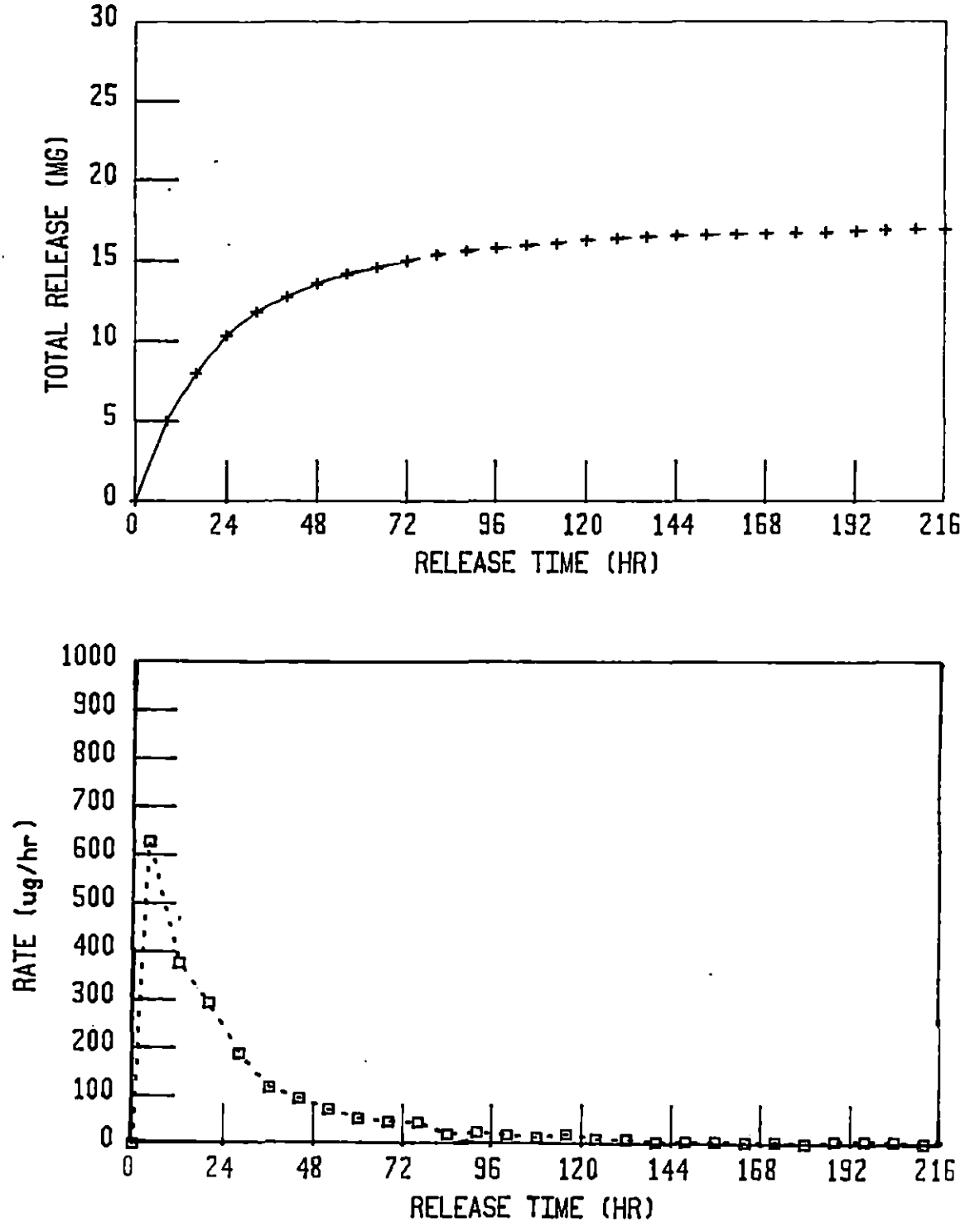

FIGURE 26. Drug release characteristics for ring device 062. Top: total release vs. time; bottom: rate vs. time

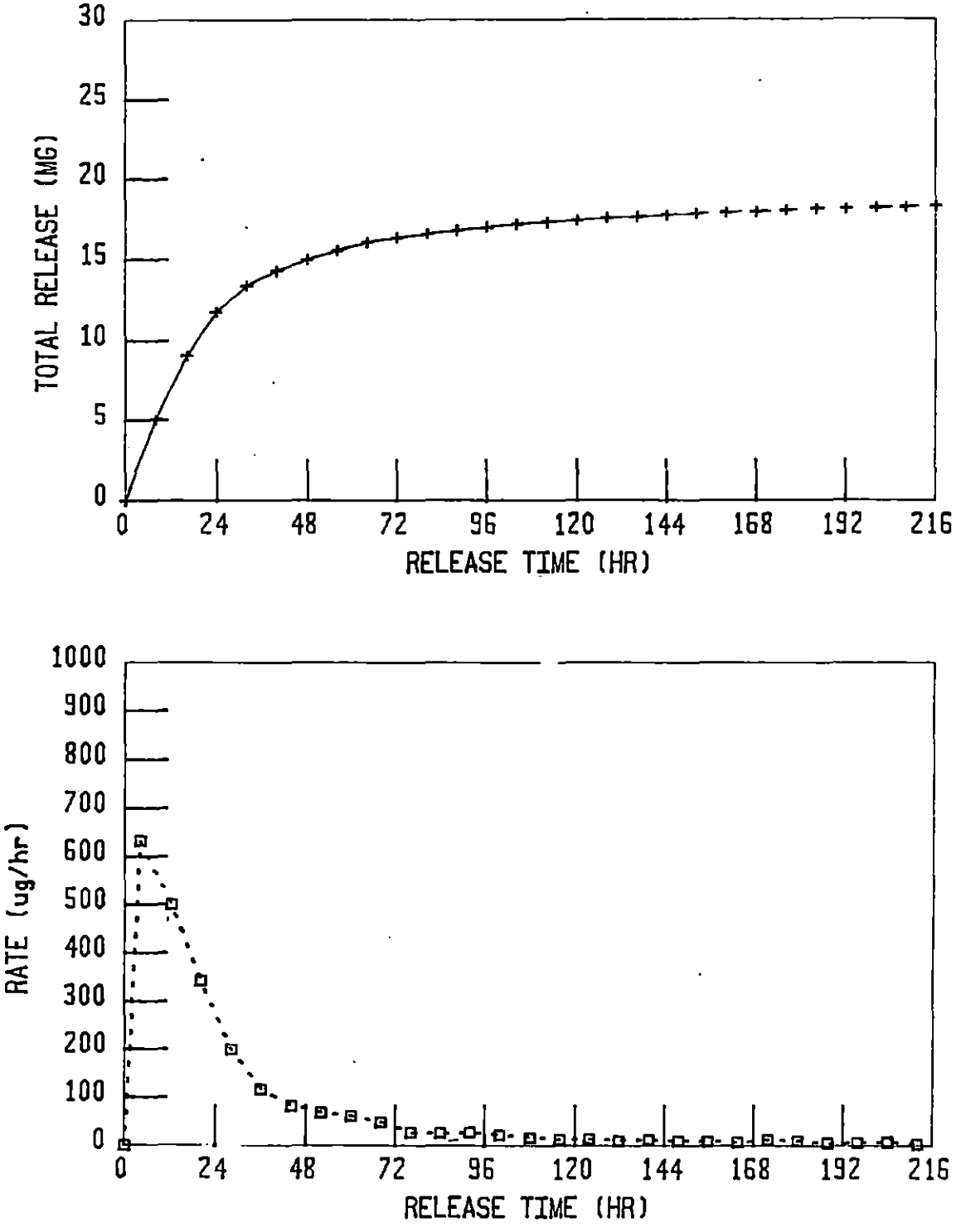

FIGURE 27. Drug release characteristics for ring device 063. Top: total release vs. time; bottom: rate vs. time

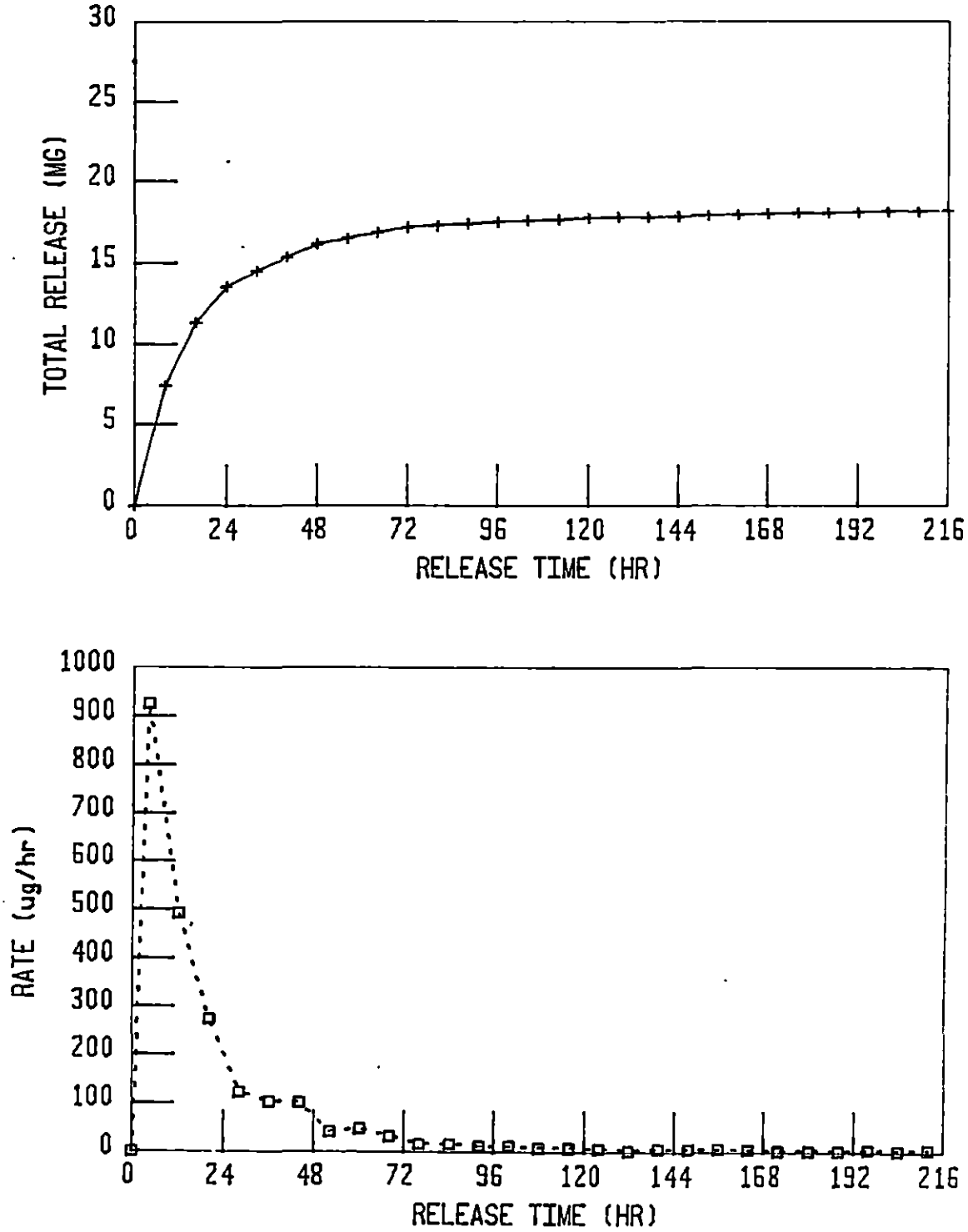

FIGURE 28. Drug release characteristics for ring device 064. Top: total release vs. time; bottom: rate vs. time

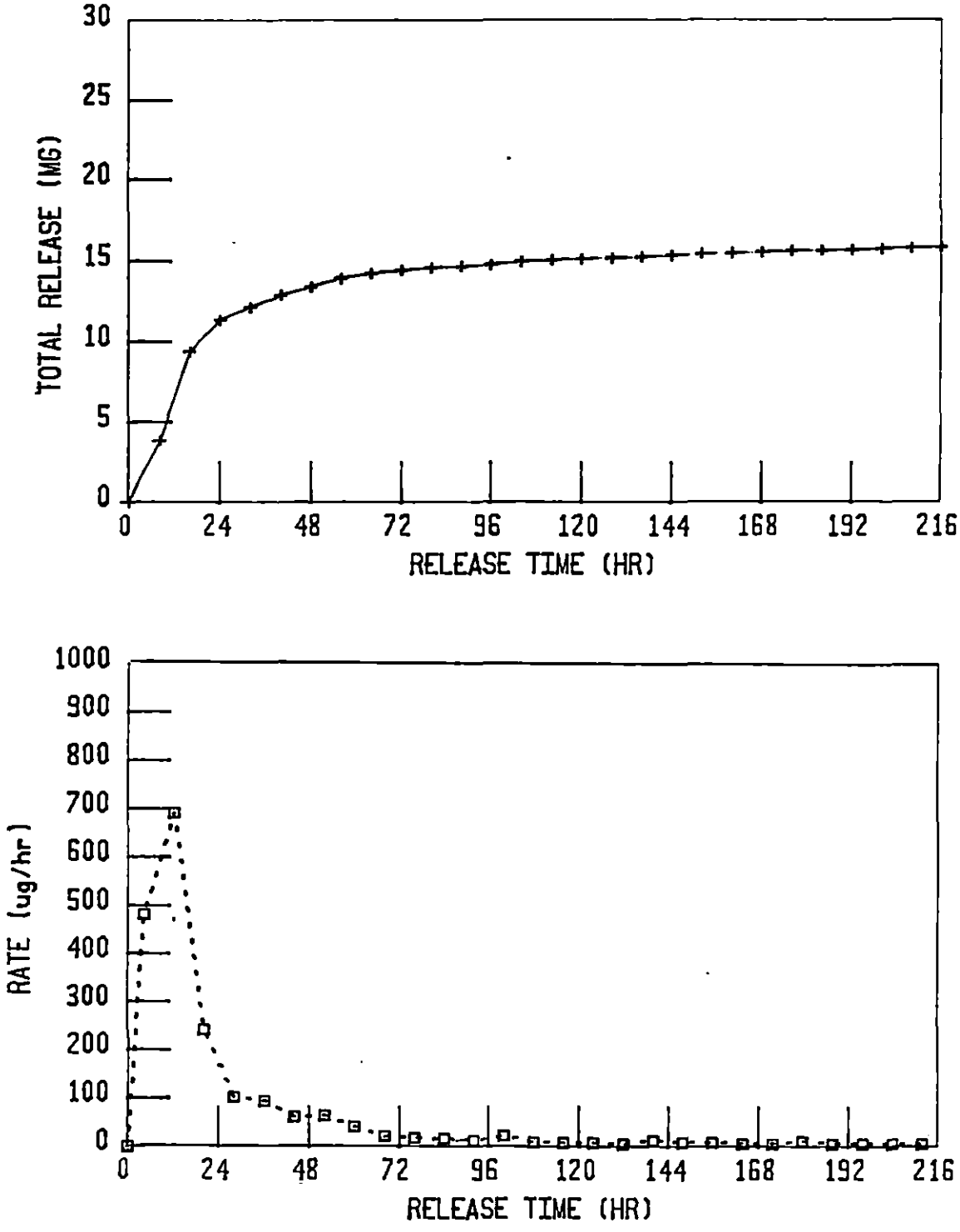

FIGURE 29. Drug release characteristics for ring device 065. Top: total release vs. time; bottom: rate vs. time

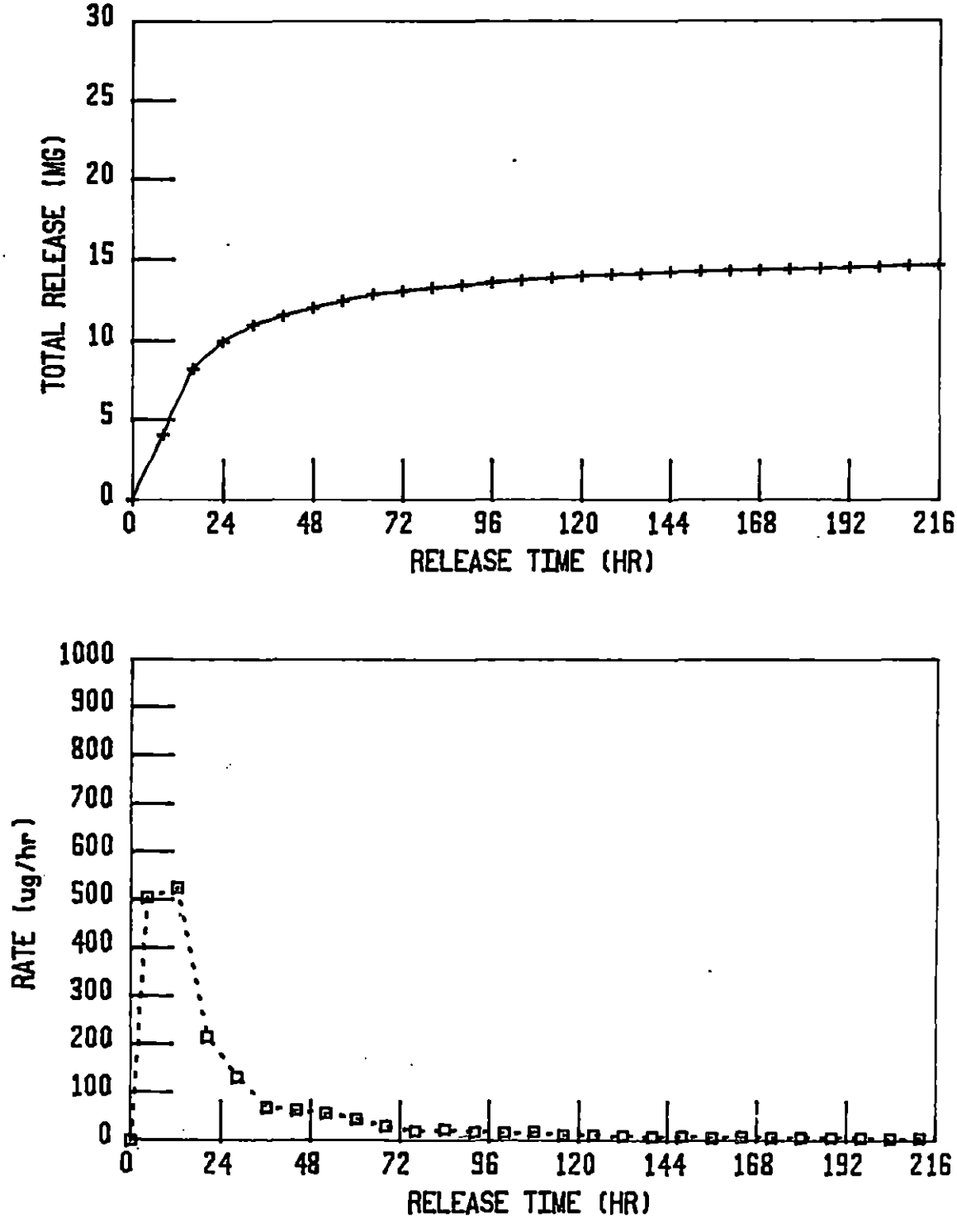

FIGURE 30. Drug release characteristics for ring device 071. Top: total release vs. time; bottom: rate vs. time

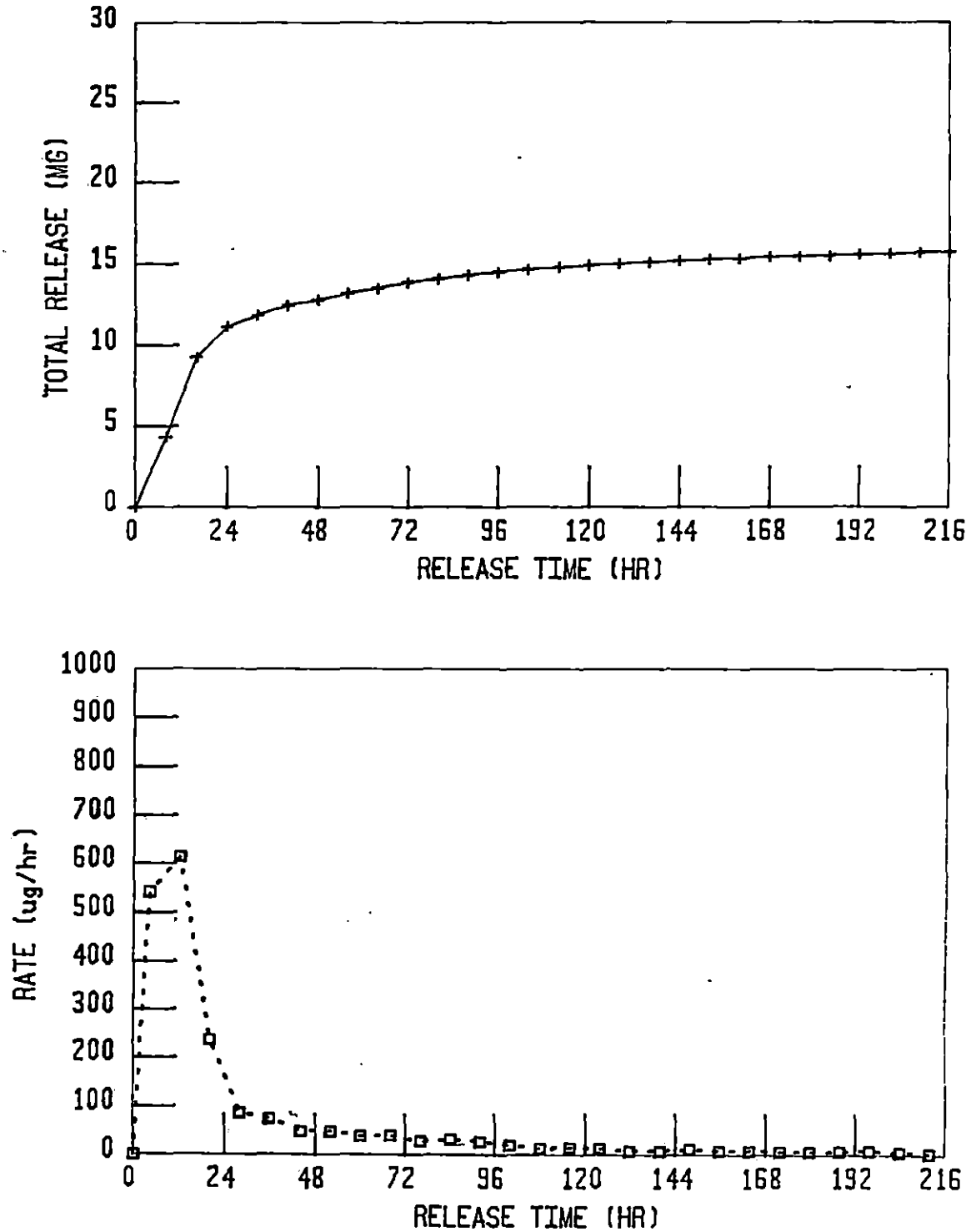

FIGURE 31. Drug release characteristics for ring device 072. Top: total release vs. time; bottom: rate vs. time

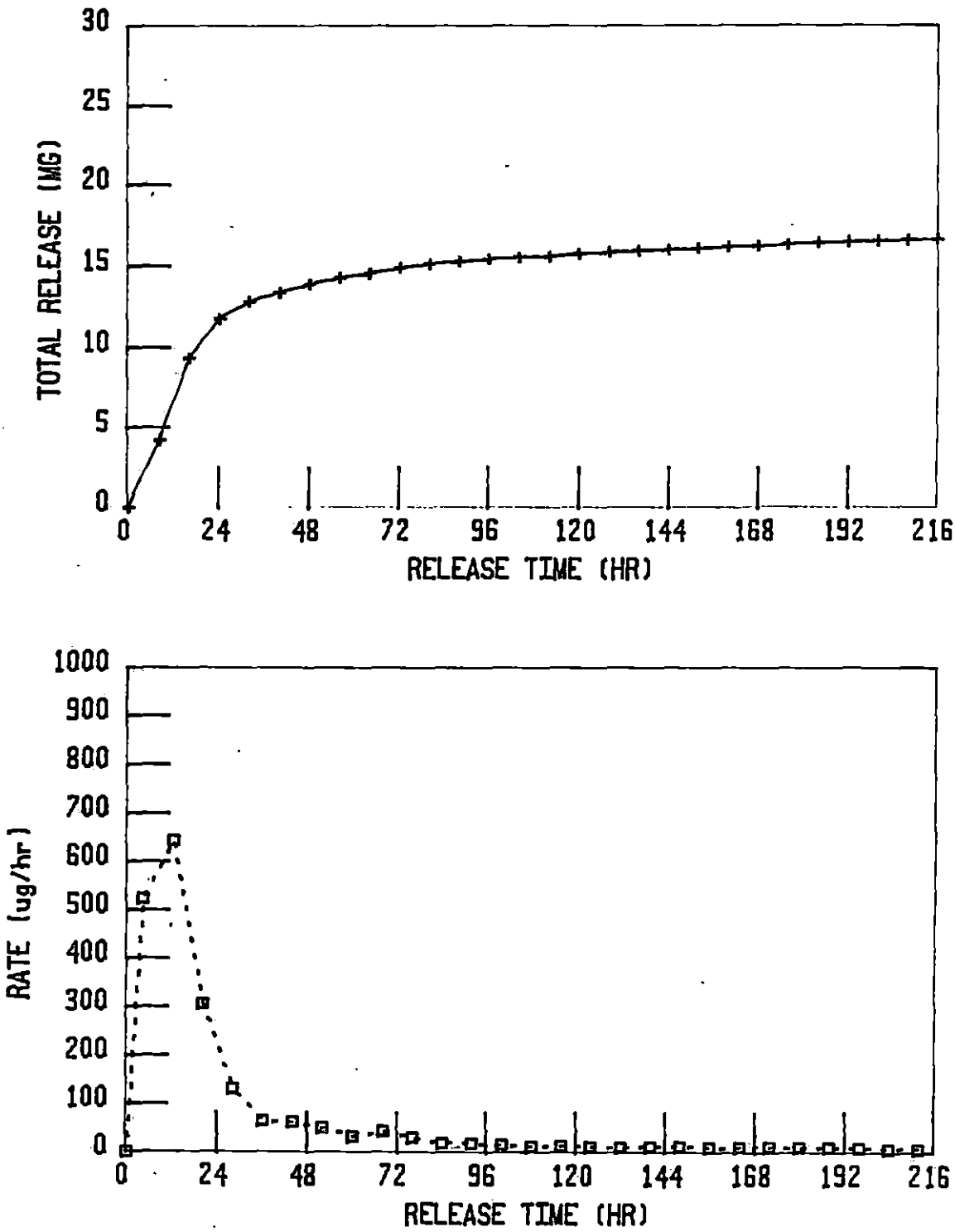

FIGURE 32. Drug release characteristics for ring device 073. Top: total release vs. time; bottom: rate vs. time

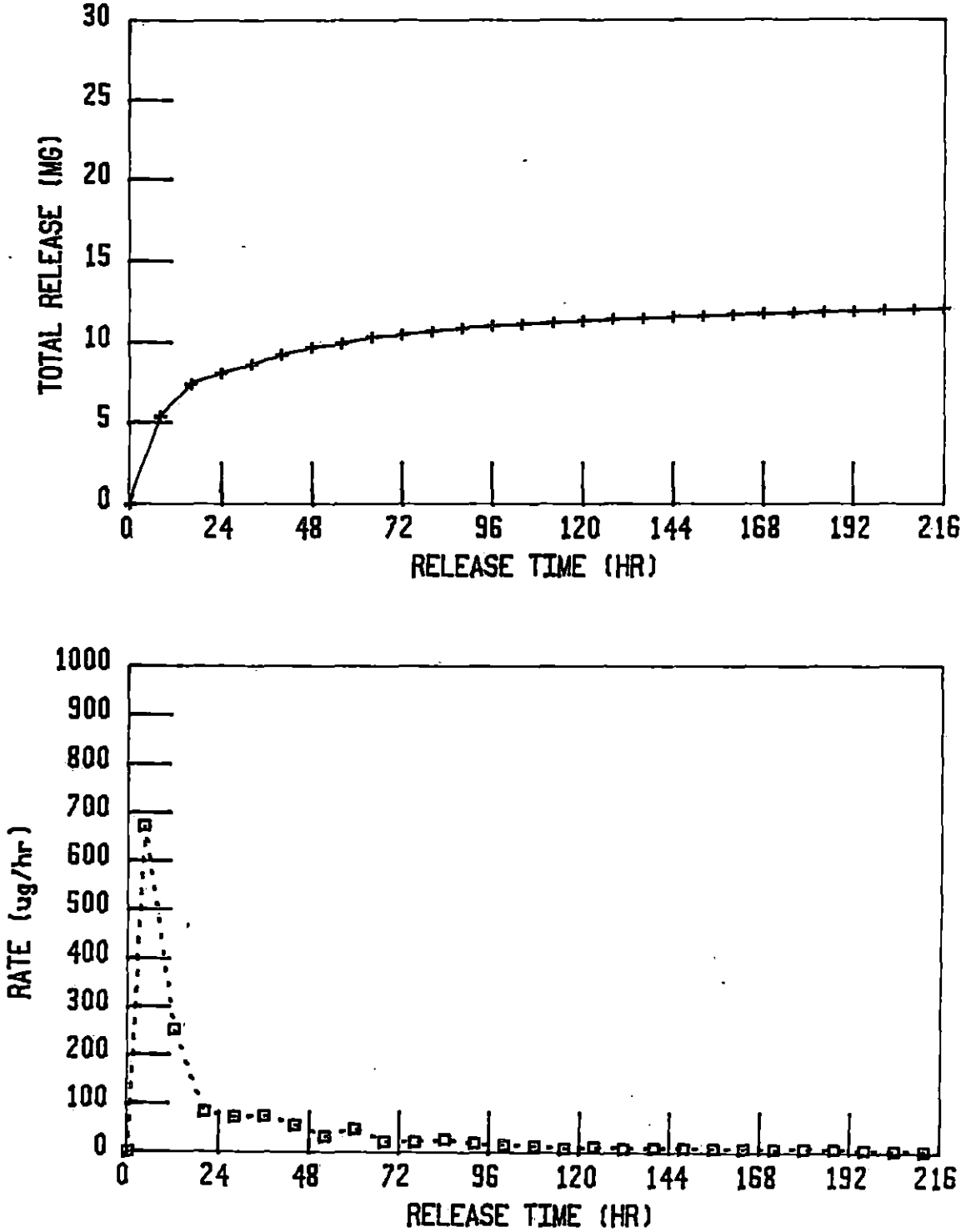

FIGURE 33. Drug release characteristics for ring device 074. Top: total release vs. time; bottom: rate vs. time

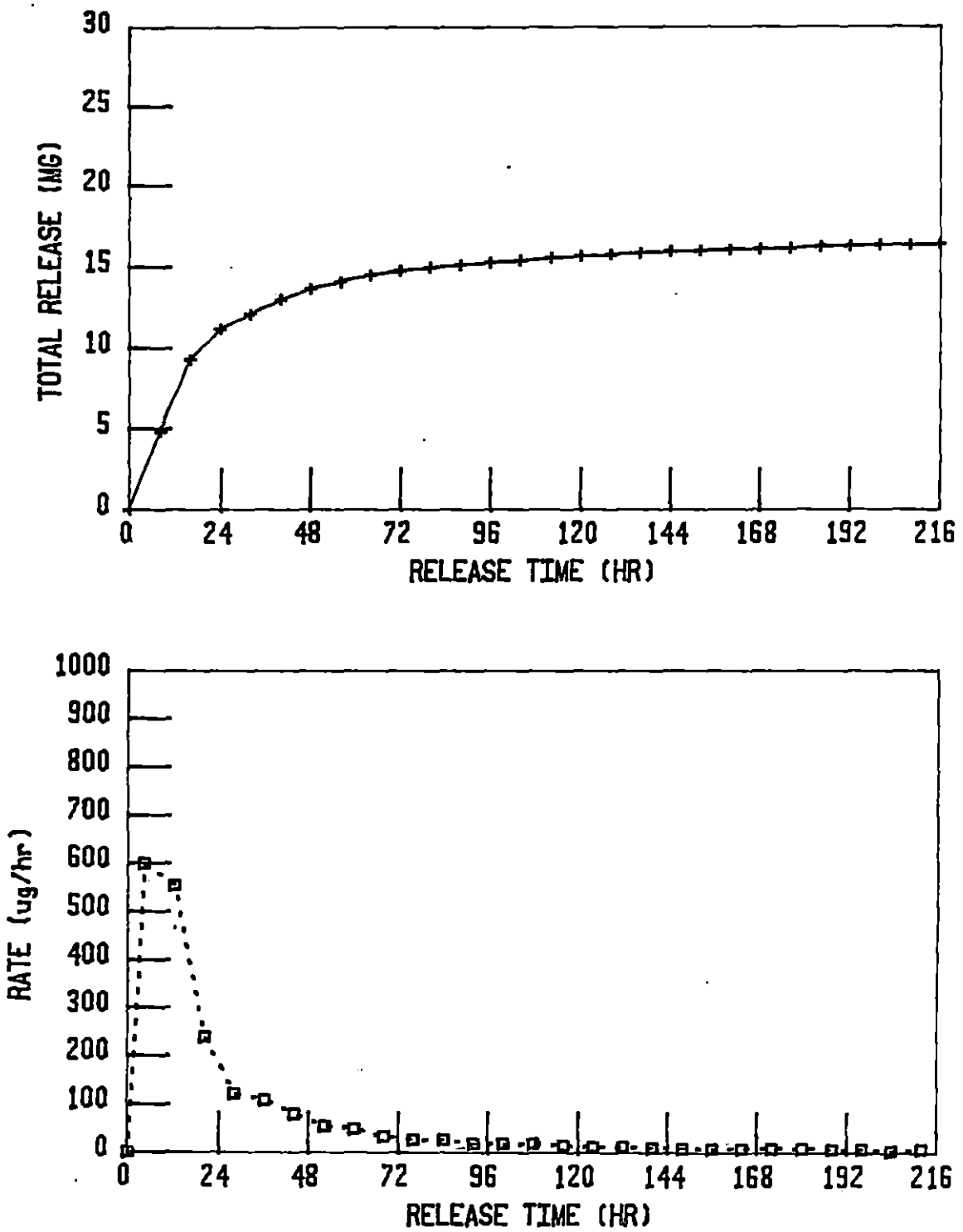

FIGURE 34. Drug release characteristics for ring device 075. Top: total release vs. time; bottom: rate vs. time

TABLE 8. Summary of the drug release characteristics of each ring device

Device No.	Initial release rate (up to 2 days) ug/hr	Release rate level after 6 days ug/hr	Cumulative release amount ug	Ratio of release amount %
011	148 - 657	7 - 27	21886	42.3
021	113 - 349	11 - 34	17412	34.9
022	156 - 445	10 - 32	20654	41.7
031	77 - 315	13 - 27	15275	30.9
032	182 - 1921	5 - 25	50262	45.9
033	63 - 469	4 - 16	14990	33.6
041	106 - 436	7 - 21	19409	40.0
042	220 - 2673	5 - 22	64222	63.1
043	162 - 657	6 - 37	25010	40.7
044	240 - 2685	6 - 29	62063	55.7
051	251 - 3631	10 - 20	68010	58.4
052	216 - 3015	6 - 19	58514	58.6
053	104 - 1275	9 - 25	26462	49.2
054	101 - 1366	5 - 20	26168	46.0
061	92 - 682	3 - 22	16363	32.2
062	97 - 626	3 - 8	16978	35.4
063	83 - 632	4 - 12	18273	34.6
064	101 - 925	3 - 7	18181	36.8
065	62 - 629	6 - 13	15851	28.7
071	64 - 525	4 - 9	14705	30.2
072	48 - 613	4 - 12	15706	28.6
073	62 - 644	6 - 11	16780	29.3
074	55 - 671	4 - 10	12080	23.9
075	80 - 599	4 - 10	16439	29.3

release rate decreased exponentially. A large drug loading device affected the release pattern in the initial period, but after this period the release rate was similar to that seen for the small loading examples. The range of ratios of the release amount to total loading amount was 28.7-36.8% for the 90:10 copolymer (50 mg loading), 45.9-63.1% for the 90:10 copolymer (100 mg loading), and 23.9-49.2% for 25:75 copolymer (50

TABLE 9. Summary of the release characteristics according to device types

Device group	Device No.	Device type (polymer) (loading) (joint)	Initial release rate (up to 2 days) ug/hr	Release rate level after 6 days ug/hr	Total release amount ug	Ratio of release amount %
I	011,021 022,031 041	90:10, 50 mg, wire	77 - 657	7 - 34	15275- 21886	30.9- 42.3
II	032,042 044,051 052	90:10, 100 mg, wire	182 - 3631	5 - 25	50262- 68010	45.9- 63.1
III	033,043 053,054	25:75, 50 mg, wire	63 - 1366	4 - 37	14990- 26462	33.6- 49.2
IV	061-065	90:10, 50 mg, PVC	62 - 925	3 - 22	15851- 18273	28.7- 36.8
V	071-075	25:75, 50 mg, PVC	62 - 671	4 - 12	12080- 16780	23.9- 30.2

mg loading). Also, group IV and V examples showed a relatively low value compared with those of group I, II and III due to having less surface area.

Post-experiment Physical Characteristics of Ring Devices

After a release experiment, the physical characteristics of the ring device were checked to evaluate the effect of having a ring device in the solution for a particular period. Table 10 summarizes the results of the post-experiment physical examinations of the ring devices.

TABLE 10. Post-experiment physical characteristics of the ring devices

Group	Characteristics of ring devices	Device No.	Comments
I	90:10 copolymer, 50 mg loading, copper wire joint	011,021 022,031 041	Surface was smooth and white color; all had longitudinal, irregular cracks and enlarged defects
II	90:10 copolymer, 100 mg loading, copper wire joint	032,042 044,051 052	A slightly yellow color; rough; had longitudinal, irregular defects as well as a badly defective portion
III	25:75 copolymer, 50 mg loading, copper wire joint	033,043 053,054	Rough surface and gray color; same original defects remained and a few holes occurred due to air bubbles.
IV	90:10 copolymer, 50 mg loading, PVC tube joint	061-065	Smooth surface and strong joint; yellowish white color right after immersion; flexible and strong; one or two holes due to air bubble; minor circular, regular cracks for one device.
V	25:75 copolymer, 50 mg loading, PVC tube joint	071-075	

All of the ring devices changed color sometime during the beginning of a release experiment (within 1-2 hours). When a ring device was seen to swell slightly, it became white and opaque (an exception was seen for 100 mg loading device which still had a yellow tint). The swelling property also increased the flexibility of ring device in the solution, but, after drying the ring device, the surface of ring device was very brittle (except for group IV and V examples). The ring device with the copper wire joint had longitudinal cracks. This would be undesirable for use in an eye. However, the ring devices with PVC joints maintained their shape after a release experiment, and showed better flexibility and rigidity. Although cracks were seen on some units, these were not serious. On the other hand, for the 25:75 copolymer series (group III

and V), a rough surface was observed (related to the difficulty of coating tubes with this copolymer formulation), but defects did not grow to larger cracks as seen in the 90:10 copolymer, Silastic series (groups I and II).

Preliminary In Vivo Testing

In the preliminary in vivo testing, five of six cattle dislodged a PVC ring device within minutes of insertion. Removal was due to concerted movements of the lower and third eyelid. These devices had weak joints. Only one of six ring devices remained in an eye over 1 week. However, no overt inflammatory reaction in the eye was seen for this 1 week case. A few micrograms of drug were detected in tear samples collected at several time periods.

As a result of the above in vivo observations of weak joint strength, additional tests of the flexibility and joint strength of ring devices were made under dry and wet conditions for PVC joints made with a new adhesive based on a mixture of copolymer and THF/PVC solution. The results for these ring devices are listed in Table 11.

As shown in the table, the wet and dry ring devices had reasonably good mechanical property characteristics such as flexibility and joint condition. The wet devices had better flexiblility than the dry ones. Even if there was a small crack due to the compressive forces used for the test, the ring shape was kept and the ring did not separate at the joint. These ring devices had a stronger joint compared with those used in the preliminary in vivo testing. These joints were established by cementing a small diameter 1 cm PVC length of tubing segment within the

TABLE 11. Flexibility test for ring devices under dry and wet conditions

Condition of device	Device No.[a]	Result of test	Remarks (force/adhesive)
Dry	M01	Crack on joint part and opposite part, but inner joint tube kept ring shape	parallel/polymer
	M02	Crack near joint part and on the side, but inner joint tube kept ring shape	vertical/polymer
	M03	No crack, very strong joint	both/polymer
Wet	M04	Stronger than dry test, crack on joint part for compressive force	both/polymer
	M05	the same as above	both/polymer
	M06	Stronger than dry test, crack on joint and part opposite the compressive force	parallel/THF and polymer

[a]All devices were made by 90:10 copolymer (50 mg loading).

two open ends of the PVC ring. The adhesive solution was a mixture of copolymer solution and a THF/ 5w/o PVC solution.

In practice, rings are preconditioned in a saline solution for about 8 hours, so that they are soft and pliable prior to insertion, and less susceptible to cracking.

DISCUSSION

Preparation and selection of ring device

The 90:10 copolymer (less hydrophilic hydrogel) was a more suitable dipcoating material than the 25:75 copolymer (more hydrophilic hydrogel) for use in preparing the matrix system. PVC ring devices appeared to be satisfactory for use as an ocular insert. They were rigid enough to retain their ring shape and were flexible enough to conform to the conjunctival sac. In preliminary in vivo testing, a ring device was retained in a cow's eye for more than 1 week. Strong joints can be achieved through the use of a strong adhesive such as the solution mixture of copolymer and THF/PVC.

No overt inflammation was observed during this in vivo experiment (Dr. Rosenbusch: personal communication)[19].

Tylosin tartrate release characteristics

The continuous release experiments provided consistent results in the determination of the release rates of the antibiotic. The cassette pump provided better flow control than did the syringe pump.

All of the ring devices showed a relatively high release rate in the initial period (up to 2 days) compared with the 2 to 9 day period. Eighty to ninety percent of the total amount of drug released over a nine day period was released within the first two days of an in vitro experiment. The release profile after two days showed an exponential

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[19] Veterinary Medical Research Institute, Iowa State University, Ames, Iowa.

decrease. The type of profile is probably due to the result of the change of the flux of drug in the coating layer of a ring device. As the thickness of the depletion zone increases, the diffusion distance increases with time (Cardinal, 1984a). Since the flux is inversely proportional to the zone thickness, the release rate will decrease with time whether the concentration gradient in the zone of depletion is linear or not.

The release kinetics from ring devices were influenced by the initial drug loading and the geometry of devices. The total drug release amount was proportional to the surface area of ring device. For example, a ring device with a diameter of 40 mm (group I and III) released more drug than a ring device with a diameter of 35-37 mm (group IV and V).

When the drug loading quantity was increased from 50mg to 100mg, the initial release rate was increased by 2 - 6 times. After the initial period, the release pattern for the 100 mg loading examples was similar to that of the 50 mg drug loading examples.

As mentioned before, the copolymer composition can influence the diffusion coefficient in the matrix system. Copolymers of HEMA and MMA lead to poorly swollen hydrogels. Since hydrogels consist of macromolecules crosslinked to form a three dimensional network, the permeability for a solute can change according to the composition of polymer. However, in this study only a slight change in release kinetics was seen in a comparison for the devices made from the two formulations. The release rate profile for a 25:75 copolymer ring device was seen to exhibit a similar rate profile to that of 90:10 copolymer devices. It had been expected that a 25:75 copolymer device would release more drug

than a 90:10 copolymer device due to different diffusion coefficients for the two materials. After 4 days, the 25:75 copolymer devices showed more uniform release profiles than did the 90:10 copolymer devices. The release rate curves based on one-hour sampling times were smoother than those seen for 8-hours sampling times.

Usually the maximum release rate for a device was noted within 8 - 10 hours after starting a release experiment. After 9 days, all of the ring devices provided a release rates in the range of 3 - 7 ug/hr. These values are above that estimated drug release rate (about 1.2 ug/hr) needed to treat bovine ocular infections.

RECOMMENDATIONS FOR FUTURE RESEARCH

Additional detailed in vivo testing will be necessary to evaluate retention of rings, eye irritation levels, effects on tearing rates, and release characteristics. The 90:10 copolymer or 25:75 copolymer (50 mg loading) formulations should provide useful characteristics. The 5 w/o PVC-tetrahydrofuran solution and copolymer solution mixture should be used to connect the ring segments.

A swab can be used to collect tears from the cow's eye. To collect sufficient tears in in vivo experiments, it is recommended that a tear sample for TLC analysis be recovered from a swab by using a small syringe (27G needle), and that the small sample then be directly spotted on the TLC plate at the time of collection. The use of a pre-wetted swab is not satisfactory as this dilutes the drug concentration in a tear solution sample and adds to the uncertainty in analytical results for the drug.

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