Tylosin tartrate release from hydrogel ocular inserts

by

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ABSTRACT

As part of an effort to develop an ocular insert controlled drug release system to treat Bovine Infectious Keratoconjunctivitis, tests were performed on hydrogel disks containing the antibiotic, tylosin tartrate. Thin layer chromatography was utilized to characterize the rates of release, and microstructural information from scanning electron microscopy was of use in determining ways to improve the release characteristics.

Disk-shaped laminates of 90:10 methyl methacrylate/2-hydroxyethyl methacrylate which contained tylosin tartrate were studied for release characteristics by a five-day in vitro (in mammalian Ringer's solution at 37°C) experiment. Three different drug loading configurations were compared in quintuplicate: poly (lactic acid)-tylosin tartrate matrix containing 4.8 mg of antibiotic in the center of the disk, 5.0 mg of tylosin tartrate powder in the center of the disk, and 50.0 mg of tylosin tartrate powder in the center of the disk. On the basis of known minimum inhibitory concentration levels for the bacteria of interest and known tear flow rates, a minimum release rate range of 1.2-2.5 ug/hr of tylosin tartrate was required. Release rates of approximately 20-30 ug/hr were observed for the controlled-release disks containing 50.0 mg of tylosin tartrate. Release rates for the other two systems were lower than 1.2 ug/hr.

Thin layer chromatography and direct densitometric scanning were utilized to quantitate the amount of tylosin tartrate in release-experiment samples. The variability of the quantitative analysis and

the linearity of the relationship between average weight of peak and tylosin tartrate amount were determined by a statistical analysis of type-one aqueous and saline solution standards with tylosin tartrate concentrations of 0.1-10.0 ug/10.0 ul. A sensitivity of 0.2 ug of tylosin tartrate was achieved. Type-one aqueous solutions produced coefficients of variance from 10.0-28.6 percent for 0.2-0.8 ug amounts and 5.6-10.5 percent for 0.9-10.0 ug amounts. Coefficient of determination calculations indicated a good linear relationship between average weight of peak and tylosin tartrate amount. The reduced viscosity of saline solutions resulted in coefficients of variance that were inversely related to the saline concentration, and larger than the values for type-one aqueous solutions.

INTRODUCTION

Statement of the Problem

Bovine Infectious Keratoconjunctivitis (BIK) is an acute contagious ophthalmia characterized by lacrimation, photophobia, corneal ulcers, and corneal opacities. BIK affects cattle worldwide. The disease is rarely fatal, but infected cattle refuse food. Consequently, major economic loss may occur as milk production, body weight, and growth diminish.

The principal treatment method consists of topical applications of antibiotic and sulfonamide eyedrops, sprays, powders, or ointments for a period of five to seven days. Lachrymal fluid rapidly removes drug from the eye; therefore, treatment is effective when medication is applied two or three times daily. This repetitious regimen is time-consuming and costly; therefore, developing a more efficient method of drug administration is of interest.

To address the BIK treatment problem, a controlled drug release system was developed and characterized for its suitability for this application. Controlled-release systems may utilize biocompatible polymers to regulate the duration and rate of drug release for extended periods of time. An ocular controlled-release system that maintains a therapeutic level of a drug currently employed to treat BIK would eliminate the repetitious regimen. In this study, hydrogels were selected as the polymer for the ocular controlled-release system.

Certain hydrogel formulations are widely utilized as soft-contact lenses and have controllable water-imbibing characteristics. Tylosin tartrate, an agricultural antibiotic that effectively eradicates causative and contributory organisms of BIK, was chosen as the treatment drug.

An acceptable ocular controlled-release system of hydrogel and tylosin tartrate would release sufficient (microgram) quantities of drug per hour into low volumes of lachrymal fluid in the animal's eyes. An important part of the research to be reported was to develop a method to characterize samples representing amounts of tylosin tartrate released into artificial lachrymal fluid. A method based on thin layer chromatography was developed and applied to quantitate tylosin tartrate release as a function of time from three types of hydrogel-drug disks.

The hydrogel-drug disk experiments were used to examine effects of differences in drug loading and the potential usefulness of a poly (lactic acid) matrix containing the drug as a type of reservoir within the hydrogel disk. These experiments were directed at evaluating the feasibility of using hydrogel ocular inserts to release a suitable antibiotic for treatment of BIK.

Literature Review

Nomenclature

BIK is commonly called pinkeye. The disease is also referred to as Infectious Bovine Keratoconjunctivitis IBK (Hughes and Pugh, 1970; Hughes and Pugh, 1975; Hughes, 1981), Infectious Bovine Kerato-Conjunctivitis (Thrift and Overfield, 1974), Bovine Infectious

Keratoconjunctivitis BIK (Pugh and Hughes, 1975; Jensen and Mackey, 1979; Blogg, 1980), and Bovine Infectious Keratitis (Baldwin, 1945). Other synonyms are infectious ophthalmia, and keratitis solaris (Hughes, 1981).

Etiology and modes of transmission

Moraxella bovis, a common agricultural bacteria (Hughes and Pugh, 1975), is capable of living through the winter (Blogg, 1980). Various strains have been identified, and the characteristics of Moraxella bovis strains associated with BIK are listed in Table 1.

TABLE 1. Characteristics of Moraxella bovis strains associated with Bovine Infectious Keratoconjunctivitis. (Pugh, 1969).

A.	Gram-negative, nonmotile diplobacillus.
B.	Usually hemolytic, smooth, circular colonies with an entire edge convex to umbonate, grayish white and slightly indented into the medium.
C.	Does not reduce nitrates to nitrites or ferment carbohydrates.
D.	Are proteolytic, oxidase positive, and produce a typical three-zone reaction when grown in litmus milk.
E.	Produce no surface growth in liquid medium but develop a coarse, flocculent sediment with little turbidity.
F.	Do not grow in Herellea agar.
G.	Produce firm easily fragmented colonies which autoagglutinate when placed in most liquid mediums.

Moraxella bovis dissociates into various forms. The rough colony type is avirulent, while the smooth colony (typical type) of Moraxella bovis induces Bovine Infectious Keratoconjunctivitis (Jackson, 1953).

Moraxela bovis interconverts between its hemolytic and nonhemolytic forms with exposure to ultraviolet light (Pugh, 1969; Pugh and Hughes, 1968). The virulent form is the primary infectious agent of BIK (Pugh and Hughes, 1968; Hughes and Pugh, 1970).

Mycoplasma bovoculi is the primary causative agent of Mycoplasmal Conjunctivitis, a different disease than BIK; however, Mycoplasma bovoculi may create a more suitable environment for Moraxella bovis (Rosenbusch and Knudtson, 1980; Rosenbusch, 1983). The Bovine Infectious Rhinotracheitis virus causes conjunctivitis, but not keratitis; the inflammed membranes are conducive to the growth of Moraxella bovis (Blogg, 1980; Blood and Henderson, 1979).

Symptoms of the naturally occurring disease are more severe than those of the experimentally induced disease; therefore, environmental conditions are a factor. BIK is most prevalent during summer and autumn, but severe outbreaks occur in winter when cattle are confined in barns and feedlots. Ultraviolet light from the sun induces the conversion of nonhemolytic forms of Moraxella bovis to hemolytic forms. Face flies (Musca autumnalis and Musca domestica), dust, and temperature extremes increase the severity of the disease by irritating the eye (Blood and Henderson, 1979; Jensen and Mackey, 1965).

The exact method of natural transmission has not been determined, but the disease can be produced by transferring Moraxella bovis into the conjunctiva of the eye (Pugh and Hughes, 1975). A cow may carry Moraxella bovis in tear and nasal secretions for one year after having

BIK without displaying visible signs of the disease (Blood and Henderson, 1979; Blogg, 1980). Tear and nasal secretions are transferred by direct contact between cattle, or by intermediaries such as wind-blown dust, animal handlers, and face flies (Blogg, 1980).

Handlers pass Moraxella bovis, but do not contract pinkeye. Face flies harbor Moraxella bovis for up to three days after contact with an infected eye. A high density of cattle in feedlots facilitates the transmission process (Blood and Henderson, 1979).

Nature of Bovine Infectious Keratoconjunctivitis

BIK occurs as a unilateral or bilateral infection. After an incubation period of one to twenty days, the conjunctiva of the infected eye begins to swell and excess tearing occurs. Muscular spasms of the eyelid and an elevated temperature are common. Ambient light causes pain; therefore, the animal seeks out dark areas, closes the eye, and refrains from eating (Blogg, 1980). Range cattle have died from starvation, drowning, and falling from high places due to impaired sight (Baldwin, 1945).

Three to four days after the initial clinical signs, the eye remains tightly closed. A pus discharge mats the fur and causes dirt to adhere to the eye region. When the eye is forced open, an opaque covering of the cornea is visible. At this stage, some mild cases of BIK begin to recover spontaneously; however, if secondary bacteria enter the conjunctiva, corneal ulcers develop. The ulcers invade the interior of the eye and cause temporary or permanent blindness. In rare cases, ulcers cause fatal meningitis (Jensen and Mackey, 1965).

With treatment, cattle that have not developed corneal ulcers recover in two to three weeks (Blogg, 1980). Cases that involve severe ulceration require five to six weeks for recovery, and permanent corneal scars are common (Jensen and Mackey, 1965). Cattle that contract BIK seem to develop an immunity against reinfection. A severe case imparts greater immunity than a mild case (Blood and Henderson, 1979; Blogg, 1980).

The effect of BIK is breed dependent. Cattle with non-pigmented eyelids are more susceptible and experience more serious reactions than those with pigmented eyelids (Blogg, 1980; Jensen and Mackey, 1965). Figure 1 illustrates the relationship between body-color pattern and BIK occurrence in the calves of a purbred Hereford herd and a mixed-breed herd.

In any particular breed, cattle under two years of age have the highest rate of infection and disease. A five-year study conducted at the Iowa State University Beef Nutrition Farm found that calves had an average annual Moraxella bovis infection rate of seventy-five percent and an average annual BIK rate of fifty-eight percent, while cows had an average annual Moraxella bovis infection rate of sixty-three percent and an average annual BIK rate of sixteen percent (Hughes and Pugh, 1970).

In a similar study, 158 Hereford calves were observed from birth until one year of age. Thirty-six percent of the bulls and twelve percent of the heifers developed BIK. At 205 days of age, the bulls that contracted BIK were an average of thirty-six pounds lighter than those without BIK. Since the bulls experienced a greater percentage of

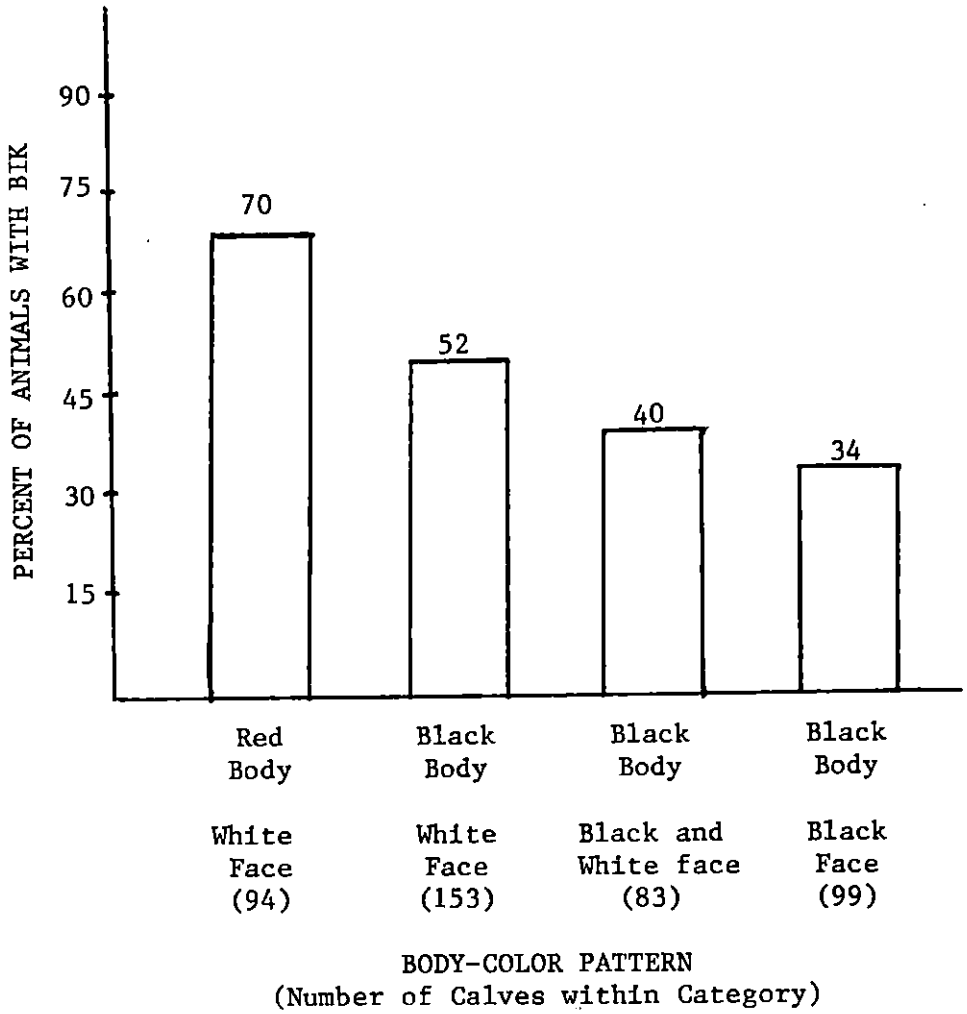

FIGURE 1. The relationship between body-color and Infectious Bovine Keratoconjunctivitis in the calves of a purebred Hereford herd and a mixed-breed herd. (Pugh et al., 1982)

BIK than the heifers, the bulls may be more susceptible to the disease or they may simply have an increased chance of being infected due to their tendency to roam throughout the herd. At one year of age, the calves were reevaluated to determine prolonged effects. The bulls that contracted BIK weighed an average of seventy pounds less than those

without BIK; however, the heifers had no significant weight difference (Thrift and Overfield, 1974).

A study with dairy cows found that milk production dropped an average of twenty-five percent during the course of the disease (Baldwin, 1945).

There is a general lack of information concerning BIK's effect on adult bulls because a number of bulls are not routinely maintained in a herd. Current research indicates that a bull's libido may be reduced while infected with BIK (Thrift and Overfield, 1974).

Present methods of treatment

Early cases of BIK are treated with antibiotic solutions and ophthalmic ointments containing chloramphenicol, oxytetracycline, penicillin-streptomycin (Jensen and Mackey, 1979; Blood and Henderson, 1979), or tylosin (Burger, 1970; Rossoff, 1974). Eyedrops are the prevalent form of ocular delivery and generally the least expensive. Most eyedrops have an aqueous medium; however, poly (ethylene glycol), poly (vinyl alcohol), hydroxypropylmethylcellulose, and poly (vinyl-pyrrolidone) are frequently added to increase viscosity (Chiou and Watanabe, 1982). Eighty percent of an eyedrop is lost from the preocular film immediately after instillation (Gelatt et al., 1979). Ointments that contain a lanolin, petrolatum, or vegetable oil base are utilized with drugs such as the tetracyclines. The base increases the pentration of drug through the corneal membrane by improving retention time (Chiou and Watanabe, 1982). Applications to both the upper and

lower conjunctival sac, three times daily, for five to seven days provides optimum results (Blood and Henderson, 1979; Jensen and Mackey, 1965).

A 30 mg dose of tylosin tartrate [$C_{45}H_{77}NO_{17}$-$C_4H_6O_6$] (Stecher et al., 1968), applied twice daily as a 50 mg/ml aqueous spray, eliminated clinical signs of BIK and Moraxella bovis from the eyes of cattle after five days (Ellis and Barnes, 1961). Sprays have retention times comparable to eyedrops. The delivery device increases unit price; however, a spray is less irritating than a drop, and less manual dexterity is required for proper application (Chiou and Watanabe, 1982).

Sampson and Gregory (1974) have shown Tylan® plus neomycin eye powder[1] to be effective in the treatment of BIK when applied once or twice daily for a duration of one to three days. Aronson et al. (1983) recommend daily applications of Tylan® plus neomycin eye powder for a seven day period to treat BIK. Retention time is similar to the other methods.

Parenteral treatment using sulphadimidine (100 mg/kg body weight) provides a therapeutic level in the tears for twelve to twenty-four hours (Blood and Henderson, 1979). Oxytetracycline and tylosin used in the same manner have also eliminated Moraxella bovis ocular infections (Hughes, 1981). Since systemically administered drugs must cross the blood-eye barrier, topical instillation is preferred in most situations (Chiou and Watanabe, 1982).

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[1] Elanco Products Co., Indianapolis, Indiana.

When corneal swelling of blood vessels is severe, sub-bulbar conjunctival injections of corticosteroids and antibiotics together with topical anesthetics and atropine improve drug absorption, reduce muscular spasms, and minimize drug loss (Blogg, 1980). Blood and Henderson (1979) recommend a 1 mg dexamethasone/2 ml mixture of penicillin-streptomycin injection; one injection is usually sufficient, but some cases require daily injections for three days. Injections are recommended only for antimicrobial drugs such as penicillins, cephalosporins, and aminoglycosides, or for application to the eye's posterior region. Topically administered drugs diffuse into the circulation and Schlemm's Canal before reaching the posterior region (Chiou and Watanabe, 1982). Sewing the third eyelid across the globe promotes healing and protects against dirt and insects (Jensen and Mackey, 1965; Blood and Henderson, 1979; Blogg, 1980).

Once ulceration occurs, corticosteriods and cortisone drugs are replaced by anticollogenases (Blogg, 1980).

Researchers have tried to develop a BIK vaccine since 1975. A vaccine has been developed for protection against a homologous infection of Moraxella bovis; however, several strains of Moraxella bovis are present in any infection. Until a vaccine is developed that provides protection against the heterologous challenge, other types of treatment must be utilized (Pugh et al., 1978; Pugh et al., 1982).

Theodorakis et al. (1983) developed a poly (lactic acid)-chloramphenicol sodium succinate ocular insert matrix designed to treat BIK. The insert was attached to the outer side of the third eyelid by

sutures or a spear, and released a therapeutic level of chloramphenicol sodium succinate for two days at an irregular decreasing rate that did not follow the square root of time law (rate proportional to $t^{-\frac{1}{2}}$).

Ocular drug delivery methods

Present methods of ocular drug delivery are summarized in Table 2. A brief comparison of eyedrops, ointments, sprays, powders, oral administrations, injections, and inserts was presented in the previous section. This section will provide more specific details for contact lenses and ocular inserts which bear on the projected application.

TABLE 2. Methods of ocular drug delivery. (Chiou and Watanabe, 1982)

Eyedrops
Ointments
Sprays
Powders
Oral Administrations
Injections
Soft Contact Lenses
Perfusion Systems
Inserts

Soft contact lenses, Bionite[2] (soaked in a drug solution), improve retention time significantly compared with previous methods and are currently utilized in human medicine (Podos et al., 1972). A hydrophilic contact lens soaked in a four percent pilocarpine nitrate

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[2] Griffin Laboratories Inc., Buffalo, New York.

solution releases approximately two-thirds of the drug in five minutes (Richardson, 1975). Although the drug concentration rapidly decreases and the release mechanism is considered uncontrolled, the effects of pilocarpine (Gelatt et al., 1979) and tetracycline (Maichuk, 1975b) are seen twenty-four hours after application. In addition to not maintaining therapeutic levels for the duration necessary for BIK treatment, Hughes and Pugh (1975) found that the nictitating membrane removed such devices from the bovine eye within two hours.

The perfusion system pumps a continuous and constant flow of drug solution to the eye through a poly (ethylene) tube inserted into the conjunctival sac. Due to its cumbersome nature and expense, it is not widely utilized (Chiou and Watanabe, 1982).

Ocular inserts for use in human medicine are fabricated from insoluble or soluble polymers and placed in the upper or lower conjunctival sac. The inserts are generally eight to ten millimeter diameter circular flat disks, or eight by four millimeter oval flat disks (Refojo, 1974). Three types of inserts have been studied and are currently utilized.

Soluble inserts of methylcellulose, hydroxypropylcellulose, poly (vinyl alcohol), poly (vinyl pyrrolidone), and poly (ethylene glycol) deliver a flow of polymer to thicken and stablize the precorneal tear film for the treatment of keratoconjunctivitis sicca (Refojo, 1974).

Bloomfield et al. (1977) found that sixty to ninety percent of the insert dissolved within five hours. The total dissolution time of these devices ranges from eight to twelve hours (Gelatt et al., 1979). This

type of insert does not release a drug; therefore, it is not applicable to the treatment of BIK.

Maichuk (1975a) produced a soluble ophthalmic drug insert composed of poly (acrylamide), ethylacetate, and vinylpyrrolidone that dissolved in thirty minutes. Various ophthalmic drugs (such as neomycin, kanomycin, atrophine, pilocarpine, idoxuridine, and methasone mixed with the polymer) are released at the dissolution rate, prolonging the availability of active substances in conjunctival and corneal tissue for thirty-four to seventy-two hours. Clinical testing with more than 500 patients showed good tolerance of the insert and therapeutic efficacy in various forms of glaucoma, keratitis, cornea ulceration, trachoma, conjunctivitis, adenovirus, and herpesvirus. This type of device is unable to maintain a therapeutic level of drug for the duration needed to treat BIK.

Ocusert[3], an ocular insert that provides control of intraocular pressure for one week on a twenty-four hour basis, consists of 0.074 mm thick outer membranes of poly (ethylene-vinyl acetate) and a pilocarpine core. A titanium dioxide annular-ring surrounds the pilocarpine core to prevent drug escape from the edges. There are two systems currently in use. Pilo-20 contains 5 mg of pilocarpine and maintains a release rate of 20 ug/hr for seven days. Its exterior dimensions are 5.7 x 13.4 mm on its axis and 0.33 mm in thickness (Figure 2). Pilo-40 contains 11 mg of pilocarpine and maintains a release of 40 ug/hr for seven days. Its

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[3] Alza Pharmaceuticals, Palo Alto, California.

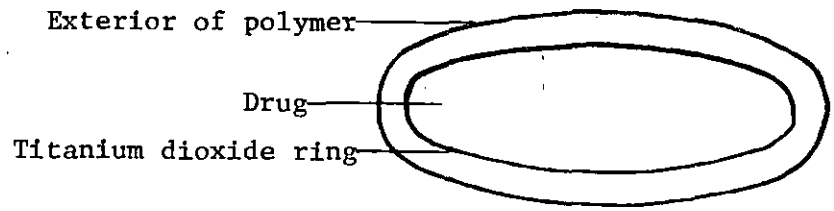

FIGURE 2. Ocusert® exterior (Top-view). (Chien, 1982)

exterior dimensions are 5.5 x 13 mm on its axis and 0.5 mm in thickness (Figure 2) (Chien, 1982). Figure 3 illustrates the release characteristics for the Pilo-20. The average release rate for the first eight hours is 64 ug/hr. After this period, a 20 ug/hr release rate is maintained for seven days. At seven days the core is no longer saturated; consequently, first order release occurs (Cowsar, 1974; Richardson, 1975).

Macoul and Pavan-Langston (1975) utilized a questionnaire format to examine the experiences of twenty-nine patients utilizing Ocusert®. The questionnaire was completed at specific times throughout a one year period. Eighty-nine percent of the responses preferred the Ocusert® system to eyedrops. None of the responses indicated a problem with insertion, 0.3 percent of the responses expressed a frequent awareness of the insert in the eye, and 6.7 percent of the responses indicated that the device dislodged from the eye.

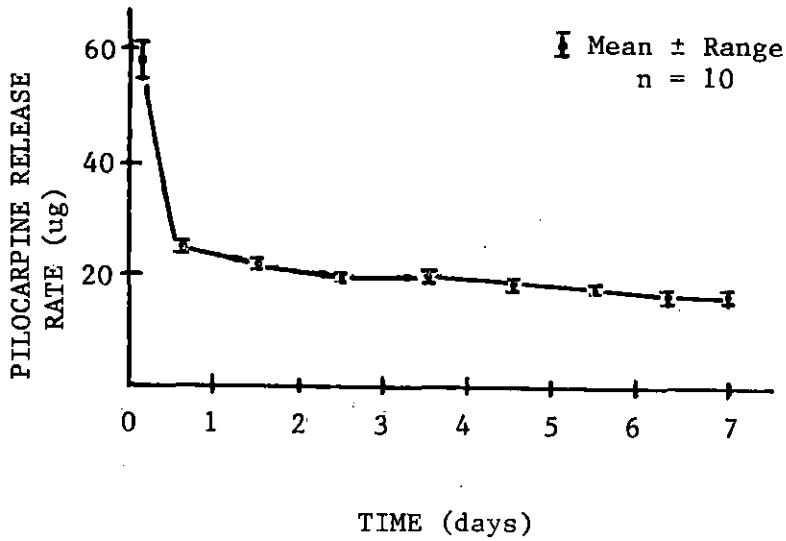

FIGURE 3. Pilo-20 release rate characteristics. (from Macoul and Pavan-Langston, 1975)

Proposed treatment method

The controlled-release system is the only method of ocular drug delivery that is capable of maintaining a therapeutic level in the eye for seven days. Controlled-release delivery systems are classified by the release rate mechanism, and diffusion-controlled systems are the most prevalent. The two types of diffusion-controlled systems are reservoir and monolithic. A nonporous or microporous polymer film surrounds the drug in the reservoir system. Zero-order release occurs when the design maintains unit thermodynamic activity immediately inside the rate-limiting membrane (Hophenberg and Hsu, 1978). Reservoir systems are not biodegradable and leaks may develop. The drug is uniformly mixed throughout the solid nonbiodegradable polymer in the monolithic system. With excess dispersed drug, release is proportional

to the square root of drug loading, and the rate slowly decreases in accordance with the square root of time rate law (Cowsar, 1974; Langer et al., 1980).

An acceptable treatment drug must effectively eradicate BIK causative and contributory organisms. Two commonly utilized drugs are penicillin and streptomycin; however, in actual practice they are not as effective as several other choices. Drugs that are effective against Moraxella bovis and Mycoplasma bovoculi are tetracycline, erythromycin, and tylosin. Of these three, the minimum inhibitory concentration (0.63 ug/ml), is lowest for tylosin. (R. F. Rosenbusch, personal communication.)[4] Thus, treatment could be accomplished with a smaller amount of tylosin within the controlled-release system than if tetracycline or erythromycin were utilized.

Tylosin (see Figure 4 for the structure), a macrolide antibiotic isolated from a strain of Streptomycetes fradiae in a soil sample from Thailand (McGuire et al., 1961; Hamill et al., 1961), is a weak base that forms soluble salts and ester compounds such as tylosin hydrochloride, tylosin tartrate, acetyltylosin, and propionyltylosin. The tylosin salts produced by the isolation and extraction process are pure enough to be utilized in that form (Korzybski et al., 1967), and tylosin tartrate is commercially available for agricultural use (Burger, 1970). Tylosin tartrate is soluble in water at concentrations greater than 300 mg/ml and is stable at room temperature in aqueous solutions

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

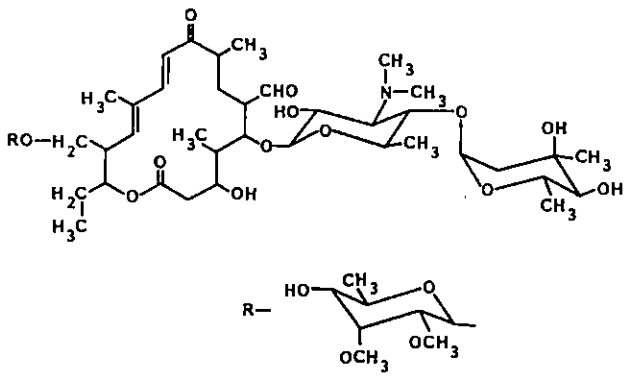

FIGURE 4. Structure of tylosin. (from Windholz et al., 1976)

(pH 4.0 - 9.0) for a least one month (Ose and Barnes, 1960). Trade names for the tylosin base are Tylan® and Tylocine® (Charles et al., 1979).

The structure of the corneal membrane consists of an aqueous layer (stroma) covered by lipid layers (epithelium and endothelium). Drugs that are both hydrophobic and hydrophilic easily penetrate corneal tissue; however, pure polar or pure nonpolar drugs do not effectively penetrate the cornea (Chiou and Watanabe, 1982). Tylosin is soluble in lower alcohols, esters, ketones, chloronated hydrocarbons, benzene and ether; therefore, it is an amphipathic compound (Windholz et al., 1976). The antibiotic is essentially nontoxic and nonirritating to the eye and conjuctival sac (Ellis and Barnes, 1961; Johnston, 1982).

An ocular controlled-release system of biodegradable material would be particularly useful since subsequent system removal would not be required.

Biodegradable materials suitable for drug delivery systems are poly (lactic acid), poly (glycolic acid), poly (ε-caprolactone) and poly (amino acids) (Bruck, 1981; Langer and Peppas, 1981).

Schindler et al. (1977) utilized in vitro methods to study the release of the steriods norgestrel, norethindrone, testosterone, progesterone, and ethynyl estradoil, from homo and copolymers of glycolide, dilactide, and ε-caprolactone cylinders 1-2 cm in length with a 0.4-2.3 mm wall thickness. These configurations produced a release rate characterized by a rapid decline in rate during the first twenty days followed by a slow decline for the next 130 days. They found that copolymers of ε-caprolactone and racemic dilactide were more permeable than poly (ε-caprolactone) and are appropriate for devices with a lifespan of less than one year. Utilizing the same steriods, Pitt et al. (1979) showed that poly (lactic acid) films were 10^4 times less permeable than poly (ε-caprolactone) films. The aqueous solubilities of the steroids at 37°C is, norgestrel 3.0 ug/ml, norethindrone 9.8 ug/ml, testosterone 30.6 ug/ml, progesterone 14.1 ug/ml and ethynyl estradiol 12.1 ug/ml (Pitt et al., 1979).

Schindler et al. (1977) found that it takes an average of eighty days for poly (dilactide) films implanted in rabbits to degrade to half of their original molecular weight. This degradation rate is 2.8 times greater than the degradation rate of poly (ε-caprolactone) measured under the same conditions (Pitt et al., 1981). Copolymers of dilactide and ε-caprolactone degrade more rapidly than either homopolymer. Figure

5 illustrates the fractional change in viscosity for the three materials versus time.

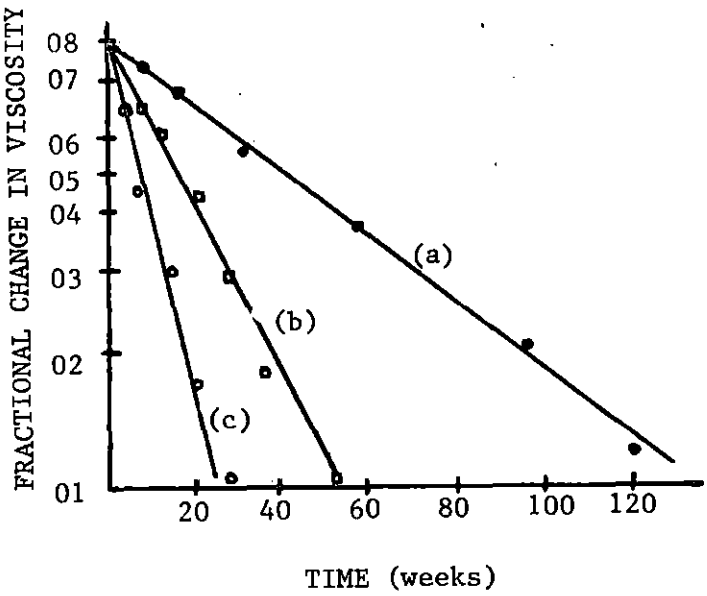

FIGURE 5. Fractional changes in the intrinsic viscosity of (a) poly (ε-caprolactone), (b) poly (dilactide), (c) poly (ε-caprolactone-co-dilactide) with time. (from Pitt et al., 1981)

The release of sulfadiazine, an antimalarial drug, from a 1.5 mm diameter spherical matrix system of poly (lactic acid) implanted subdermally in rats decreased in accordance with the square root of time law (Wise et al., 1979). The aqueous solubility of sulfadiazine is 0.13 mg/ml (Windholz et al., 1976).

Poly (amino acids) are too weak for utilization as sutures and solubilize in a few days; however, the materials may be effective in

short duration, low strength drug release applications (Kronenthal, 1975).

These references illustrate that biodegradable polymers are capable of providing near zero-order release from reservoir devices and square root of time law release from matrix devices for relatively low water-soluble drugs with molecular weights around 300. Therefore, it may be possible to release tylosin tartrate with its greater water solubility and higher molecular weight from similar devices. The literature search revealed no ocular applications of the biodegradable materials, and showed that the most common ophthalmic materials were silicon rubber, poly (methyl methacrylate) [poly (MMA)], and hydrogels (Refojo, 1974).

Silicon rubber has been utilized extensively for surgical procedures within the globe of the eye. As a contact lens or ocular insert, its high oxygen permeability permits the cornea to obtain required oxygen from the atmosphere; however, its hydrophobic nature causes eye irritation. Manufacturing difficulties are also a limitation to the production of silicone rubber contact lenses (Refojo, 1974).

Poly (MMA) (Figure 6) is a high optical quality, light weight, nonirritating material with excellent molding and machining characteristics. Contact lenses of poly (MMA) are relatively hydrophobic, absorbing 1.5 percent water by weight (Refojo, 1974). The methyl methacrylate monomer content should not exceed 0.75 percent (Estevey and Ridley, 1966), since it is moderately toxic when absorbed into the body; however, a 2-HEMA/MMA copolymer hydrogel stored in an

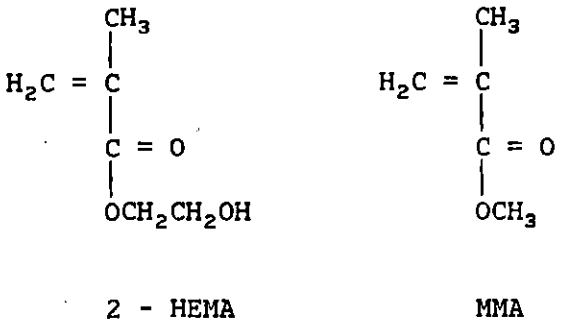

FIGURE 6. Structure of 2-hydroxyethyl methacrylate (2-HEMA) and methyl methacrylate (MMA). (from Langer and Peppas, 1981)

aqueous medium for a period of time is likely to consist only of polymer network and the swelling medium (Refojo, 1969).

Hydrogels are water-swollen, water-insoluble, polymeric materials with an equilibrium water content of up to ninety percent (Ratner and Hoffman, 1976; Pedley et al., 1980). Poly (2-hydroxyethyl methacrylate) [poly (2-HEMA)] is the most frequently utilized material due its stability under varying pH, temperature and toxicity conditions. It has an equilibrium water content of forty percent that can be reduced by copolymerization with methyl methacrylate [(MMA)] or increased by copolymerization with N-vinyl pyrrolidone [(NVP)], methacrylic acid [(MA)], or poly (vinyl pyrrolidone) [poly (VP)] (Pedley et al., 1980). Figure 7 illustrates equilibrium water content variation of MMA/2-HEMA copolymers. Hydrogel drug delivery systems are effective for antibiotic release into areas with primary or secondary infection since they permit protracted drug release at optimum concentration to the immediate environment (Pedley et al., 1980).

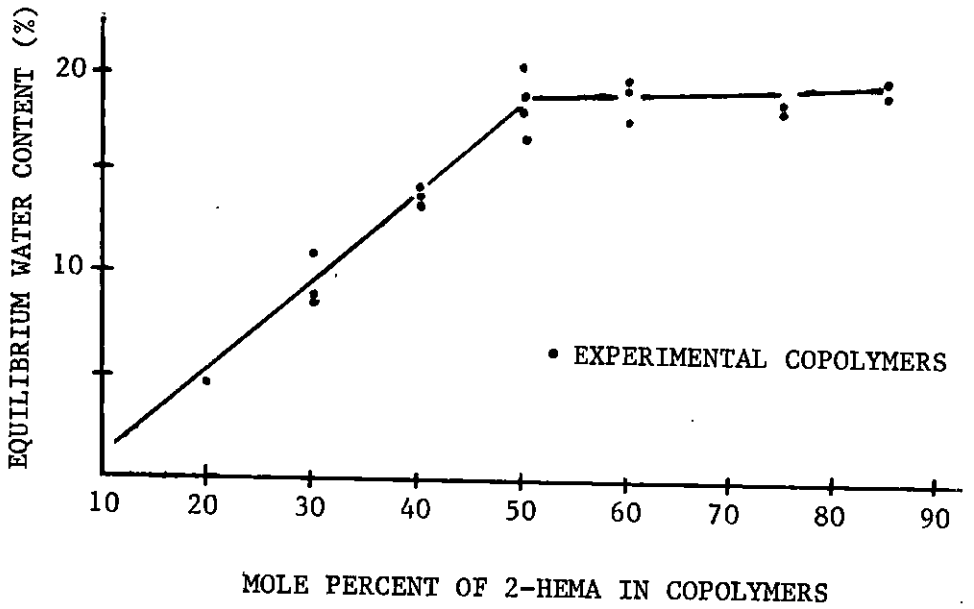

FIGURE 7. Equilibrium water content of MMA/2-HEMA copolymers. (from Cowsar et al., 1976)

Poly (2-HEMA) (Figure 6) has an equilibrium water content of forty percent (Pedley et al., 1980). It is extensively utilized by the soft-contact lens industry due to its biocompatibility, and excellent machining and molding characteristics (Refojo, 1974). Poly (2-HEMA) disks produced no reaction when implanted within the corneal stroma for two months (Langer et al., 1981).

Several reported drug release systems have poly (2-HEMA) or poly (2-HEMA)/poly (MMA) copolymers as the control membrane. Poly (2-HEMA) tubes (3 mm inside diameter, 5 mm outside diameter and 2.54 cm long) were filled with a cyclazocine polymer blend containing 140.6 gm of cyclazocine (M.W. 271.39). Zero-order release of the highly water-soluble narcotic antagonist into an agitated, 37°C, phosphate buffer

solution of pH 7.4 at a rate of 1 mg/day for five months was achieved (Abrahams and Ronel, 1975). Cardinal et al. (1980) studied the release of a 100 mg progesterone (M.W. 314.45) silicone oil blend from poly (2-HEMA) tubes (2.85 cm long, 1.2 mm wall thickness) into agitated, 23°C, deionized water. The release rate dropped from 0.15-0.04 mg/day for the first twenty days and remained zero-order at 0.04 mg/day until experiment termination thirty days later. Ebert et al. (1980) produced monolithic devices of poly (2-HEMA), prostaglandin E_1, and heparin that provided a release rate capable of reducing surface thrombosis for a period of seventy-two hours. The release characteristics followed the square root of time rate law.

Various sized 50:50 MMA/2-HEMA copolymer rectangular slabs with a sixty-two or eighty weight percent load of sodium fluoride were dip-coated with a 70:30 MMA/2-HEMA copolymer to provide a coating with a thickness ranging from 0.11-0.28 mm. A synthetic-saliva, constant-temperature flow system apparatus was utilized for the diffusion experiment. Zero-order release rates of 0.02-1.0 mg/day for sixty days were obtained. The copolymer-sodium fluoride core provides a medium of fixed geometry and water content in which the fluoride salt dissolves before passing through the outer membrane. This maintains unit thermodynamic activity and prevents rapid release should the system fail (Cowsar et al., 1976).

Olanoff and Anderson (1979) utilized 15 mm diameter trilaminar devices consisting of a tetracycline--63:37 MMA/2-HEMA matrix core

covered with 98:2 MMA/2-HEMA coatings of 0.053-0.14 mm thickness to release tetracycline (M.W. 444.43; water solubility 1.7 mg/ml). The zero-order release rate of tetracycline into Ringer's solution was found to be a function of general device geometry, coating membrane thickness, disk surface area, level of core reservoir drug loading and membrane coating copolymer composition. Zero-order release rates in the range of 0.54-28.9 ug/day were reported. The outer coating was more hydrophobic than the inner core and controlled drug diffusion. The more hydrophilic core maintained the constant thermodynamic activity of the drug at the core-coating material junction as required for zero-order release.

The desired ocular controlled-release system is required to maintain a minimum tylosin concentration of 0.63 ug/ml for seven days. The instantaneous volume of lachrymal fluid in the bovine may be approximated as 500 ul. (R. F. Rosenbusch, personal communication.)[5] Utilizing a catherization method of collecting lachrymal fluid from cattle, Hoffman and Spadbrow (1978) obtained mean flow rates with a range of 0.18-1.86 ml/hr; Slatter and Edwards (1982) obtained mean flow rates of 1.96 ± 1.84 ml/hr (± s.d.). The ocular insert release rate requirements will vary due to different possible lachrymal fluid flow rates. However, the range of interest is known.

The hydrogel reservoir and monolithic/reservoir systems described above have achieved zero-order release characteristics and are composed of acceptable ophthalmic materials. The wide range of zero-order

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

tetracycline (M.W. 444.43; water solubility 1.7 mg/ml) release (0.54-28.9 ug/day) obtained by Olanoff and Anderson (1979) indicates that reservoir and monolithic/reservoir systems with a 98:2 MMA/2-HEMA control membrane may be utilized to provide zero-order release of tylosin tartrate (M.W. 1066.14; water solubility 300 mg/ml). Olanoff and Anderson (1979) and Olanoff et al. (1979) produced the 98:2 MMA/2-HEMA copolymer by polymerizing a 90:10 MMA/2-HEMA molar feed ratio of monomers. The composition of the copolymer was determined by nuclear magnetic resonance analysis. This study duplicated the copolymerization procedures utilized by Olanoff and Anderson (1979); therefore, a similar copolymer product is expected.

Quantitative analysis methods for tylosin tartrate

The absorbancy (absorbancy=absorbance x 10000/concentration (ug/ml)) of a one percent solution of tylosin tartrate in a cell with a one centimeter path is 255 at a wavelength of 290 nm. (B. Goodlow, personal communication.)[6] Therefore, spectrophotometry may be used to determine tylosin tartrate concentration in an otherwise unvarying solvent. Hoffman and Spadbrow (1978) found that the protein concentration in lacrymal fluid varied inversely with the flow rate and had a range of 2.94-12.35 mg/ml. Thus, spectrophotometry is not an acceptable method of measuring tylosin tartrate concentration in lacrymal fluid.

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[6] Sigma Chemical Company, Technical Service Representative, St. Louis, Missouri.

Thin layer chromatography (TLC) eliminates interference by other similarly acting agents since it is based on chemical and physical properties and not pharmacological properties; therefore, it is of possible interest. Debackere and Baeten (1971) utilized Silica Gel 254 TLC plates[7] and the developing and visualization process shown in Figure 8 to detect tylosin tartrate at concentrations of 2-4 ppm in water, blood plasma, urine, milk, tissue homogenates, and feed. The quantitative analysis of tylosin tartrate was performed by eluting the spots from the plate and conducting a spectrophotometric analysis of the eluted sample. This method of TLC analysis is time consuming, and technological advances since this study may permit a more sensitive, less time consuming analysis.

Commercially manufactured TLC plates have uniformly dispersed stationary phases of reproducible thickness. This development, and the simultaneous improvements in densitometer instrumentation permit quantitative analysis of samples directly on TLC plates. The relative standard deviation due to instrument variation is less than one percent, and quantitative determinations can achieve reproducibility of ± 2 percent (Touchstone and Dobbins, 1978). A densitometer trace of zones with increasing sample weight, constant volume, and constant zone diameter results in a linear relationship between peak area and weight (Stahl and Jork, 1968). Table 3 summarizes the various peak area measurement techniques. The first four methods were available in

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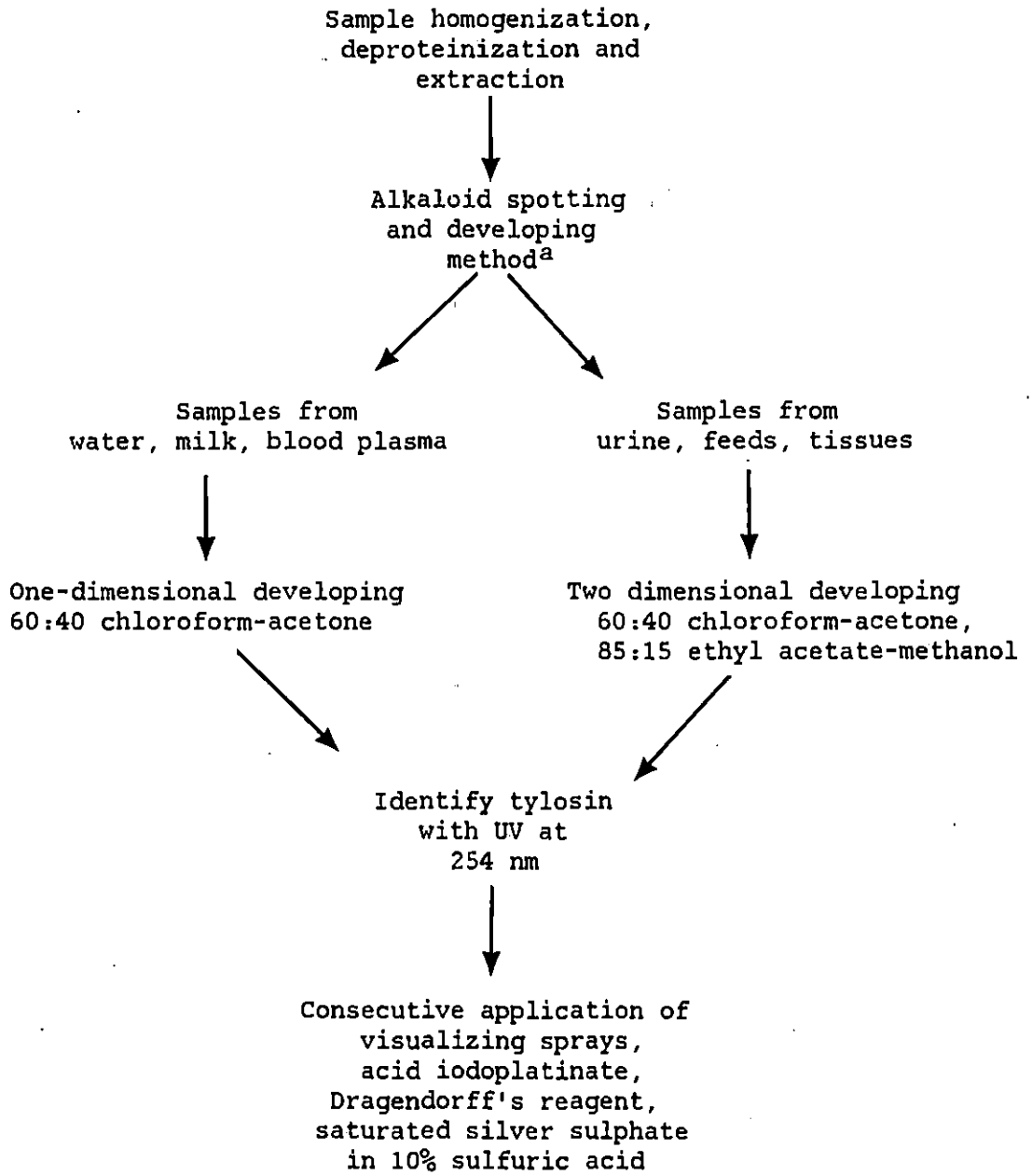
[7] Merck and Company Inc., Rahway, New Jersey.



[a](Debackere and Laruelle, 1964)

FIGURE 8. Thin layer chromatography developing and visualizing procedure for detecting tylosin tartrate. (Debackere and Baeten, 1971)

performing the work to be reported; selection of the "cut and weigh" method permitted good relative precision and provided a method of coping with irregularly shaped peaks. Touchstone and Dobbins (1978) found that peak height to width at half-height ratios of 1-10, and R_f values in the range of 0.3-0.7 improved the accuracy of the "cut and weigh" method. R_f is defined as

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

where the preadsorbent layer-stationary phase interface is the zero reference. Within the 0.3-0.7 R_f range, the sample area per unit of solute is most uniform; therefore, the densitometric analysis is most accurate.

TABLE 3. Relative precision of peak-area measurement techniques. (from Snyder and Kirland, 1974)

Method	Relative precision 1 s.d. (%)
Planimeter	3
Triangulation	3
Cut and Weigh	2
Height x 1/2 width	2
Ball and disk integrator	1
Electronic digital integrator	0.5
Computer	0.25

Early TLC work utilized a stationary phase that was more polar than the mobile phase (normal phase); however, a nonpolar, hydrocarbonaceous stationary phase and relatively more polar mobile phase (reverse phase) is useful for the separation of nonpolar compounds such as hydrocarbons, lipids, fatty acids, carotenoids, steroids, triglycerides, vitamins, and cholesterol esters (Sherma, 1981). The reversed phase plate is therefore of interest to provide a separation of tylosin from the other components of lachrymal fluid. (H. M. Stahr, personal communication.)[8] The hydrophobic stationary phase of the reversed phase plate limits the choice of spotting solvent to those that will produce small initial zones; unfortunately, these solvents may not solubilize the sample. An inert preadsorbent layer comprising the first two or three centimeters of the TLC plate acts as a blotter and reduces unfavorable spotting solvents to strong acids and bases (Sherma, 1982). Table 4 summarizes additional advantages of preadsorbent TLC plates compared with conventional plates. The preadsorbent layer is of particular importance to this study because it permits direct spotting of aqueous tylosin-saline solutions, eliminating the extraction process.

The improved resolution and sensitivity of detection permit more precise direct quantitative analysis with a densitometer, and the improved reproducibility of R_f values reduces scanning time.

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[8] Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.

TABLE 4. Advantages of preadsorbent TLC compared with conventional TLC. (from Sherma, 1982)

Rapid application of high volumes and dilute solutions
Automatic formation of uniform, narrow streaks at the layer interface
Improved resolution
Improved sensitivity of detection
Improved reproducibility of R_f values
Improved precision of quantitation by scanning
Less required sample cleanup because of retention of salts and certain insoluble interfering organic compounds in the preadsorbent
Analytes that may become irreversibly sorbed on active silica gel during drying of applied spots can be successfully chromatographed since substances do not contact the silica gel until they have passed through the preadsorbent, and then only in dissolved form
Very dilute solutions can be applied by immersion of the preadsorbent in the sample solution

The preadsorbent reversed phase TLC plate chosen for this application is the Whatman $LKC_{18}F$[9]. The union of the preadsorbent layer and octadecylsilane reversed phase TLC has resulted in a ten to thirty percent increase of resolving power over normal phase conventional plates (Sherma, 1982).

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[9] Whatman Chemical Separation Inc., Clifton New Jersey.

Whatman $LKC_{18}F$ TLC plates are coated with a silicon oil powder during the manufacturing process, and this powder produces a random pattern of brown and black spots when the TLC plate is visualized with sulfuric acid--methanol spray and heat. Full development of the TLC plates in methanol, before spotting, washes the silicon oil powder from the surface; consequently, a uniform, white-background is achieved on a visualized TLC plate. (H. M. Stahr, personal communication.)[10]

Whatman Chemical Separation Inc. (1981) recommends an 80:20 methanol/water solution as a starting point for the selection of a developing solution.

Charring, spraying a developed, preheated (110°C) silica gel TLC plate with concentrated sulfuric acid, is a common method of visualizing antibiotics. This process produces dark zones against a white background (Wagman and Weinstein, 1973). However, Whatman $LKC_{18}F$ TLC plates have octadecylsilane chemically bonded to the silica gel; consequently, charring techniques produce background discoloration (Sherma, 1981). Sherma (1981) recommends a uniform 90:10 methanol/sulfuric acid spray followed by 110-170°C heat for two to five minutes as a visualization method. He found spraying preferable to dipping as the former produced a lighter background.

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[10] Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.

PROCEDURES AND MATERIALS

Production of 90:10 MMA/2-HEMA Copolymer Films

The following materials were added in the order listed to the one-liter Erlenmeyer flask of the experimental apparatus shown in Figure 9: 570.0 ml of ethanol, 380.0 ml of type-one water[1], 6.1 ml of 2-HEMA[2], 46.6 ml of MMA[3], 0.2507 gm of sodium persulfate[4], and 0.1253 gm of potassium persulfate[5].

The flask was sealed with a rubber stopper and the liquid contents were bubbled with nitrogen for thirty minutes. After thirty minutes, slight positive nitrogen pressure was maintained on the system for the ten day copolymerization reaction carried out at room temperature (21-23°C). On day ten, the white copolymer precipitate and solvent were added to a four-liter beaker containing three liters of type-one water. Suction filtration of the copolymer was completed with a Buchner funnel and 7.0 cm, 1-qualitative filter paper[6]. After the initial filtration,

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[1] American Society for Testing Materials definition; 0.1 mg/l maximum total matter, 0.06 micromho/cm maximum electrical conductivity at 25°C, 16.67 megaohm.cm minimum electrical resistivity at 25°C, 60 minutes minimum color retention time of potassium permanganate, no detectable soluble silica.

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

[3] Adlrich Chemical Company Inc., Lot #041557, Milwaukee, Wisconsin.

[4] Aldrich Chemical Company Inc., Lot #0608HK, Milwaukee, Wisconsin.

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

[6] Ward R. Balston Limited, London, England.

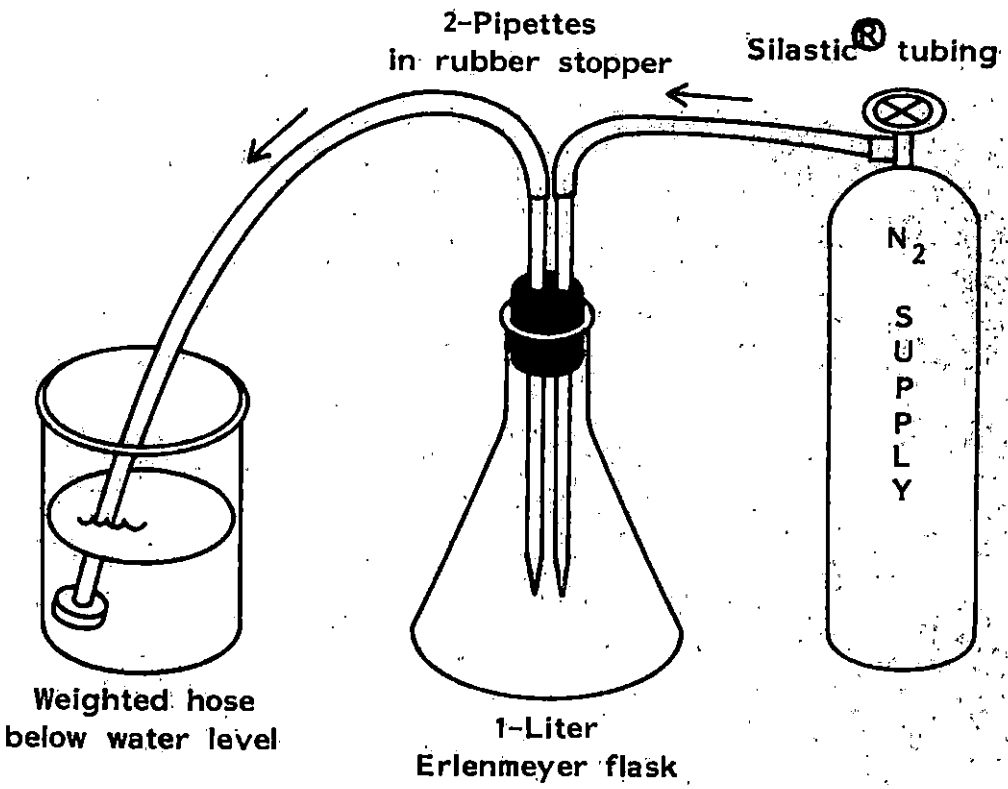

FIGURE 9. Experimental apparatus for 90:10 MMA/2-HEMA copolymer production

the copolymer in the funnel was washed four times with 25 ml amounts of type-one water before being placed into a 190 x 100 mm Pyrex glass container for drying. The storage container was covered with a filter paper[7] top which prevented contaminants from entering during the five day drying period (drying temperature of 50°C[8] 25 in Hg vacuum[9]).

Copolymer films (90:10 MMA/2-HEMA) were produced by the following process. Two grams of 90:10 MMA/2-HEMA copolymer, 12.0 ml of acetone, and 8.0 ml of dimethylformamide[10] were added to a 50 ml Erlenmeyer flask in the order listed. The flask was covered with a weighted watch glass (75 gm) and placed onto a preheated magnetic stirrer. Mixing and heating rates were adjusted to produce 200 rpm and 33-35°C values, respectively. After ten hours of mixing and heating, the solution was dispersed in approximately equal amounts onto three, siliconized[11], 75 x 50 mm plain-glass microscope slides. The films were produced by slowly pouring the solution from the Erlenmeyer flask in the pattern shown in Figure 10. Solution did not cover the entire slide; therefore, it could seek its own level. The poured films were immediately covered with a petri dish top, and were kept at room temperature (21-23°C) for the

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[7] Whatman Limited, Type 1-qualitative, London, England.

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

[9] The Welch Scientific Company, Duo Seal® Vacuum Pump, Model 1402, Skokie, Illinois.

[10] J. T. Baker Chemical Company, Lot #35107, Phillipsburg, New Jersey.

[11] PCR Research Chemicals, Inc. Prosil-28, Gainesville, Florida.

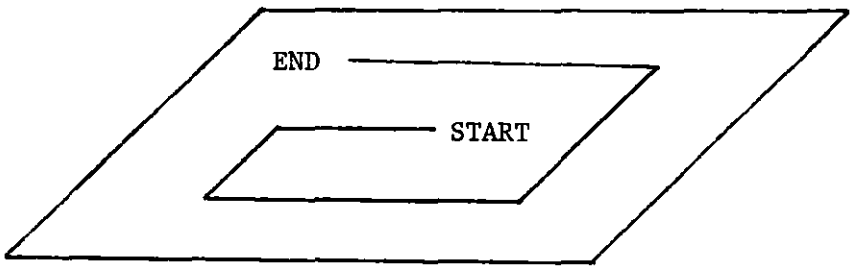

FIGURE 10. Pouring pattern for 90:10 MMA/2-HEMA films

evaporation process. Forty-eight hours after pouring, the cured films were separated from their glass slide by immersion into type-one water. The films floated free of the glass slide within five minutes. The separated films were soaked for ninety-six hours in a 600 ml glass beaker containing that same amount of type-one water. At twenty-four hour intervals, the type-one water was changed.

After the four-day soaking period, the films were removed from the beaker and immediately cut with a corkborer into 17.9 mm diameter disks. The disks were stored at room temperature (21-23°C) in a glass petri dish. After twenty-four hours, the disks were dry. The thickness of the dry disks was directly measured with a micrometer[12] and scanning electron microscopy[13] was utilized to obtain microstructural

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[12] L. S. Starrett Company, Model EDP 50940, Athol, Massachusetts.

[13] Japanese Electron Optics, Limited, Model U3, Tokoyo, Japan.

information. The disks were stored at room temperature in 15 x 10 cm polyethylene storage bags until needed.

Fabrication of Poly (Lactic Acid)-Tylosin Tartrate Matrix by the Cold Process

Rhine et al. (1980) produced a poly (ethylene/vinyl acetate)-protein matrix with uniform particle dispersion. Their fabrication procedures were adapted to produce a poly (lactic acid)-tylosin tartrate matrix.

One hundred milligrams of poly (lactic acid)[14], 100 mg of tylosin tartrate[15] and 5.0 ml of methylene chloride[16] were added in the order listed to a 25 ml Erlenmeyer flask. The flask was covered with a weighted watch glass (75 gm) and placed onto a magnetic stirrer. Mixing for five minutes at 100 rpm produced a solution. A 100 x 15 mm disposable petri dish was packed with granulated dry ice, and a siliconized, 75 x 50 mm plain-glass microscope slide was precooled for five minutes by placing it on top of the sealed petri dish. During precooling, the slide was covered with a second slide to prevent frost formation.

When the five minute mixing and precooling period elapsed, 4.4 ml of solution were pipetted onto the precooled, siliconized, glass slide by the pattern shown in Figure 11. The slide remained on the petri dish

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[14] Polysciences Inc., Lot #23062, Warrington, Pennsylvania.

[15] Sigma Chemical Company, Lot #89C-0315, St. Louis, Missouri.

[16] Fisher Scientific Company, Lot #721513, Fair Lawn, New Jersey.

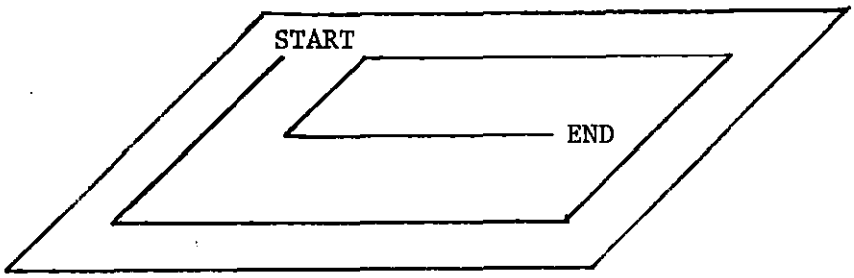

FIGURE 11. Pouring pattern for poly (lactic acid)-tylosin tartrate matrix

for ten minutes; the first three minutes it remained uncovered, and the last seven minutes it was covered with a disposable petri dish top. After ten minutes, the slide was transferred to a freezer (-20°C) for forty-eight hours. Upon removal from the freezer, forceps were utilized to pull the poly (lactic acid)-tylosin tartrate matrix from the siliconized glass slide. The matrix was transferred to a 160 x 255 mm glass desiccator and kept at room temperature (21-23°C) under a 1 mm Hg water-flow vacuum for forty-eight hours.

After forty-eight hours in the desiccator, the matrix was removed and immediately cut with a corkborer into eight 16.2 mm diameter-disks. Each disk was weighed[17], and its thickness was determined by placing a disk between two 3 x 1 inch plain-glass microscope slides of known thickness and measuring the change of thickness with a micrometer.

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[17] Mettler Instrumente AG, Model H31AR, Zurich, Switzerland.

Microstructural information was obtained by scanning electron microscopy. The disks were kept at room temperature in 15 x 10 cm polyethylene storage bags until needed.

Fabrication of Poly (Lactic Acid)-Tylosin Tartrate Matrix by the Room Temperature Process

One hundred milligrams of tylosin tartrate, 0.1 gm of poly (lactic acid) and 9 ml of methylene chloride were added in the order listed to a 25 ml Erlenmeyer flask. The flask was covered with a weighted watch glass (75 gm) and placed onto a magnetic stirrer. A solution was produced after mixing at 100 rpm for five minutes.

When the five minute mixing period elapsed, 4.4 ml of the solution were pipetted onto a siliconized, 75 x 50 mm plain-glass microscope slide by the pattern shown in Figure 11. The slide was covered with a petri dish top for the twenty minute room temperature (21°C) evaporation process. The dry film was pulled from the slide with forceps and was kept at room temperature in 15 x 10 cm polyethylene storage bags until needed.

Fabrication of Controlled-Release Systems

Three types of trilaminar systems were produced in quintuplicate. Table 5 indicates the composition of each layer. The following procedure was utilized to join the outer layers together. One gram of 90:10 MMA/2-HEMA copolymer, 6.0 ml of acetone, and 4.0 ml of

TABLE 5. Composition of trilaminar controlled-release systems

Controlled-release system sample number	Composition of inner layer	Composition of outer layer
32A 32B 32C 32D 32E	16.2 mm diameter poly (lactic acid)-tylosin tartrate[a] matrix containing approximately 4.84 mg of tylosin tartrate[a]	17.9 mm diameter disk of 90:10 MMA/2-HEMA
33A 33B 33C 33D 33E	Tylosin tartrate[a] (5 mg loading)	17.9 mm diameter disk of 90:10 MMA/2-HEMA
34A 34B 34C 34D 34E	Tylosin tartrate[a] (50 mg loading)	17.9 mm diameter disk of 90:10 MMA/2-HEMA

[a]Sigma Chemical Company, Lot #89C-0315, St. Louis, Missouri.

dimethylformamide were added in the order listed to a 50 ml Erlenmeyer flask. The flask was covered with a weighted watch glass (75 gm) and placed onto a preheated magnetic stirrer. Mixing and heating rates were adjusted to produce 200 rpm and 33-35°C values, respectively. A cloudy solution formed in six hours.

Figure 12 illustrates the assembly process. The bottom 90:10 MMA/2-HEMA disk was placed glass side down on a 75 x 50 mm plain-glass

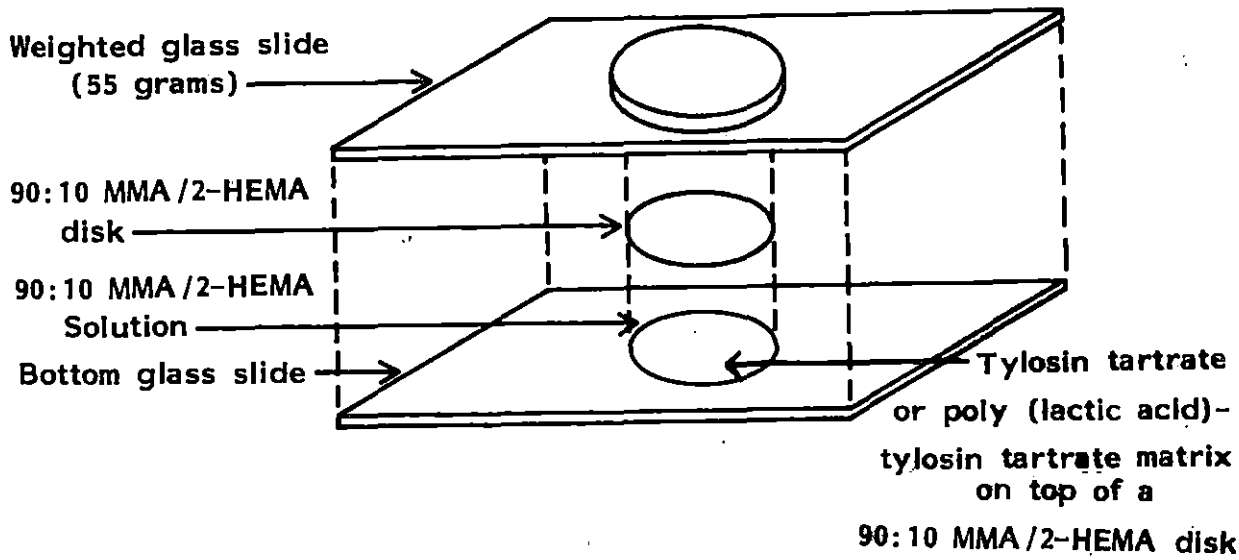

FIGURE 12. Controlled-release system assembly diagram

microscope slide. Tylosin tartrate was weighed directly onto the 90:10 MMA/2-HEMA disk for controlled-release system samples 33A-E and 34A-E. By comparison, a 16.2 mm diameter disk of the poly (lactic acid)-tylosin tartrate matrix (from the cold process) was placed on top of the 90:10 MMA/2-HEMA disk for controlled-release system samples 32A-E. The 90:10 MMA/2-HEMA solution produced above was dispersed along the outer perimeter of the 90:10 MMA/2-HEMA disk using a disposable 1cc syringe and 20G-1 needle. A second 90:10 MMA/2-HEMA disk was applied glass side up, and a weighted 75 x 50 mm plain-glass microscope slide (55 gm) covered the trilaminar system. Solvent evaporation at room temperature

(21-23°C) was completed in twenty-four hours. The edges of the completed controlled-release systems were examined for perforations at 40X with a stereomicroscope[18], and completed disks were stored in glass petri dishes at room temperature until utilized in the tylosin tartrate release-experiment forty-eight hours later.

Tylosin Tartrate Release-Experiment

Each of the fifteen controlled-release systems was placed into a 20 ml scintillation vial containing 2.0 ml of mammalian Ringer's solution[19]. The lids were lined with aluminum foil to prevent contamination of the samples from the glue used on the conventional cap liners, and the tightly sealed vials were placed into a shaking water bath[20] operating at 60 rpm and 37°C. At the time intervals shown in Table 6, the 2.0 ml of mammalian Ringer's solution was removed from each vial using a 3 ml disposable syringe and 22G-1 needle, and placed into a one-dram glass vial. A fresh 2.0 ml amount of mammalian Ringer's solution was added to each 20 ml scintillation vial by directing the flow into the bottom corner of the vial away from the insert. Each 20 ml scintillation vial had a separate syringe for collection; however, a single syringe was utilized for addition of mammalian Ringer's solution.

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[18] Nikon, Model 90783, Tokoyo, Japan.

[19] 8.60 gm sodium chloride, 0.30 gm potassium chloride, 0.33 gm calcium chloride, combined in one-liter volumetric flask and filled with type-one water.

[20] Fisher Scientific Company, Model 127, Pittsburgh, Pennsylvania.

TABLE 6. Collection times for the tylosin tartrate release-experiment

Sample number	Time of collection from beginning of experiment (hrs)	Sample number	Time of collection from beginning of experiment (hrs)
1	4	11	60
2	8	12	72
3	12	13	76
4	24	14	80
5	28	15	84
6	32	16	96
7	36	17	100
8	48	18	104
9	52	19	108
10	56	20	120

The one-dram glass vials containing the collected samples were placed into an oven[21] at 50°C and the liquid was totally evaporated.

TLC Spotting, Developing, Visualizing, and Quantitative Procedures

Whatman $LKC_{18}F$, 20 x 20 cm, TLC plates[22] were fully developed in a standard developing chamber[23] containing methanol[24]. The developed

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[21] GCA/Precision Scientific, Model 28, Chicago, Illinois.

[22] Whatman Chemical Separation Inc., Lot #002513, 002360, 002061, 002280, Clifton, New Jersey.

[23] Whatman Chemical Separation Inc., Type CDC-12, Clifton, New Jersey.

[24] Fisher Scientific Company, Lot #734176, Fair Lawn, New Jersey.

plates were air-dried at room temperature for a period of at least four days.

Ten microliter aqueous or saline solutions of tylosin tartrate were applied to the TLC plate with a Drummond 0-10 ul micropipette. Spotting was accomplished by depressing the plunger until an approximately 3 ul drop formed at the end of the glass dispenser tube. By touching the edge of the drop to the preadsorbent layer of the TLC plate, the drop was transferred to the plate.

Room temperature air which was passed through a drying tube of Drierite® was subsequently blown across the TLC plate's surface to facilitate the evaporation process.

When the spots were completely dried, each TLC plate was individually developed a distance of 8 cm in a standard developing chamber containing an eighty-five percent methanol and fifteen percent type-one water solution. Fresh solution was produced in 100 ml amounts and was utilized for each developing session. The chamber was equilibrated for one hour before developing TLC plates, and one side of the chamber was lined with filter paper[25] to maintain chamber equilibrium. TLC plates were developed with the gel side facing the liner. Developed plates were air-dried at room temperature before beginning the visualization procedure.

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[25] Whatman Chemical Separation Inc., Type 3MM-0.3 millimeter thickness, Clifton, New Jersey.

Visualization of the developed plate was accomplished by spraying[26] ten percent (by volume) sulfuric acid[27] in methanol[28] at a rate of 15 ml/min for fifteen seconds, and placing the sprayed plate into a 100°C oven for ten minutes. After ten minutes of heating, the plate was kept at room temperature for fifteen minutes as fading of the tylosin tartrate sample occurred. After the initial fading, the tylosin tartrate samples maintained their intensity for several hours. Quantitative analysis was conducted during the 15-120 minute period following heating.

A Kontes fiber optic scanner, model 800[29], was utilized to directly measure tylosin tartrate dark-spot intensity by cross scanning the TLC plate (perpendicular to the development direction). The output signal of the densitometer was transmitted to a linear plotter[30] which produced a trace of peaks for subsequent evaluation of areas. The lower limit of a peak area was determined by connecting the baseline on either side of the peak with a straight line. Each TLC plate was analyzed twice; consequently, there were two peaks for each tylosin tartrate spot. The

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[26] Kontes, Model K-422550, Vineland, New Jersey.

[27] Fisher Scientific Company, Lot #732068, Fair Lawn, New Jersey.

[28] Fisher Scientific Company, Lot #734176, Fair Lawn, New Jersey.

[29] Kontes Scientific Instrument Group, Vineland, New Jersey. Scan rate 2cm/min; dual-beam reflectance mode; phosphor coated disk #9660750 (red filter), emission peak 615nm, bandwidth 10nm; attenuator adjusted to produce peak height to half-height width ratios of one to ten.

[30] Linear Instruments Corporation, Model 255/MM, Irvine, California.

peaks were cut-out and weighed. The arithmetic mean of the two weights was utilized in the computer analysis.

Upon completion of the densitometric analysis, R_f was determined.

Evaluation of TLC Quantitative Analysis of Tylosin Tartrate

A melting point determination for tylosin tartrate was conducted. Tylosin tartrate/type one water standards were produced by the following procedure. One hundred milligrams of tylosin tartrate and 10 ml of type-one water were added to a 15 ml ground glass test tube. The contents were initially mixed for three minutes with the Vortex-Genie Mixer[31] (setting 5) to form a solution and were then placed into a dry, shaking water bath unit (setting 8) for three hours to ensure uniform dispersion. Subsequently, a 1:100 dilution was performed, and this solution was mixed in the same manner. The solution concentration was verified by a UV spectrophotometric analysis[32]. This analysis was conducted by measuring the absorbance (at 290 nm) of the tylosin tartrate type-one aqueous solution against a reference of pure type-one water.

The original solution and the 1:100 dilution were utilized to prepare the tylosin tartrate standards listed in Table 7. All dilutions were subjected to the mixing procedure outlined above, before subsequent dilutions were conducted.

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[31] Scientific Industries Inc., Bohemia, New York.

[32] Varian/Instrument Division, Model 219, Palo Alto, California.

TABLE 7. Tylosin tartrate standard concentrations (ug per 10 ul of solution).

0.10	0.50	0.90	4.0	8.0
0.20	0.60	1.0	5.0	9.0
0.30	0.70	2.0	6.0	10.0
0.40	0.80	3.0	7.0	

Two separate experimental procedures were conducted. Seven, 10 ul-amounts of a single standard were applied to a TLC plate at 2.0 cm intervals. This was repeated for each standard listed in Table 7. The method utilized to obtain the average weight of peak from a tylosin tartrate standard applied to a TLC plate may be found in the section entitled, TLC Spotting, Developing, Visualizing, and Quantitative Procedures. The average weight of peak for each of the seven standards on a TLC plate was analyzed with a computer statistical analysis program (SPSS Inc., 1983). Maximum, minimum, mean, standard deviation, range and scattergram data were calculated.

Standards were separated into three groups, 0.10-0.70, 0.80-5.0, and 4.0-10.0 for the second experiment. Ten microliters from each of the seven standards in a group was spotted onto a TLC plate at 2 cm intervals. Five TLC plates of each group were produced. The method utilized to obtain the average weight of peak from a tylosin tartrate standard applied to a TLC plate may be found in the section entitled, TLC Spotting, Developing, Visualizing, and Quantitative Procedures. The

average weight of peak for each of the seven standards on a TLC plate was analyzed with a computer statistical analysis program (SPSS Inc., 1983). The coefficient of determination (r^2), standard error, regression line equation, and scatterplot data were calculated.

Determination of the Tylosin Tartrate Concentration in the Release-Experiment Samples

The release-experiment samples were dried and sealed in one-dram glass vials with teflon screw caps at the beginning of this procedure. An Eppendorf pipette was utilized to add type-one water in 100 ul amounts until a solution was formed. Ten microliters from each sample were spotted onto a TLC plate at 1 cm intervals; thus, nineteen samples were on a single TLC plate. The spotting, developing, and visualizing process are outlined in the section entitled, TLC Spotting, Developing, Visualizing, and Quantitative Procedures. The sample spots of tylosin tartrate were visually compared with a TLC plate of tylosin tartrate standards to determine the need for subsequent dilutions. The dilution process was repeated until a tylosin tartrate concentration below 5.0 ug/10 ul was obtained for each release-experiment sample. This dilution process utilized 100 ul, 500 ul, and 1000 ul Eppendorf pipettes.

Quantitative analysis of the samples was conducted by applying a 10 ul amount of five samples and five standards to a TLC plate at 1.5 cm intervals. Tylosin tartrate standards were selected to correspond with the visually determined sample concentration. The method utilized to obtain average weight of peak from the tylosin tartrate spots on a TLC

plate may be found in the section entitled, TLC Spotting, Developing, Visualizing, and Quantitative Procedures. The average weight of peak for each of the five standards on a TLC plate was analyzed with a computer statistical analysis program (SPSS Inc., 1983). The coefficient of determination (r^2), standard error, regression line equation, and scatterplot data were calculated. The average weight of peak for each of the five release-experiment samples was substituted into the regression line equation to calculate tylosin tartrate concentration.

The above procedure utilized known concentrations of tylosin tartrate in a type-one aqueous solution to calculate the regression line equation. However, the average weight of peak substituted into the regression line equation to determine the amount of tylosin tartrate released was based on saline solutions of varying concentration. The following procedures were utilized to determine the effect of saline concentration on the TLC quantitative analysis method. Tylosin tartrate concentrations of 1.0, 2.0, 3.0, 4.0, and 5.0 ug/10 ul were produced utilizing type-one water and mammalian Ringer's solutions. Ten microliters from each of the ten solutions were applied to a Whatman $LKC_{18}F$ TLC plate at 1.5 cm intervals, and the TLC plate was developed and visualized by the method previously described. A densitometer analysis was conducted to determine pattern intensity variation due to differences in saline concentration of the application solvent.

The variability of R_f and of spot diffusion due to different salt concentrations in the application solvents was examined by applying 10 ul samples with varying saline concentrations, but identical tylosin tartrate amounts, to a Whatman $LKC_{18}F$ TLC plate. The saline concentrations were produced by the following procedure. Two milliliter mammalian Ringer's solutions (with the tylosin tartrate concentrations shown in Table 8) were placed into one-dram vials and totally evaporated at 50°C. The salts and tylosin tartrate were resolubilized by adding type-one water in the amounts shown in Table 8. Ten microliters of the three saline concentrations and a type-one water solution containing the same concentration of tylosin were applied in duplicate to a Whatman $LKC_{18}F$ TLC plate at 1.5 cm intervals, and the TLC plate was developed, visualized, and analyzed by the method previously described.

The average weight of peak for each of the eight samples on a TLC plate was analyzed using a computer statistical analysis program (SPSS Inc., 1983). Maximum, minimum, mean, standard deviation, range and scattergram values were calculated.

TABLE 8. Preparation of application solvents with various salt concentrations

Vial number	Tylosin tartrate in mammalian Ringer's solution (ug/ml)	Amount of type-one water added to dry vial (ul)	Amount of tylosin tartrate on a TLC plate (ug)
1	10	100	2
2	30	300	2
3	50	500	2
4	15	100	3
5	45	300	3
6	75	500	3
7	20	100	4
8	60	300	4
9	100	500	4

RESULTS

Before thin layer chromatography could be utilized to detect the amount of tylosin tartrate in controlled-release experiment samples, the suitability and reliability of the process had to be established. Therefore, the first topics to be discussed are the TLC process and the justification for the utilization of particular solutions and operational parameters. Next, the spot patterns of tylosin tartrate aqueous and saline solutions are compared and contrasted by examining spot shape, spot size, and R_f.

This report utilized direct densitometric scanning of the TLC plate to quantify the amount of tylosin tartrate in a controlled-release experiment sample. In order to conduct such a procedure, the variability and linearity of the peak weight to tylosin tartrate relationship for aqueous solutions and the variability for saline solutions must be known. The results of the three experiments utilized to determine these values are discussed.

The second area of emphasis in this report is the production of a controlled-release system that may be utilized for the treatment of BIK. Three types of devices were studied in quintuplicate. The discussion begins with the production of the control membrane and inner drug layer, and details scanning electron microscopy results of microstructural properties. Next, the controlled-release system assembly procedure is reviewed, and results of a pre-experiment examination of the systems with a stereomicroscope are given.

Finally, the details of the controlled-release experiment are reviewed, and the tylosin tartrate summary release profiles are presented in graphic and tabular form (complete data utilized to produce the profiles are located in the Appendices). The release profiles are evaluated in relation to the appropriate lachrymal flow rate and the results of a post-experiment stereomicroscopic examination.

TLC Spotting, Developing, Visualizing, and Quantitative Analysis Process for Tylosin Tartrate

Full development (based on methanol) of 20 x 20 cm Whatman $LKC_{18}F$ TLC plates took approximately one hour, and provided a uniform, white-background upon visualization.

Ten microliter amounts of application solution were utilized for all cases. The 10 ul solution was applied in approximately 3 ul amounts because the drop remained at the end of the micropipette glass dispenser and could be transferred completely to the TLC plate. Larger drops tended to form along the side of the micropipette glass dispenser and would not transfer completely to the TLC plate. The room temperature air flow permitted the 10 ul amount to be applied in approximately three minutes.

Developing solution of eighty-five percent methanol, fifteen percent type-one water produced R_f values in the 0.3-0.7 range. The R_f results will be discussed in a subsequent section.

The ten percent (by volume) sulfuric acid in methanol visualization spray produced a white background and a brown tylosin tartrate pattern. Increased spraying or heating periods caused the background to darken. Conversely, the lower concentrations of tylosin tartrate were not visible with shorter spraying and heating periods.

The densitometer was operated in the dual-beam, reflectance mode. Figure 13 shows densitometer traces of the same three samples on a Whatman $LKC_{18}F$ TLC plate for the transmission, reflectance, and reflectance/transmission modes. The transmission mode consistently produced a more erratic baseline than the other two modes. The reflectance and reflectance/transmission modes both produced smooth baselines; however, the reflectance mode provided additional damping of inflection points on the peaks. Therefore, the dual-beam reflectance mode was selected for densitometric analysis.

The densitometer attenuator-control and the plotter input-control were adjusted to provide a smooth baseline and to be within a ratio of peak height to width at half-height of one to ten. Figures 14 - 16 illustrate the peak traces for 0.2-0.8 ug, 1.0-4.0 ug, and 5.0-10.0 ug of tylosin tartrate applied as a type-one aqueous solution. The peaks are uniformly shaped and the baselines are smooth.

On some TLC plates, the visualized pattern for tylosin tartrate amounts near 0.2 ug lacked intensity. Thus, it was not always possible to maintain a smooth baseline and the desired ratio. In these cases, the smooth baseline was compromised somewhat in order to obtain a trace

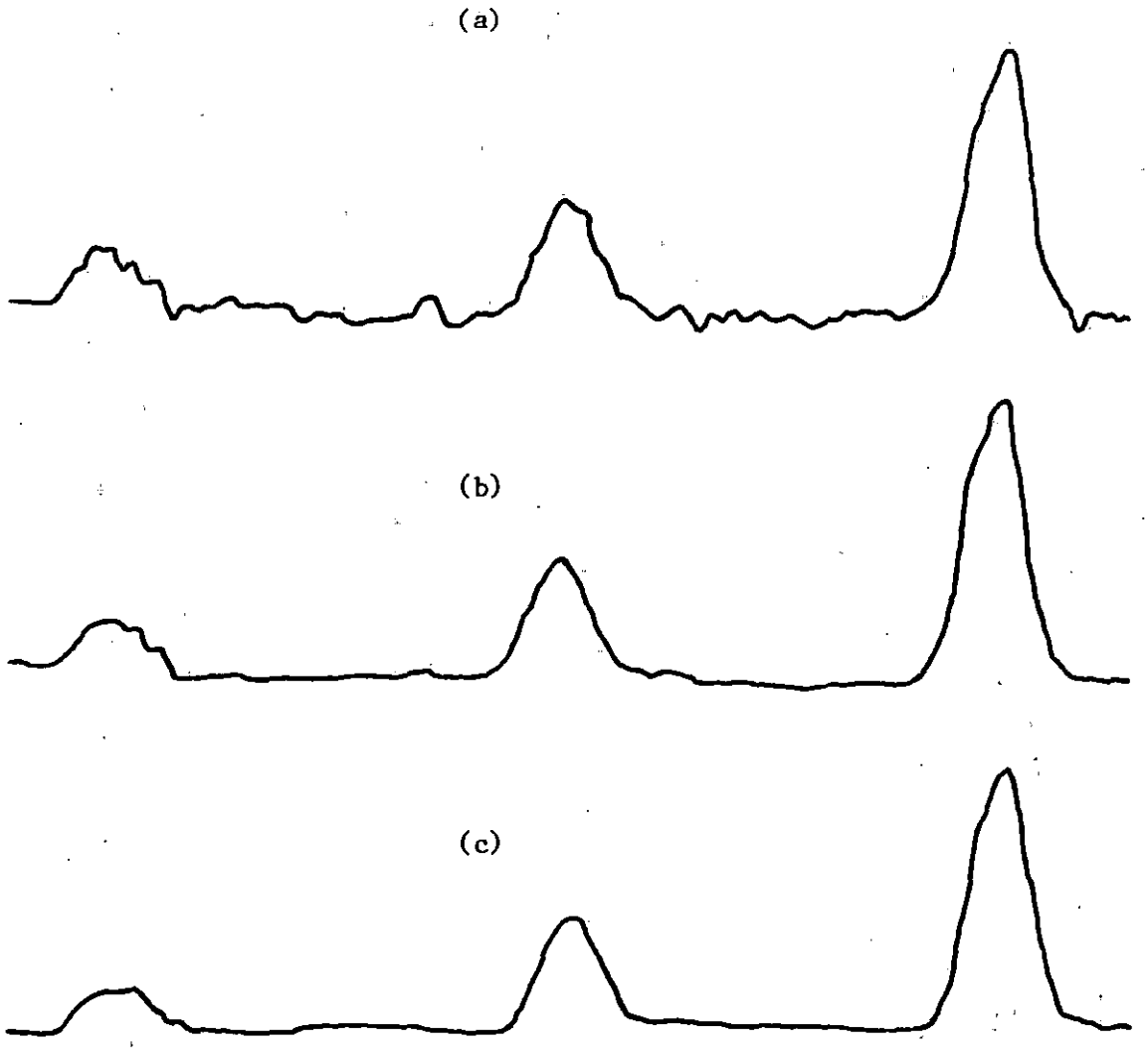

FIGURE 13. Densitometer trace characteristics of (a) transmission mode, (b) transmission/reflectance mode, (c) reflectance mode

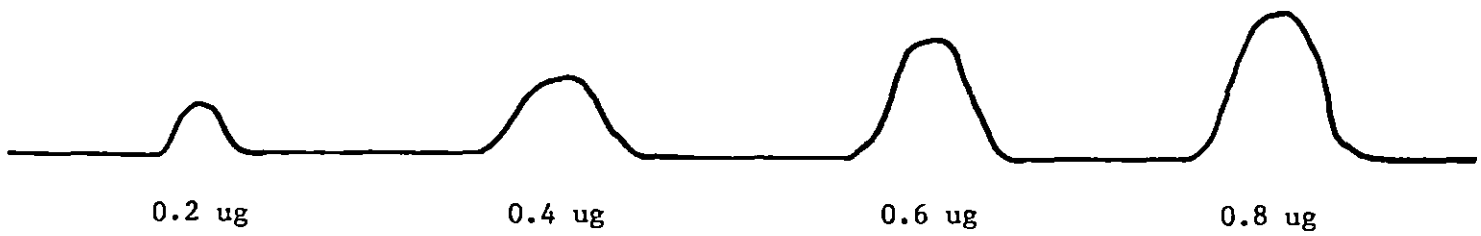

FIGURE 14. Densitometer trace for 0.2-0.8 ug of tylosin tartrate applied as a type-one aqueous solution. Densitometer attenuator-control 8, plotter input-control 100mV

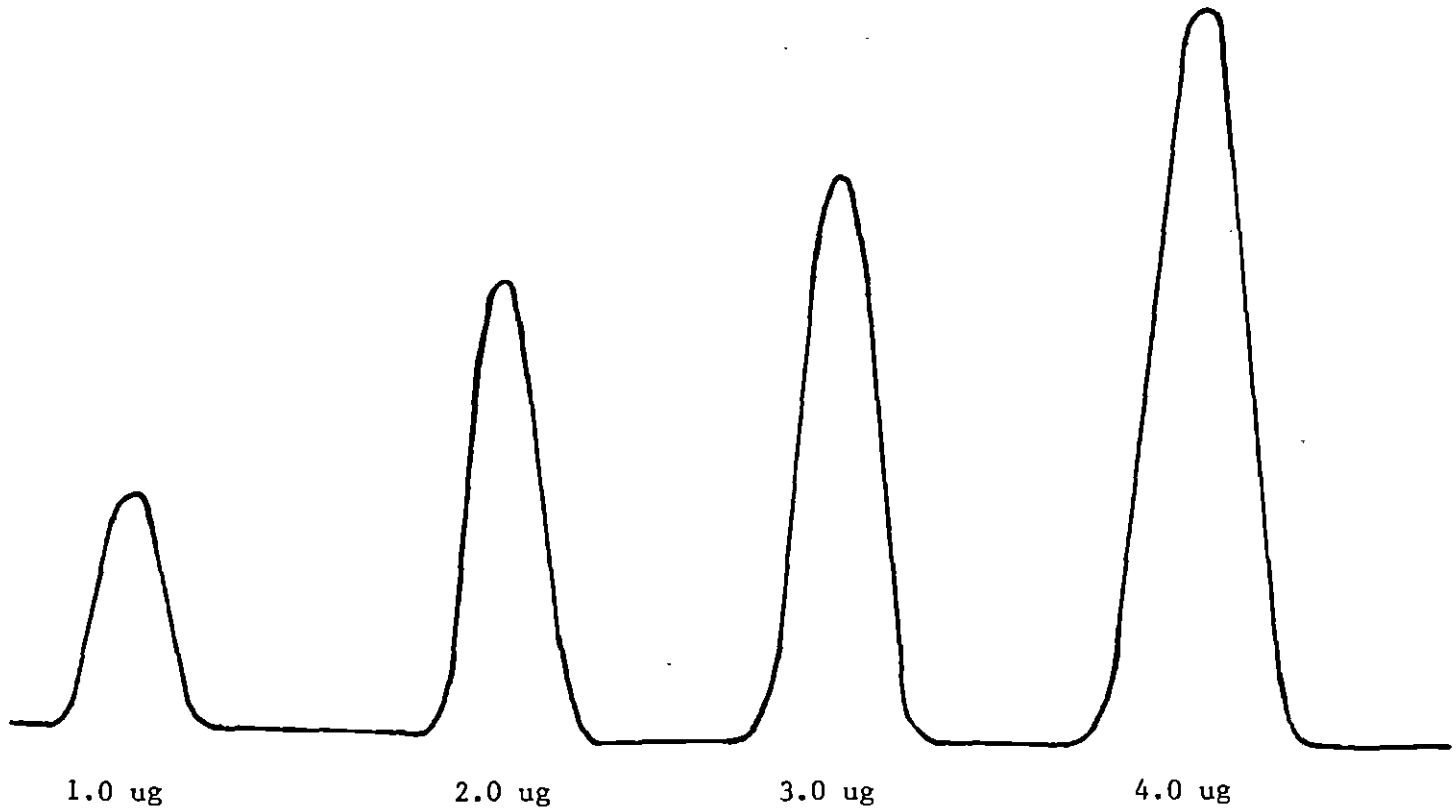

FIGURE 15. Densitometer trace for 1.0-4.0 ug of tylosin tartrate applied as a type-one aqueous solution. Densitometer attenuator-control 8, plotter input-control 100mV

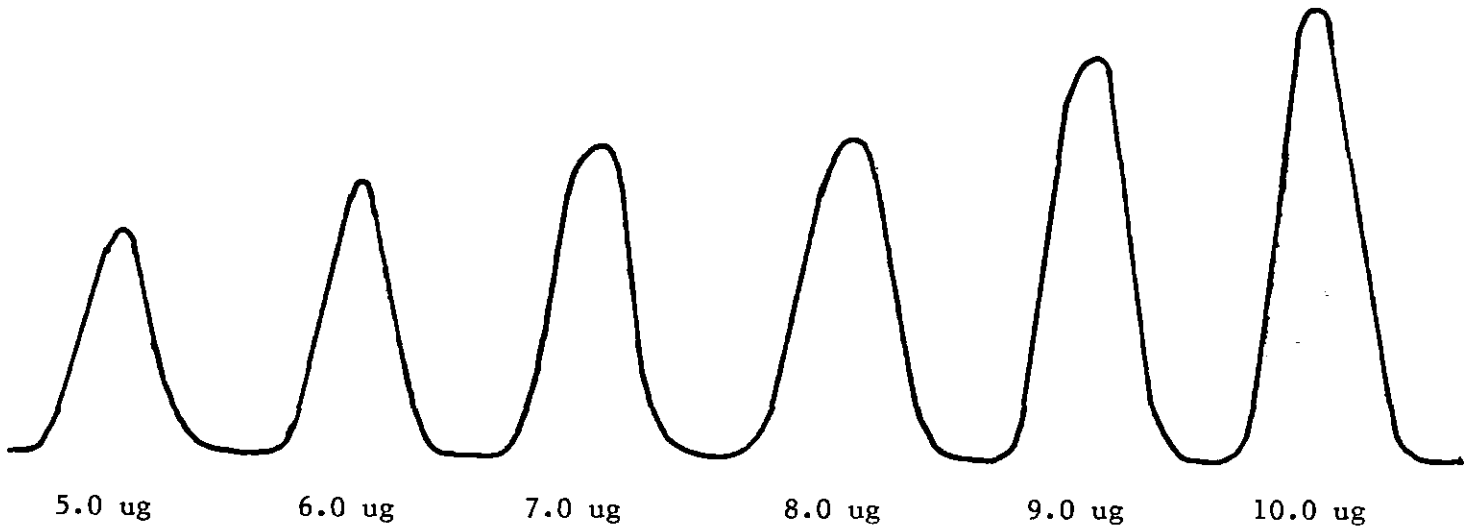

FIGURE 16. Densitometer trace for 5.0-10.0 ug of tylosin tartrate applied as a type-one aqueous solution. Densitometer attenuator-control 16, plotter input-control 100mV. Baseline distances reduced to accommodate peaks.

near the desired peak height to width at half-height ratio. Figure 17 shows a densitometer trace for this type of sample. The peaks have irregular shapes, and baseline fluctuations introduce uncertainty in the selection of the peak lower limit. Figure 18 illustrates the baseline location for these types of samples. The location results in approximately equal areas of fluctuation above and below the baseline. Fluctuations resulting from visible imperfections on the plate were discounted from this process.

Densitometer scan-speed and plotter paper-speed were adjusted to conserve paper, yet produce a stable baseline recording between peaks. The peak width was also affected by these settings.

Densitometric analysis of all plates was performed in duplicate to reduce errors from densitometer variation, chart paper differences, and the "cut and weigh" process.

Tylosin Tartrate Type-One Aqueous Solution TLC Pattern

Tylosin tartrate in a type-one aqueous solution on Whatman $LKC_{18}F$ TLC plates produced the pattern (brown) shown in Figure 19. A 0.1 ug quantity of tylosin tartrate was visible one of the twelve times that it was applied to six different TLC plates; however, 0.2-10.0 ug quantities were always visible.

Figure 19 illustrates that each amount of tylosin tartrate in the range of 0.2-0.7 ug was visualized as a dark spot and a less intense band at greater R_f; 0.8-10.0 ug amounts were visualized in order of

FIGURE 17. Densitometer trace of tylosin tartrate in type-one aqueous solution for visualized patterns of low intensity. Densitometer attenuator-control 32, plotter input-control 10mV

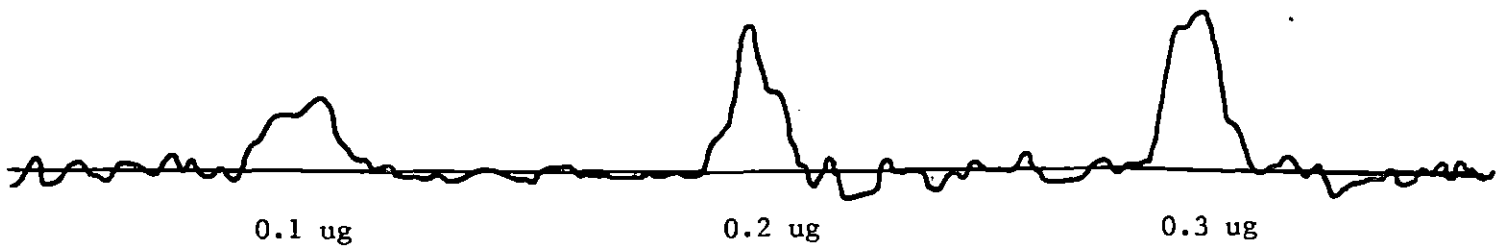

FIGURE 18. Baseline location for visualized patterns of low intensity. Densitometer attenuator-control 32, plotter input-control 10mV

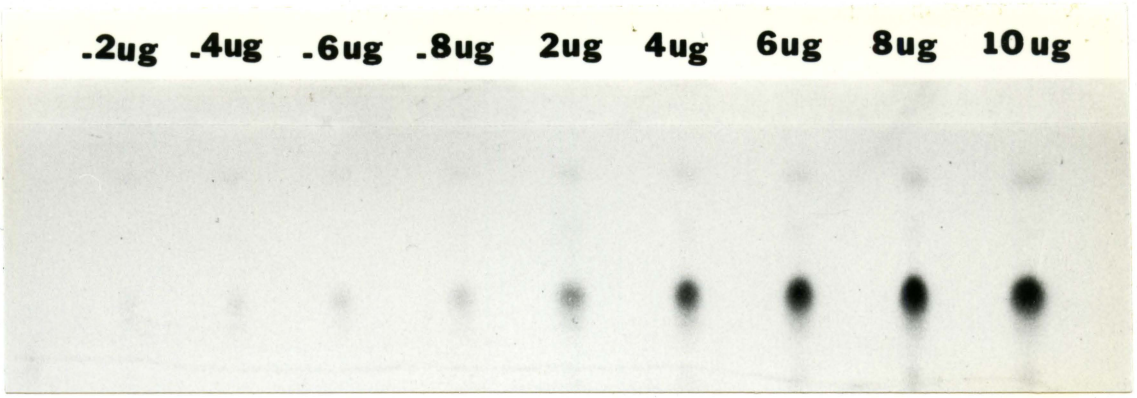

FIGURE 19. Tylosin tartrate pattern on a Whatman $LKC_{18}F$ TLC plate, applied as a type-one aqueous solution.

increasing R_f, as a dark spot, a light, diffuse spot, and a band of medium intensity. Tailing of the dark spot occurs at 5.0-10.0 ug quantities of tylosin tartrate. The formation of a comet-like trail (tailing) will occur with any sample if sufficient material is applied to the TLC plate.

Tylosin Tartrate Saline Solution TLC Pattern

The saline concentration of the application solution influences spot diameter. Figure 20 is a photograph of a Whatman $LKC_{18}F$ TLC plate with 1.0-5.0 ug tylosin tartrate samples applied as 10 ul amounts of type-one aqueous solutions (left) and 10 ul amounts of mammalian Ringer's solutions (right). The tylosin tartrate samples applied with the mammalian Ringer's solution produce diffuse, (brown) spot-patterns. The distance between the dark spot and light, diffuse spot and the light, diffuse spot and band of medium intensity are reduced for the mammalian Ringer's solution samples.

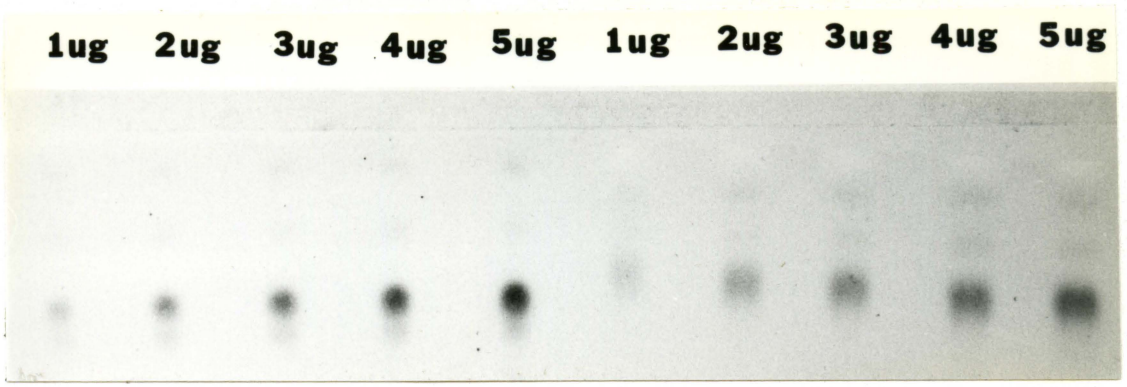

FIGURE 20. Variation of tylosin tartrate pattern with application solution. Type-one aqueous solution (left) and mammalian Ringer's solution (right)

Tylosin Tartrate Type-One Aqueous Solution R_f

Table 9 contains the R_f values for seven identical amounts of tylosin tartrate in a type-one aqueous solution, applied to a Whatman $LKC_{18}F$ TLC plate. R_f values of the tylosin tartrate groups on Whatman $LKC_{18}F$ TLC plates are listed in Table 10. The data indicate that the R_f values are quite reproducible. This reproducibility expedites the densitometric scanning process since it permits an entire plate to be cross-scanned without intermediate alignment.

In all cases, the dark spot R_f's are within the 0.3-0.7 range suggested by Touchstone and Dobbins (1978).

Tylosin Tartate Saline Solution R_f

The variation of R_f due to different application solution saline concentrations was examined by applying various saline concentrations

TABLE 9. R_f values of seven identical amounts of tylosin tartrate on a Whatman $LKC_{18}F$ TLC plate, applied as a type-one aqueous solution

TLC plate	Amount of tylosin tartrate (ug)	Dark spot (R_f)	Diffuse spot (R_f)	Band (R_f)
1	0.2	0.57	----	0.86
2	0.3	0.58	----	0.88
3	0.4	0.54	----	0.89
4	0.5	0.57	----	0.93
5	0.6	0.57	----	0.90
6	0.7	0.56	----	0.83
7	0.8	0.57	0.71	0.86
8	0.9	0.57	0.71	0.86
9	1.0	0.57	0.73	0.86
10	2.0	0.58	0.72	0.84
11	3.0	0.57	0.71	0.85
12	4.0	0.58	0.69	0.85
13	5.0	0.57	0.71	0.86
14	6.0	0.58	0.72	0.85
15	7.0	0.58	0.73	0.87
16	8.0	0.58	0.72	0.87
17	9.0	0.57	0.71	0.88
18	10.0	0.57	0.70	0.85

TABLE 10. R_f values of tylosin tartrate groups on Whatman $LKC_{18}F$ TLC plates, applied as a type-one aqueous solution

TLC plate	Range of tylosin tartrate (ug)	Dark spot (R_f)	Diffuse spot (R_f)	Band (R_f)
1	0.1-0.7	0.56	----	0.79
2	0.1-0.7	0.59	----	0.88
3	0.1-0.7	0.58	----	0.87
4	0.1-0.7	0.56	----	0.88
5	0.1-0.7	0.57	----	0.86
6	0.8-5.0	0.57	0.72	0.88
7	0.8-5.0	0.54	0.70	0.88
8	0.8-5.0	0.55	0.72	0.87
9	0.8-5.0	0.54	0.72	0.86
10	0.8-5.0	0.56	0.71	0.87
11	4.0-10.0	0.56	0.72	0.85
12	4.0-10.0	0.57	0.71	0.87
13	4.0-10.0	0.57	0.71	0.86
14	4.0-10.0	0.56	0.72	0.87
15	4.0-10.0	0.56	0.72	0.87

with an identical tylosin tartrate amount to a Whatman $LKC_{18}F$ TLC plate. The R_f values obtained from this procedure are listed in Table 11.

TABLE 11. R_f variation due to saline concentration differences of the application solution

TLC plate	Amount of tylosin tartrate (ug)	Amount of type-one water added to the salts of 2 ml of Ringer's solution (ul)	Dark spot (R_f)	Diffuse spot (R_f)	Band (R_f)
1	2.0	100% type-one water	0.56	0.70	0.91
1	2.0	100.0	0.55	0.66	0.85
1	2.0	300.0	0.69	0.83	0.94
1	2.0	500.0	0.73	0.85	0.94
2	3.0	100% type-one water	0.59	0.70	0.89
2	3.0	100.0	0.61	0.72	0.87
2	3.0	300.0	0.70	0.83	0.95
2	3.0	500.0	0.74	0.87	0.98
3	4.0	100% type-one water	0.56	0.70	0.88
3	4.0	100.0	0.54	0.66	0.78
3	4.0	300.0	0.64	0.78	0.90
3	4.0	500.0	0.68	0.80	0.94

There are no significant differences in R_f between the one hundred percent type-one water and 100.0 ul saline solutions. However, the R_f's of the 300.0 ul and 500.0 ul saline solutions are consistantly greater than those of the corresponding one hundred percent type-one water solutions. The R_f's of the 500.0 ul solutions are greatest in all

cases. In order to complete a densitometer cross-scan of these plates, realignment was necessary at the 300.0 ul and 500.0 ul saline solutions. The dark spot R_f's are above the 0.3-0.7 range for two of the 500.0 ul saline solutions.

Tylosin Tartrate Saline Solution Spot Diffusion

Saline concentration of the application solution influences the tylosin tartrate spot diameter. This phenomenon was examined by applying various saline concentrations with an identical tylosin tartrate amount to a Whatman $LKC_{18}F$ TLC plate. Table 12 is a summary of the dark spot diameters. The spot diameters tend to increase as the viscosity of the resultant solution decreases.

The one hundred percent type-one water and 100.0 ul saline solutions have comparable spot diameters. Spot diameters increase for the 300.0 ul saline solutions and are greatest for 500.0 ul saline solutions. The spot diameters of the mammalian Ringer's solution samples in Figure 20 are comparable to the values of the 500.0 ul saline solutions in Table 12. In Figure 20, an amount of drug is present in 10 ul of type-one water at five different concentrations, and these are spotted on the TLC plate (left side). Also, the same levels of drug are present in 10 ul of Ringer's solution which are spotted on the TLC plate (right side). In Table 12, the solutions are dried down in a one-dram vial, and then specific amounts of type-one water are added to the salts and drug deposit. For the 500.0 ul cases in Table 12, spot diameters

TABLE 12. Variation of dark spot diameter due to saline concentration differences of the application solution.

TLC plate	Amount of tylosin tartrate (ug)	Amount of type-one water added to the salts of 2 ml of Ringer's solution (ul)	Dark spot diameter (mm)
1	2.0	100% type-one water	2.5
1	2.0	100.0	3.0
1	2.0	300.0	3.5
1	2.0	500.0	5.0
2	3.0	100% type-one water	2.5
2	3.0	100.0	3.0
2	3.0	300.0	4.0
2	3.0	500.0	4.5
3	4.0	100% type-one water	2.5
3	4.0	100.0	3.0
3	4.0	300.0	3.5
3	4.0	500.0	4.5

are recorded and are similar to those for the Ringer's solution cases in Figure 20. Thus, the saline concentrations for the Ringer's solution samples spotted and indicated in Figure 20 were lower (by 1/4) than that of the 500.0 ul cases of Table 12, and the spot diameters were about the same (approximately 4.8 mm compared to 4.5 to 5.0 mm from Table 12). Therefore, dilution experiments greater than 500.0 ul were not conducted or reported for the series listed in Table 12.

Statistical Evaluation of the TLC Quantitative Analysis of Tylosin Tartrate

Variability and reliability of the TLC quantitative analysis of tylosin tartrate type-one aqueous solutions

The variability and reliability of the TLC quantitative analysis of tylosin tartrate were evaluated by the application of seven identical amounts of tylosin tartrate, in a type-one aqueous solution, to a Whatman $LKC_{18}F$ TLC plate and subsequent characterization. The average weight of peak data obtained from the TLC quantitative analysis are contained in Appendix A.

A statistical analysis of the data contained in Appendix A is shown in Table 13. Due to variations among the TLC plates, the results from different plates may be compared only if the data are normalized. The coefficient of variance (standard deviation/mean) is a normalized value. An examination of the coefficient of variance (Table 13) indicates that there is less variability for the 0.9-10.0 ug range than the 0.2-0.8 ug range. This pattern is expected since a steady reduction in contrast between sample-spot and background occurs as the amount of tylosin tartrate decreases.

Linearity of the relationship between average weight of peak and tylosin tartrate amount for type-one aqueous solutions

TLC quantitative analysis relies upon the linear relationship between average weight of peak and tylosin tartrate amount for the quantitation of unknown amounts of tylosin tartrate. The linearity of

TABLE 13. Variability and reliability of the TLC quantitative analysis of tylosin tartrate.

TLC plate	Amount of tylosin tartrate (ug)	Maximum average weight of peak (gm)	Minimum average weight of peak (gm)	Mean average weight of peak (gm)	Standard deviation of average weight of peak (gm)	Coefficient of Variance
1	0.2	0.011	0.004	0.007	0.002	0.286
2	0.3	0.011	0.008	0.010	0.001	0.100
3	0.4	0.024	0.013	0.018	0.004	0.222
4	0.5	0.024	0.017	0.021	0.003	0.143
5	0.6	0.033	0.019	0.027	0.005	0.185
6	0.7	0.023	0.013	0.017	0.003	0.176
7	0.8	0.014	0.009	0.011	0.002	0.182
8	0.9	0.023	0.017	0.019	0.002	0.105
9	1.0	0.015	0.010	0.013	0.001	0.077
10	2.0	0.019	0.017	0.018	0.001	0.056
11	3.0	0.056	0.049	0.052	0.003	0.058
12	4.0	0.016	0.012	0.014	0.001	0.071
13	5.0	0.031	0.022	0.028	0.003	0.105
14	6.0	0.036	0.030	0.033	0.002	0.061
15	7.0	0.037	0.030	0.035	0.003	0.086
16	8.0	0.049	0.037	0.044	0.004	0.091
17	9.0	0.071	0.060	0.067	0.004	0.060
18	10.0	0.095	0.073	0.087	0.007	0.080

the relationship was evaluated by dividing the tylosin tartrate type-one aqueous solution standards into three groups. Each group of standards was applied to five Whatman $LKC_{18}F$ TLC plates. The average weight of peak data obtained from the TLC quantitative analysis are contained in Appendix B.

Regression line calculations were conducted utilizing the data in Appendix B. The statistical summary in Table 14 shows the linearity of the experimental data in Appendix B.

TABLE 14. Linearity of the relationship between average weight of peak and tylosin tartrate amount.

TLC plate	Range of tylosin tartrate (ug)	Coefficient of determination (r^2)	Standard error x 10^{-3}
1	0.1 - 0.7	0.984	0.915
2	0.1 - 0.7	0.673	2.014
3	0.1 - 0.7	0.898	1.007
4	0.1 - 0.7	0.726	2.614
5	0.1 - 0.7	0.707	2.165
6	0.8 - 5.0	0.967	7.288
7	0.8 - 5.0	0.995	2.577
8	0.8 - 5.0	0.991	2.161
9	0.8 - 5.0	0.981	2.311
10	0.8 - 5.0	0.985	6.034
11	4.0 - 10.0	0.992	1.121
12	4.0 - 10.0	0.951	3.052
13	4.0 - 10.0	0.986	1.122
14	4.0 - 10.0	0.931	2.606
15	4.0 - 10.0	0.997	0.627

The coefficient of determination (r^2) in Table 14 indicates how well the data fit a linear regression calculation. An r^2 value of one denotes a perfect linear relationship, and an r^2 value of zero means that no linear relationship exists. The data in Table 14 indicate greater deviation from a linear relationship for the 0.1-0.7 ug group

than for the other two groups. This correlates well with the results of the variability and reliability study discussed above.

Variability and reliability of the TLC quantitative analysis of tylosin tartrate saline solutions

The saline concentration of the application solution affects spot diameter and R_f, which in turn influence the densitometric analysis. The variability and reliability of the TLC quantitative analysis of tylosin tartrate due to different saline concentrations were examined by applying various saline concentrations with an identical tylosin tartrate amount to a Whatman $LKC_{18}F$ TLC plate. The average weight of peak data obtained from the TLC quantitative analysis are in Appendix C.

A statistical analysis of the data in Appendix C is shown in Table 15. The coefficients of variance in Table 15 are greater than the coefficients of variance for identical amounts of tylosin tartrate in Table 13.

The coefficients of variance in Table 15 indicate the maximum variability of a dry release-experiment sample solubilized with 500.0 ul of type-one water. The data in Appendix C indicate a direct relationship between peak weight variability and amount of type-one water. Therefore, less variability is expected for release experiment samples resolubilized with smaller amounts of type-one water.

TABLE 15. Variability and reliability of the TLC quantitative analysis of tylosin tartrate for 500.0 ul saline solutions.

TLC plate	Amount of tylosin tartrate (ug)	Maximum average weight of peak (gm)	Minimum average weight of peak (gm)	Mean average weight of peak (gm)	Standard deviation of average weight of peak (gm)	Coefficient of Variance
1	2.0	0.014	0.008	0.011	0.002	0.182
2	3.0	0.019	0.014	0.017	0.002	0.118
3	4.0	0.024	0.016	0.019	0.002	0.105

Tylosin Tartrate Release-Experiment

Production of the 90:10 MMA/2-HEMA copolymer films

After the ten hour mixing and heating period, a cloudy solution that occasionally contained gel material formed. The dried films were clear, with some regions of small air bubbles. Although flexible, the films broke abruptly when bent to an angle of approximately ninety degrees. Films soaked in water became cloudy and were easily cut into disks with a corkborer; by comparison, dry films broke when cut. A total of 175 disks were produced from film regions without air bubbles.

Disk thickness ranged from 0.097-0.419 mm. Thirty disks of minimum thickness, but within a ± 10% tolerance, were required for the release-experiment. Thirty-one disks with a 0.223 ± 10% mm thickness were available; therefore, they were selected as the control membranes.

Figure 21 shows the film surface cast in contact with glass. The surface is smooth with no visible pores. The film surface cast in contact with air is also smooth with no visible pores (Figure 22).

Production of the poly (lactic acid)-tylosin tartrate matrix

A poly (lactic acid)-tylosin tartrate matrix was utilized for the inner layer of controlled-release systems 32A-E. Scanning electron microscopy revealed that tylosin tartrate formed a layer between the poly (lactic acid) film and the siliconized, plain-glass slide (room temperature production). Figure 23 shows the surface cast in contact with glass of the matrix that was prepared at room temperature, and the micrograph in Figure 24 is the corresponding surface of a poly (lactic acid) film produced in the identical manner, but without tylosin tartrate. Immersion of the poly (lactic acid)-tylosin tartrate matrix, in a 37°C mammalian Ringer's solution for thirty hours, removed the tylosin tartrate layer (Figure 25). The cold production method appears to keep the tylosin tartrate within the matrix. Figure 26 shows the surface cast in contact with glass of a poly (lactic acid)-tylosin tartrate matrix produced by the cold method, and Figure 27 is the same surface after a thirty-hour immersion in 37°C mammalian Ringer's solution.

This SEM analysis indicated that the cold production poly (lactic acid)-tylosin tartrate matrix would be the more favorable drug reservoir; therefore, it was utilized in controlled-release systems 32A-E.

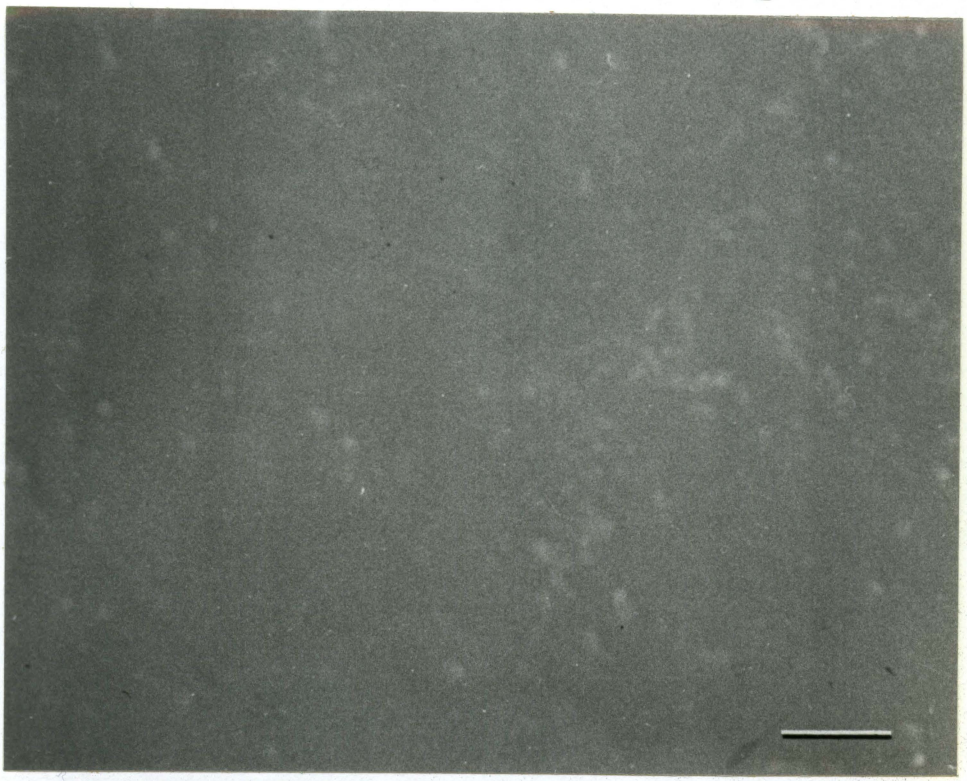

FIGURE 21. 90:10 MMA/2-HEMA cast film. Surface in contact with glass (scale bar=40 um). 25 keV.

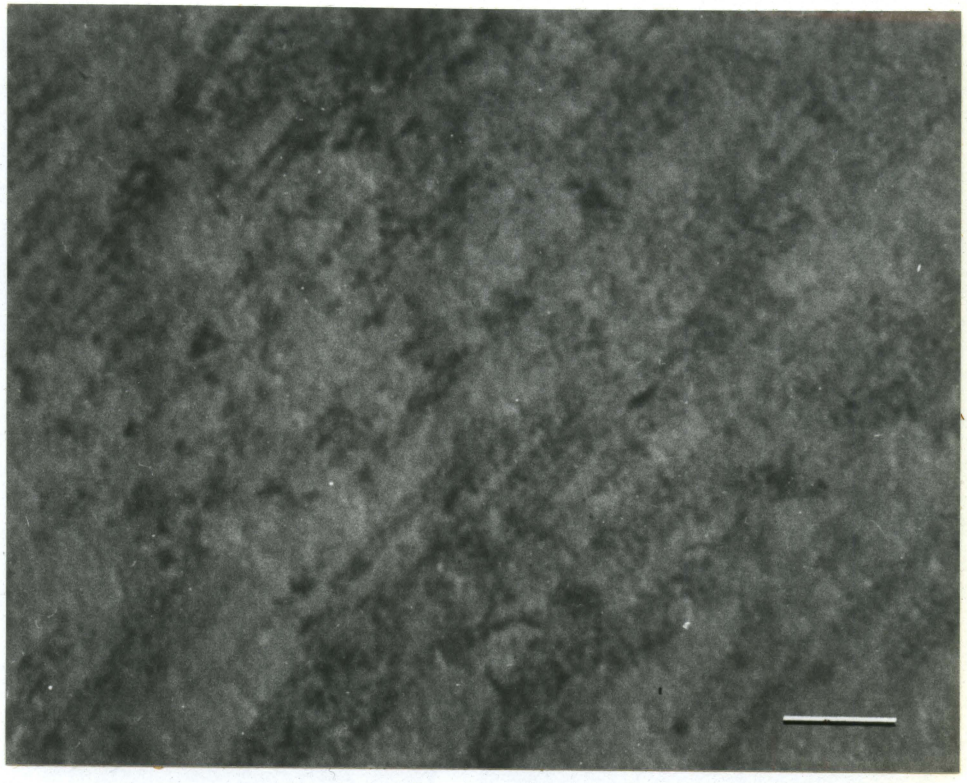

FIGURE 22. 90:10 MMA/2-HEMA cast film. Surface in contact with air (scale bar=40 um). 25 keV.

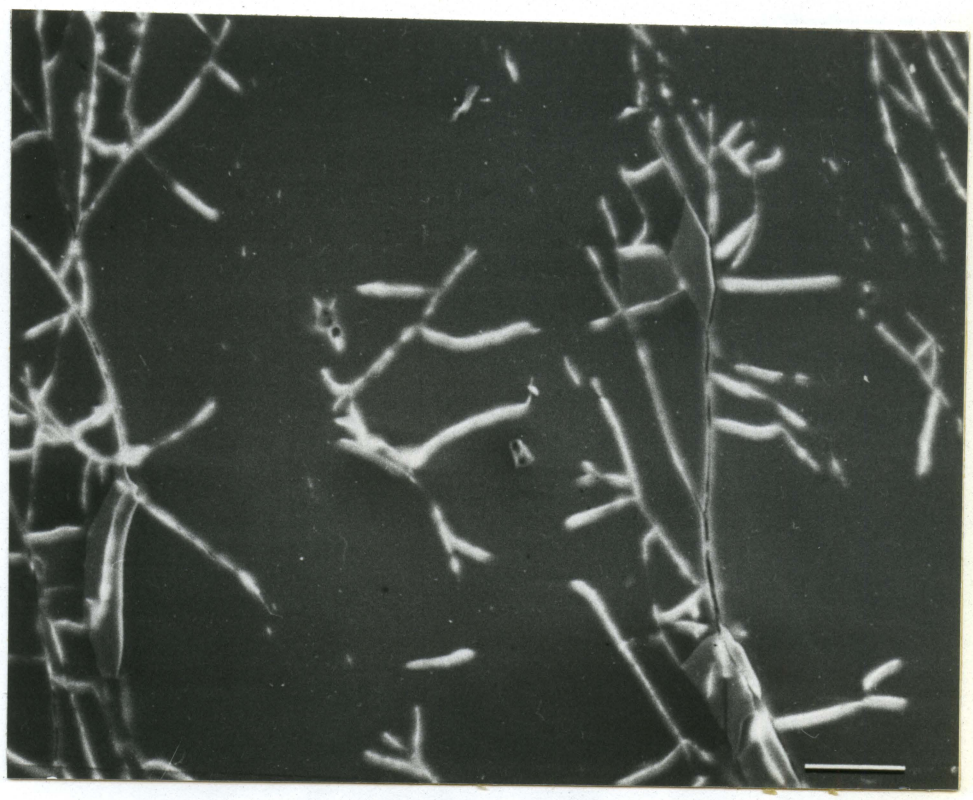

FIGURE 23. Poly (lactic acid)-tylosin tartrate matrix cast at room temperature. Surface in contact with glass (scale bar=100um). 25 keV.

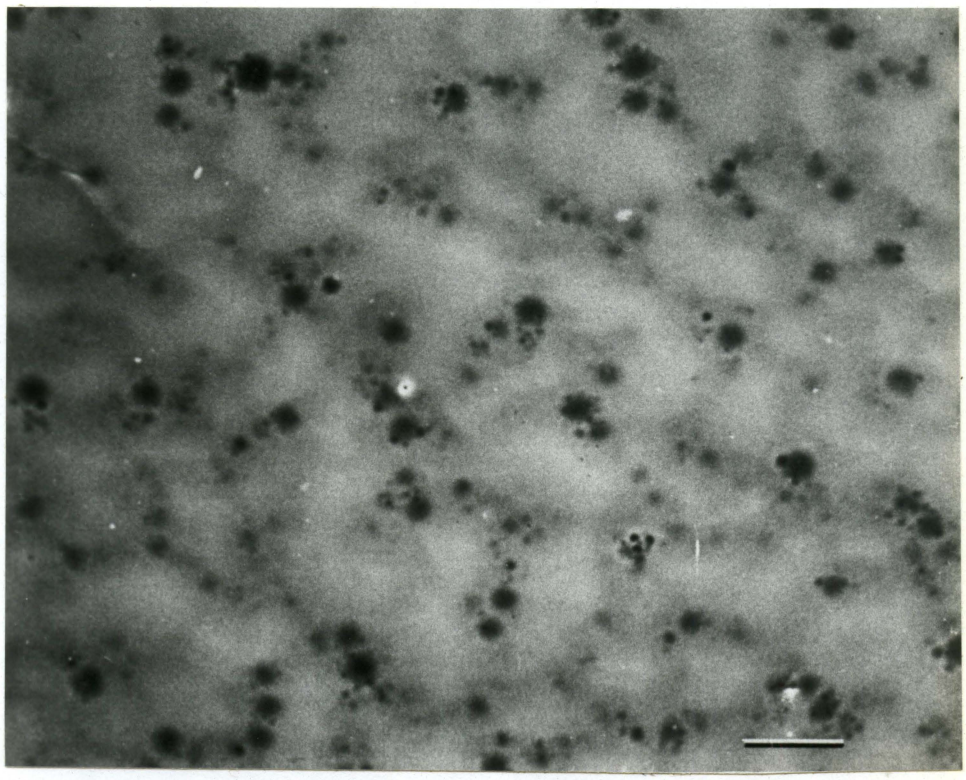

FIGURE 24. Poly (lactic acid) film cast at room temperature. Surface in contact with glass (scale bar=100 um). 25 keV.

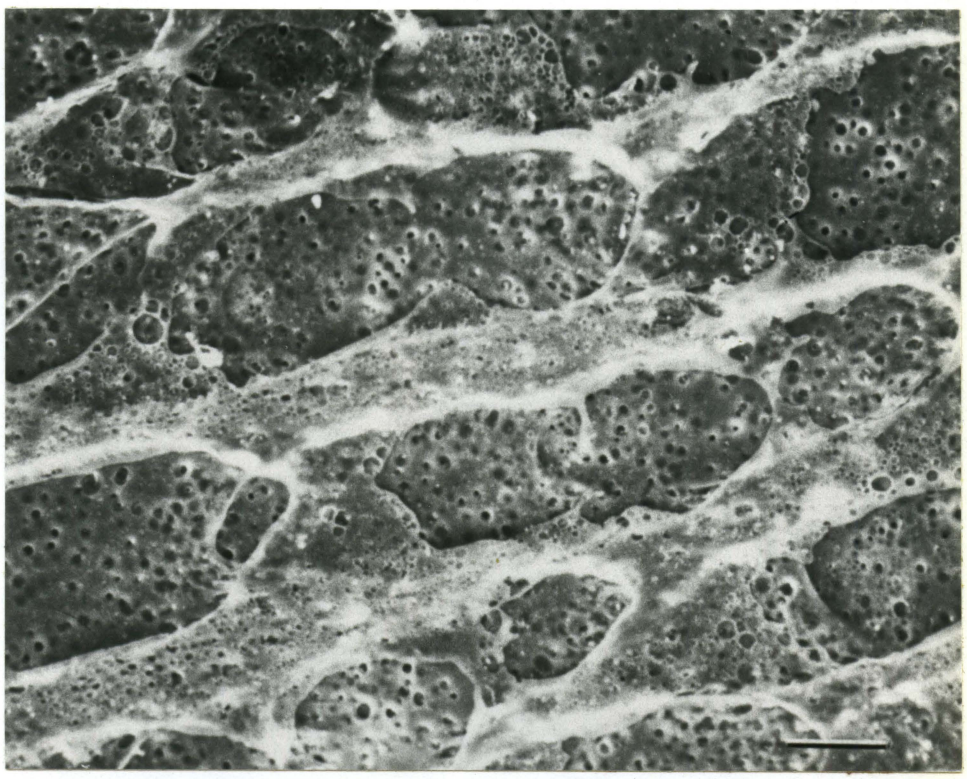

FIGURE 25. Poly (lactic acid)-tylosin tartrate cast matrix (prepared at room temperature) after immersion in a Ringer's solution at 37°C for thirty hours. Surface cast in contact with glass (scale bar=100 um). 25 keV.

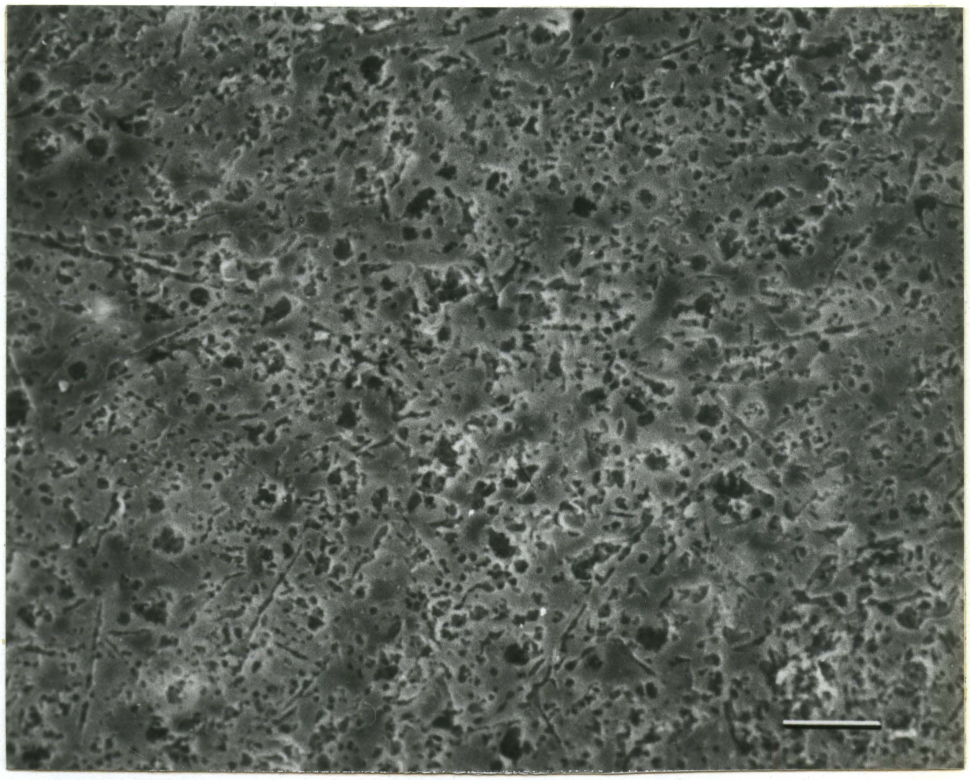

FIGURE 26. Poly (lactic acid)-tylosin tartrate cast matrix (cold production method). Surface in contact with glass (scale bar=100 um). 25 keV.

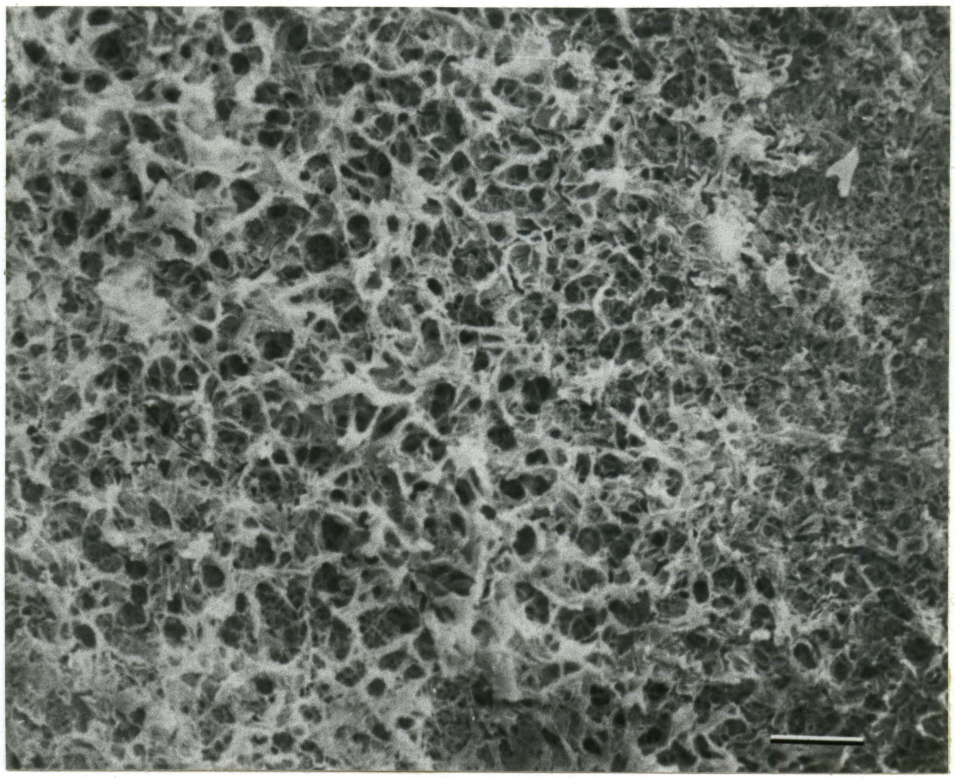

FIGURE 27. Poly (lactic acid)-tylosin tartrate cast matrix (cold production method) after immersion in a Ringer's solution at 37°C for thirty hours. Surface cast in contact with glass (scale bar=100 um). 25 keV.

The white films were highly flexible. They were cut into disks with a corkborer, and with care, they did not tear. The eight disks were 0.127 ± 10% mm thick, and the weight of the five utilized for controlled-release systems 32A-E were 0.0084, 0.0082, 0.0081, 0.0084, and 0.0082 gm, respectively.

Production of the controlled-release systems

The 90:10 MMA/2-HEMA copolymer solution effectively joined the systems together since no perforations were found during the 40X stereomicroscopic examination. The fifty-five gram weight applied to the top slide held the edges of the 90:10 MMA/2-HEMA films together during production. In some cases the joining solution flowed into the system, and in others the solution flowed out. Table 16 summarizes the post-production system characteristics. Controlled-release systems 32A-E and 33C were flat while the remaining systems were saucer-shaped due to the mass of tylosin tartrate located in the center of the device. Tylosin tartrate mixed to varying degrees with the joining solution of systems 34A-E due to the physical size limitation of the device.

Tylosin tartrate release-experiment

The experimental process was conducted as explained in the procedures section. At the beginning of the experiment, the controlled-release systems were totally dry; however, at the first collection period and throughout the remainder of the experiment, mammalian Ringer's solution filled the bubbles of 33A-B, D-E, and 34A-E. Fluid

TABLE 16. Characteristics of the controlled-release systems after production.

Controlled-release system	Characteristics
32 A-E	Systems are flat with a total thickness equal to the thickness of the three layers.
33 A-B, D-E	Systems are flat with the exception of a 5.0 mm diameter bubble in the center of the devices containing tylosin tartrate. The bubble creates a saucer-shape with approximately 1.0 mm center thickness and 0.5 mm edge thickness.
33 C	System is flat with a total thickness of approximately 0.8 mm. The tylosin tartrate is mixed with the joining solution.
34 A-E	Systems are flat with the exception of a 9.0 mm bubble in the center of the device containing tylosin tartrate. The bubble creates a saucer-shape with approximately 2.0 mm center thickness and 1.0 mm edge thickness. Tylosin tartrate mixed with the joining solution.

was not visible in 32A-E and 33C. The controlled-release systems did not appear to swell; rather, fluid filled the available space within the devices.

Samples collected in accordance with the procedure were totally evaporated in approximately seventy-two hours. Thin layer chromatography was utilized to detect and quantify the amount of tylosin

tartrate in each sample. The dark spot of the tylosin tartrate pattern was utilized for the densitometer scan. Due to dark spot tailing at 5.0-10.0 ug quantities of tylosin tartrate, all samples from the tylosin tartrate release-experiment were diluted to a tylosin tartrate concentration below 5 ug/10 ul before scanning. Appendices D-R contain data from controlled-release systems 32A-34E, respectively. The data include regression line data number, total volume of release-experiment sample, weight of peak #1, weight of peak #2, average weight of peak, amount of tylosin tartrate in the 10 ul volume applied to the TLC plate, and the amount of tylosin tartrate in the total sample volume. In the release-experiment analysis, known concentrations of tylosin tartrate in type-one aqueous solution were utilized to calculate the regression line equation. These data are contained in Appendix S, and are related to the data in Appendices D-R by the regression line data number.

The cumulative tylosin tartrate release data are plotted in Figures 28-42 and the results are summarized in Table 17. A period of zero-order release occurred for most of the controlled-release systems. This region is shown in Figures 28-31 and 33-42 by a straight line. Data points are not plotted for those samples that failed to produce spots on the TLC plate although lines are shown for ranges including these samples. Some of these sampling periods are too close to provide samples with detectable amounts of drug. The figures where this situation occurs exhibit a low slope, and it is likely that the variance on any particular sampling may yield a case where the drug is not able

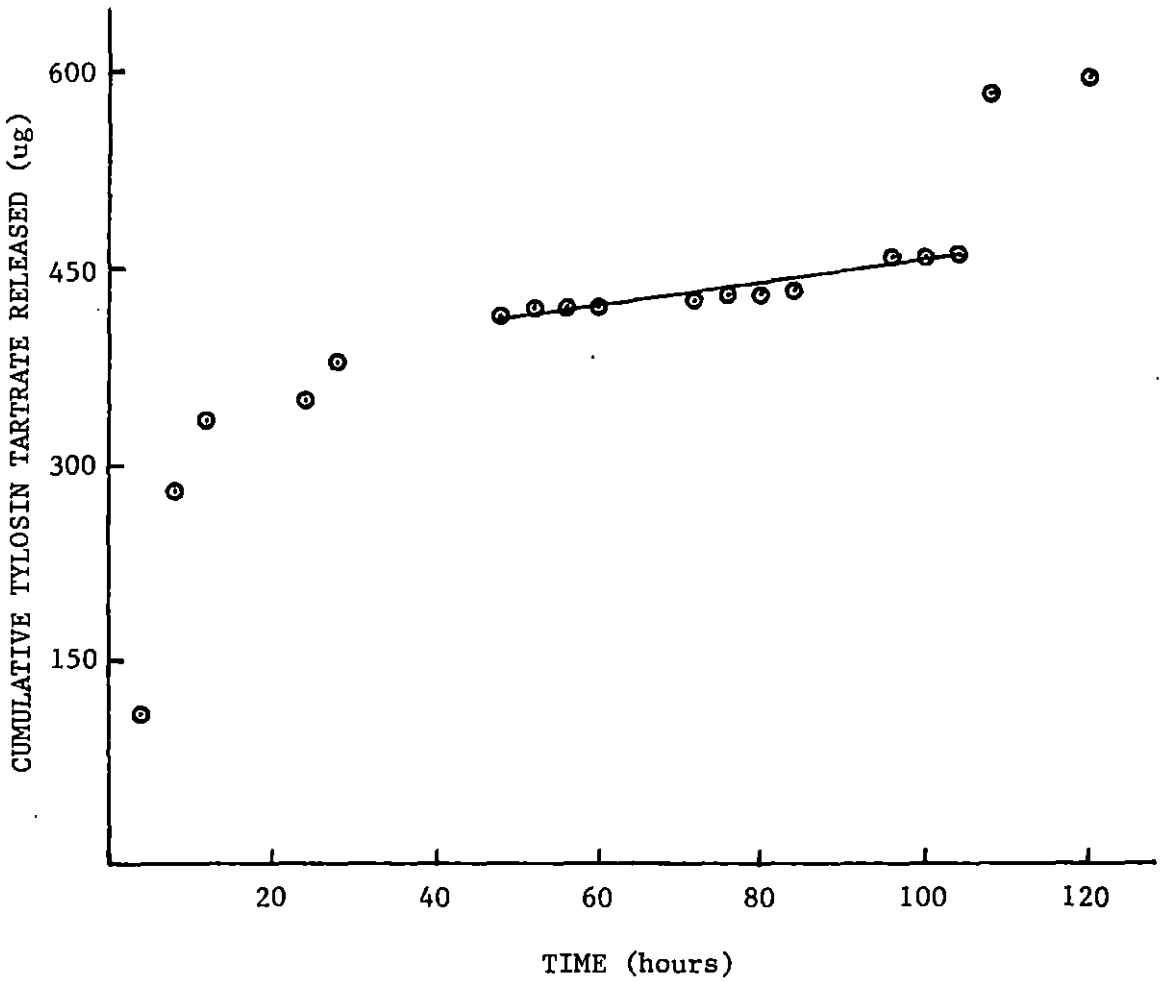

FIGURE 28. Cumulative tylosin tartrate released versus time for system 32A. No spot developed on the TLC plate for the samples collected at 32 and 36 hours

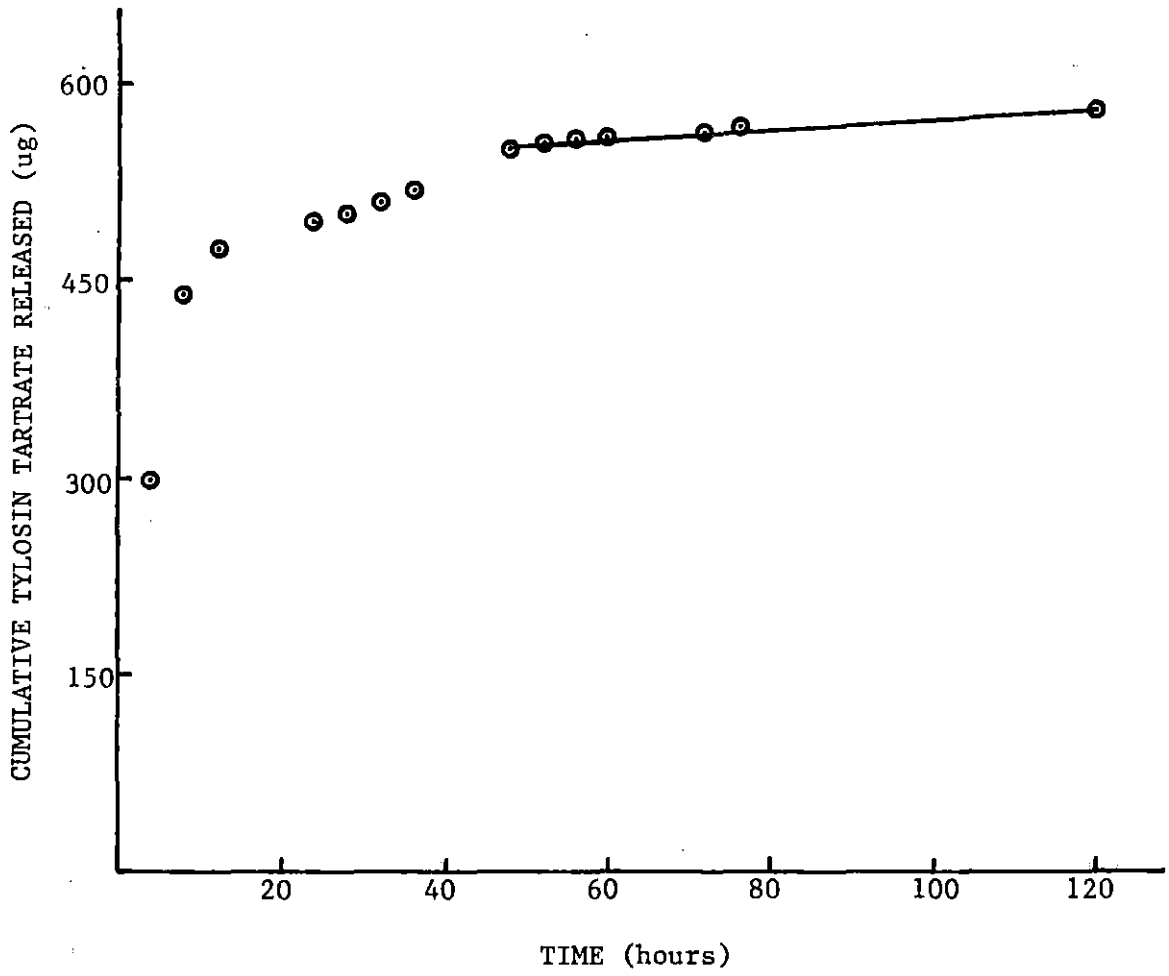

FIGURE 29. Cumulative tylosin tartrate released versus time for system 32B. No spot developed on the TLC plate for the samples collected at 80, 84, 96, 100, 104, and 108 hours

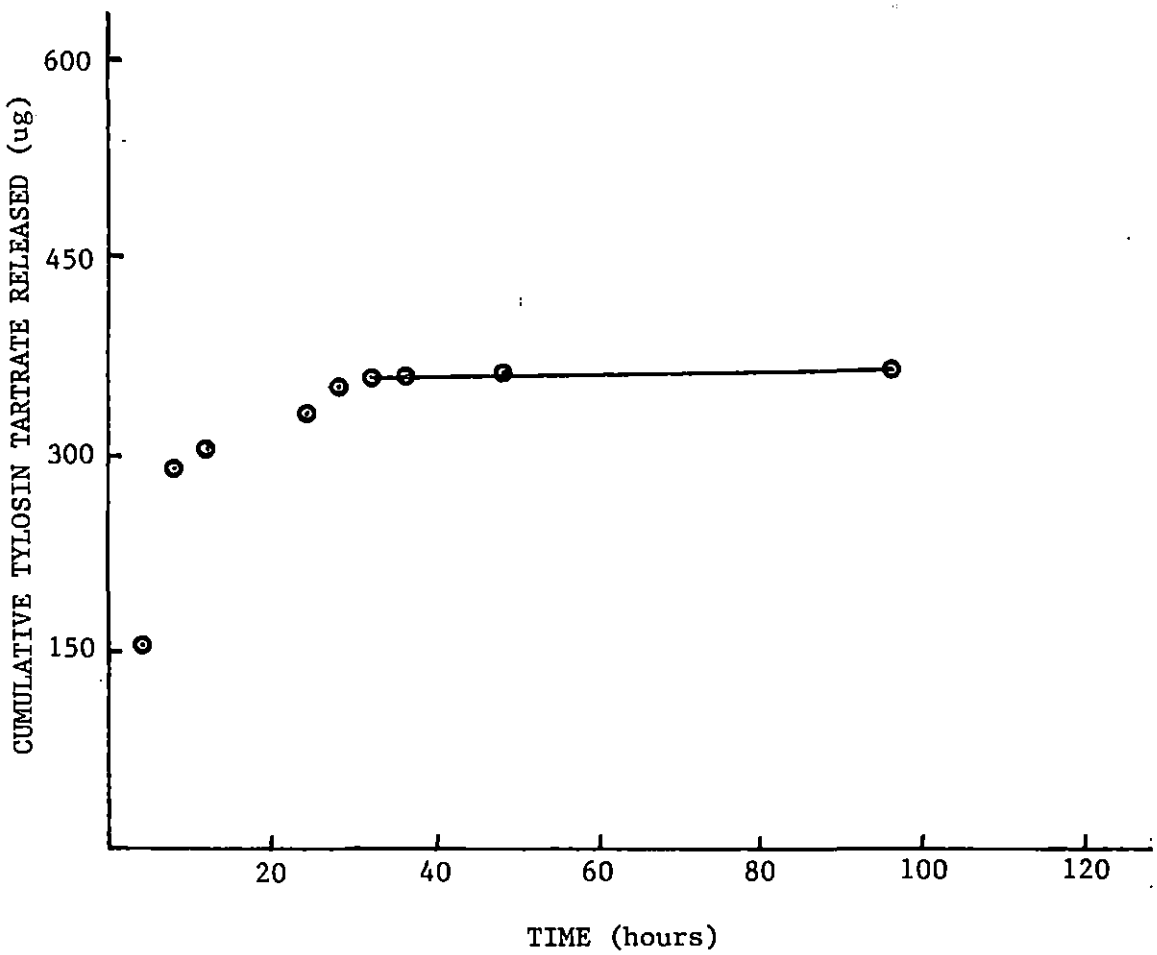

FIGURE 30. Cumulative tylosin tartrate released versus time for system 32C. No spot developed on the TLC plate for the samples collected at 56, 60, 72, 76, 80, 84, 100, 104, 108, and 120 hours

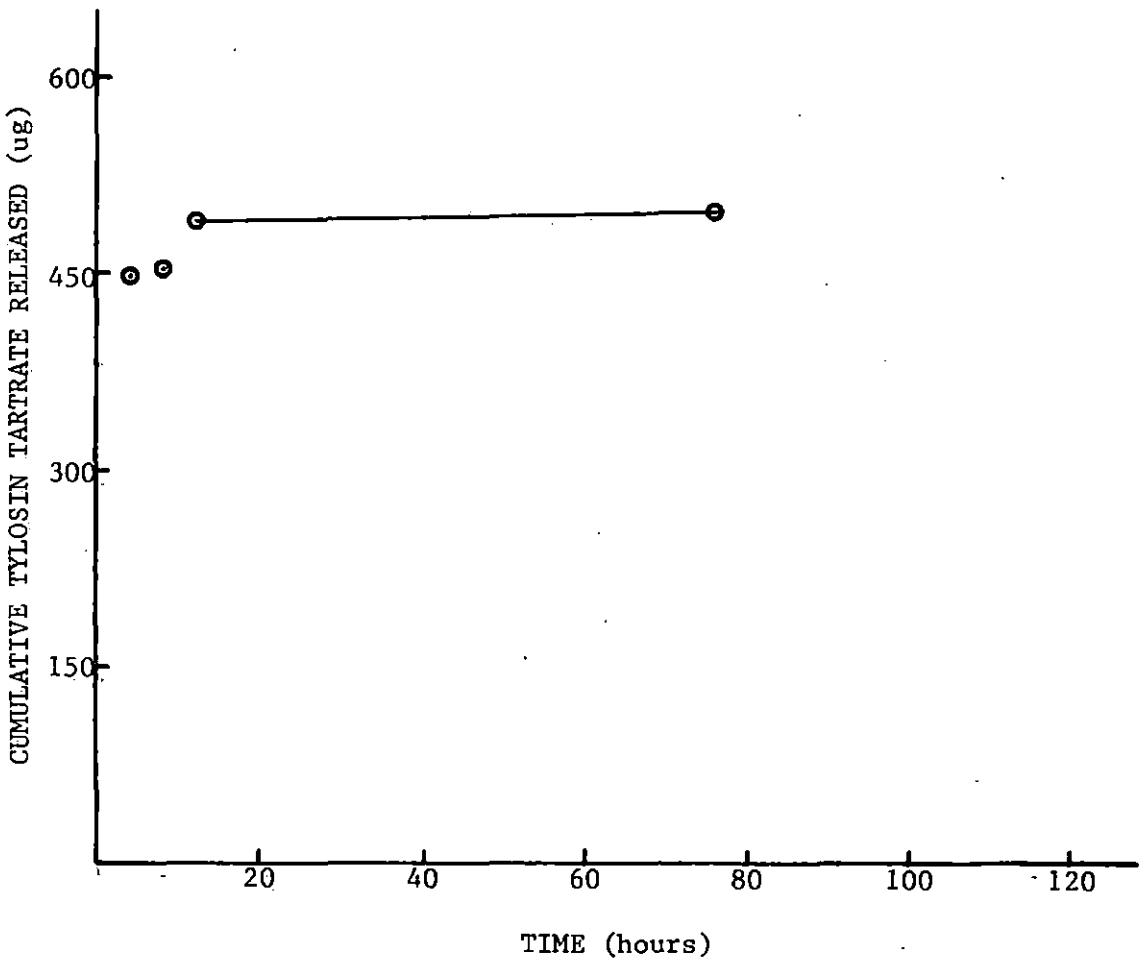

FIGURE 31. Cumulative tylosin tartrate released versus time for system 32D. No spot developed on the TLC plate for the samples collected at 24, 28, 32, 36, 48, 52, 56, 60, 72, 80, 84, 96, 100, 104, 108, and 120 hours

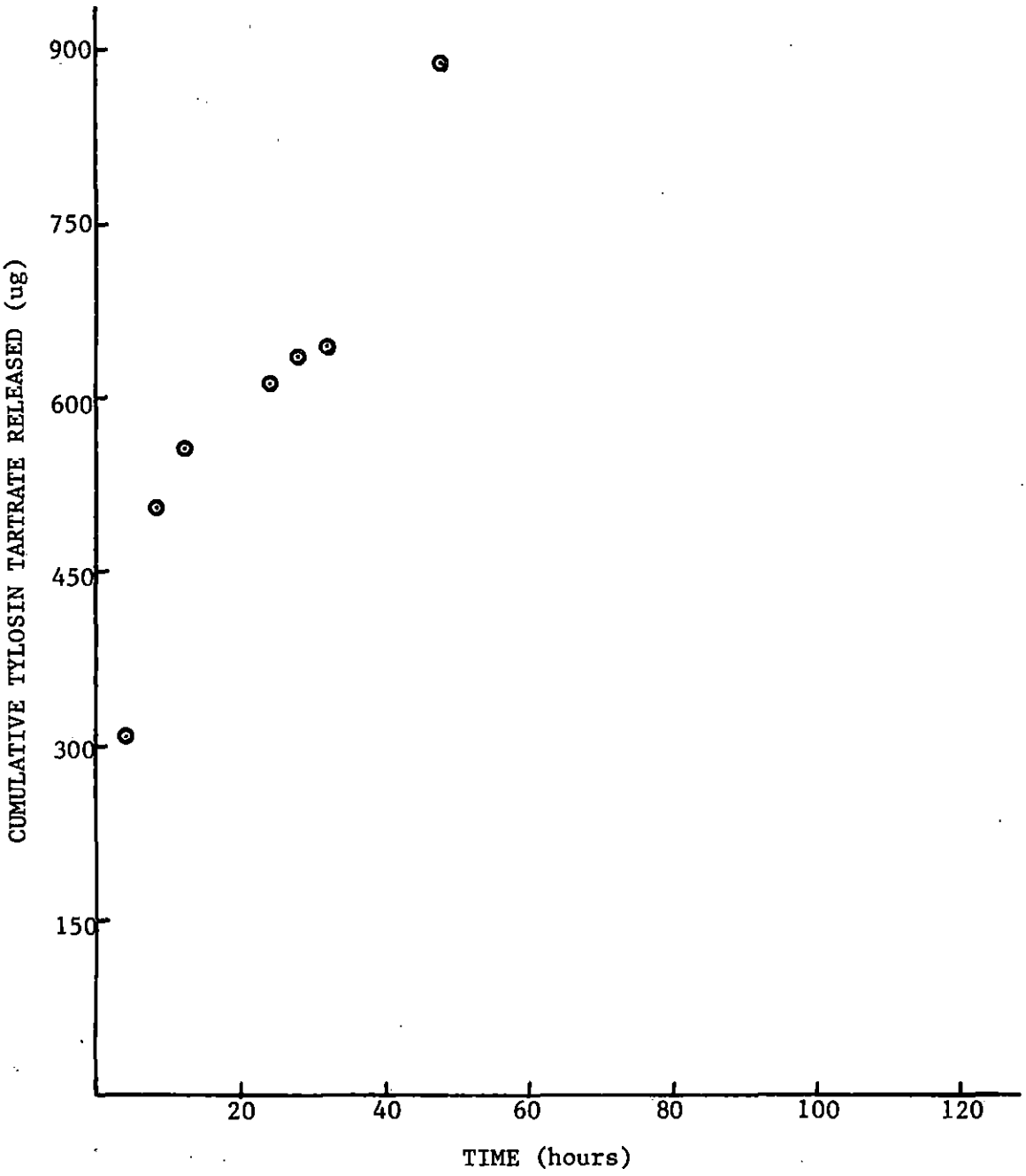

FIGURE 32. Cumulative tylosin tartrate released versus time for system 32E. No spot developed on the TLC plate for the samples collected at 36, 52, 56, 60, 72, 76, 80, 84, 96, 100, 104, 108, and 120 hours

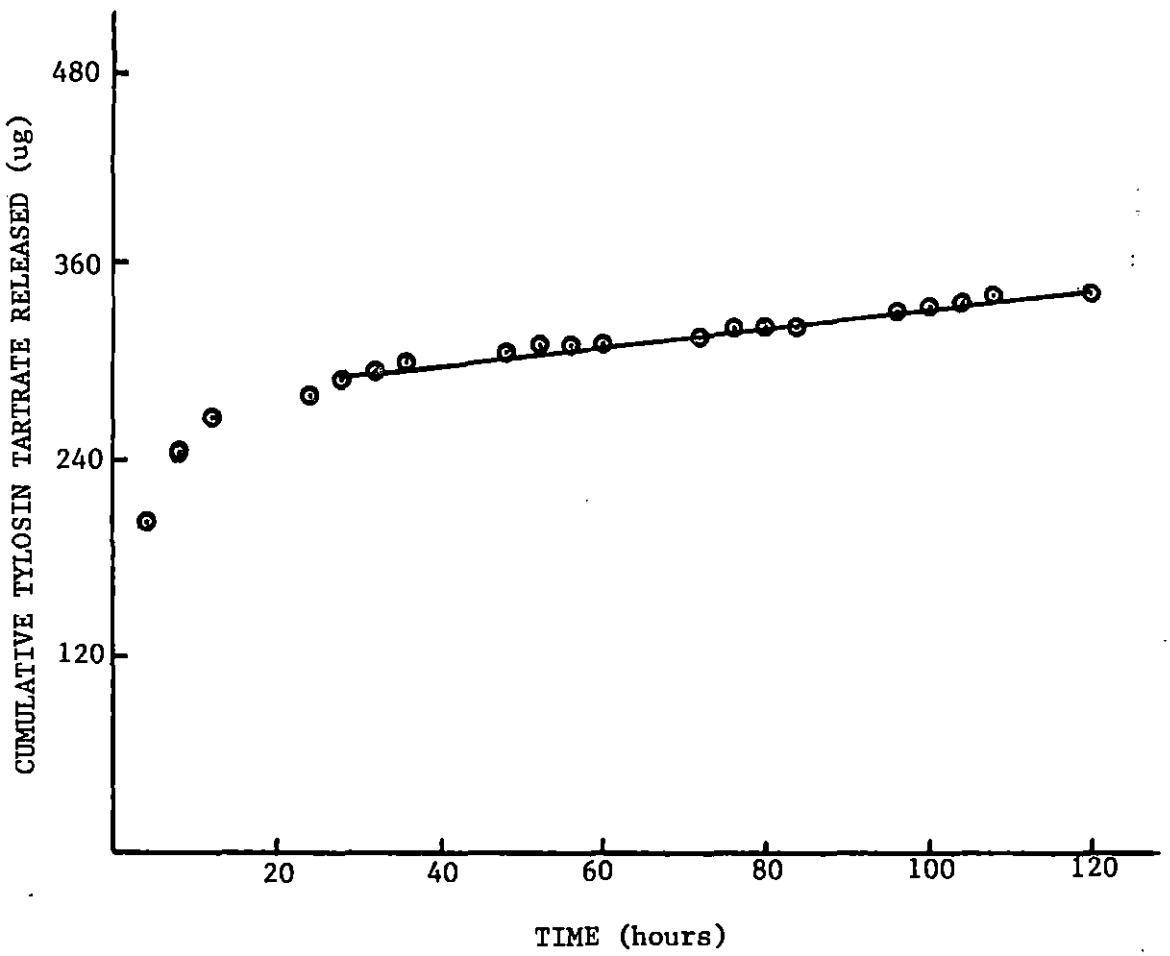

FIGURE 33. Cumulative tylosin tartrate released versus time for system 33A

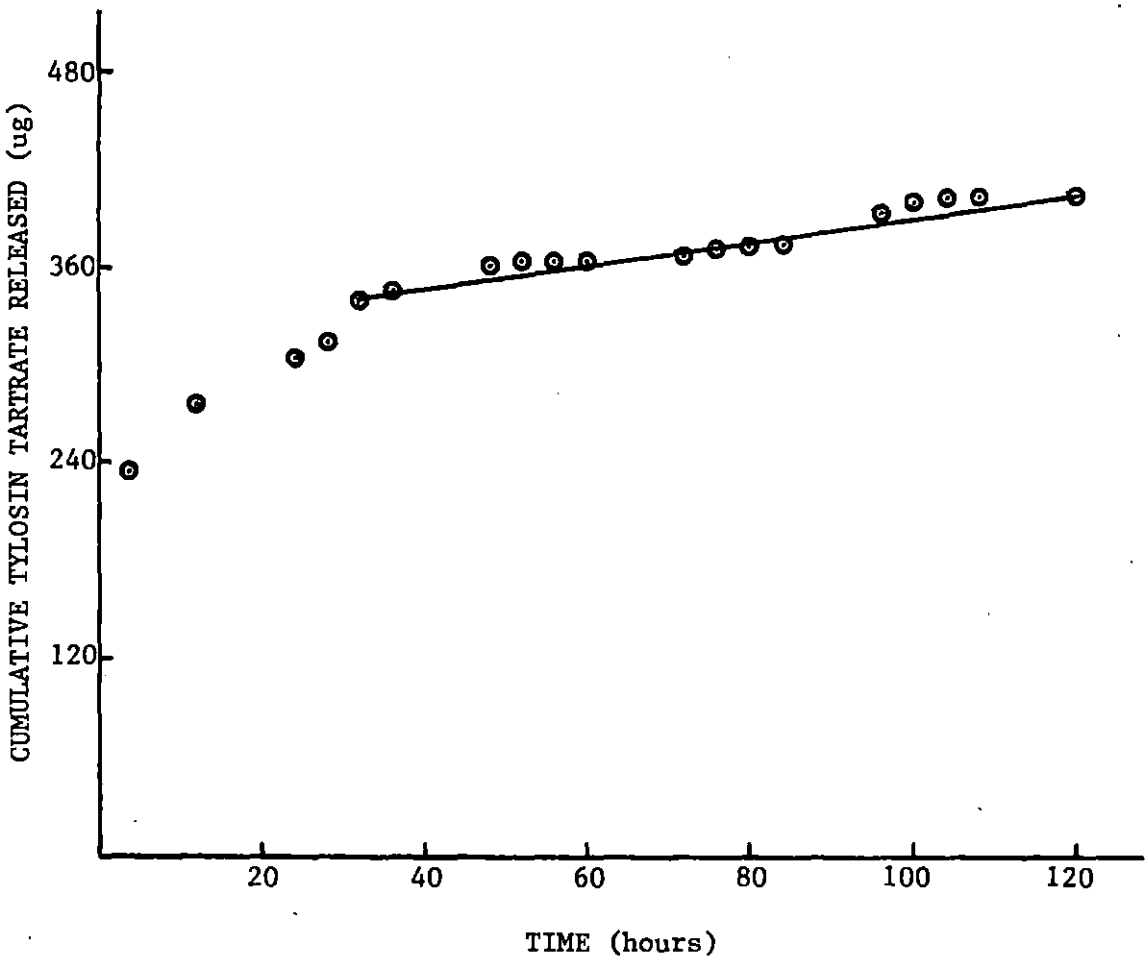

FIGURE 34. Cumulative tylosin tartrate released versus time for system 33B. No spot developed on the TLC plate for the sample collected at 8 hours

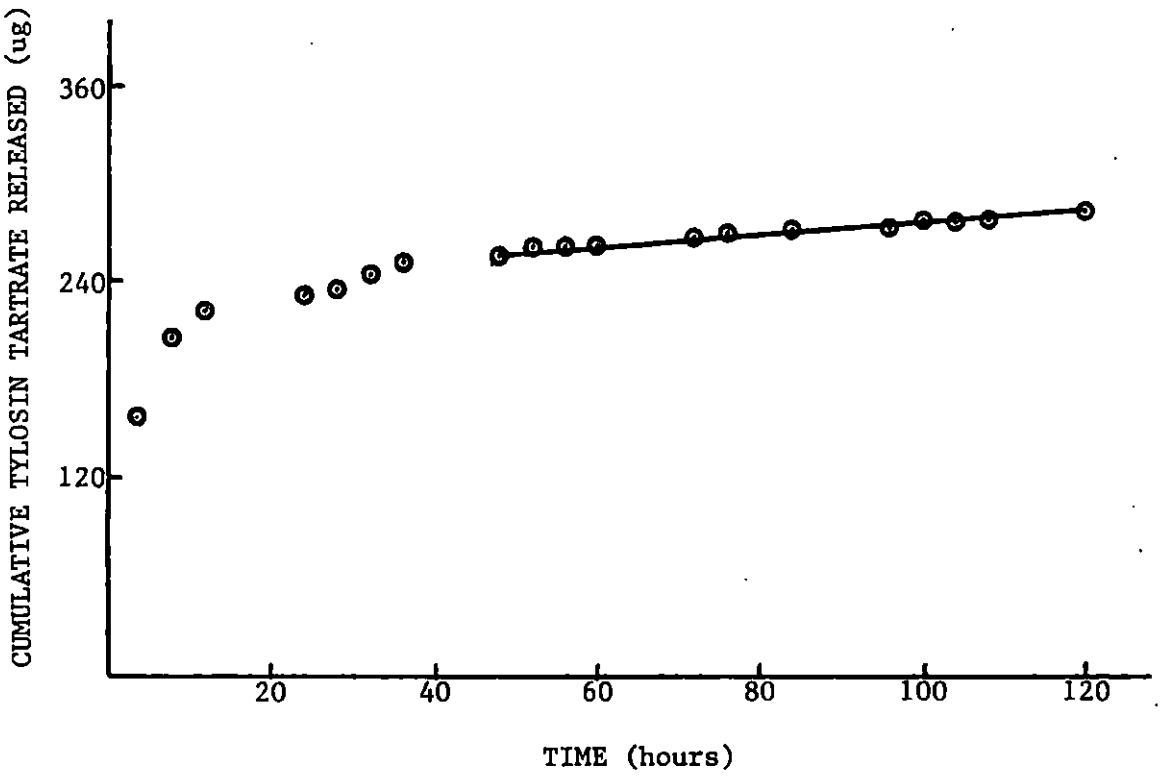

FIGURE 35. Cumulative tylosin tartrate released versus time for system 33C. No spot developed on the TLC plate for the sample collected at 80 hours

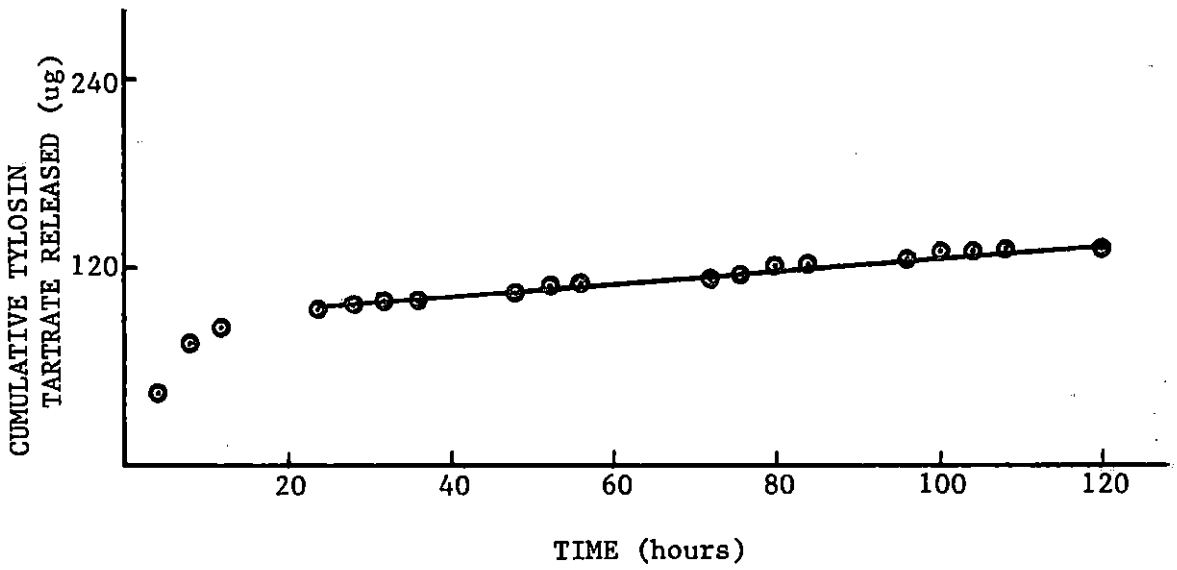

FIGURE 36. Cumulative tylosin tartrate released versus time for system 33D. No spot developed on the TLC plate for the sample collected at 60 hours

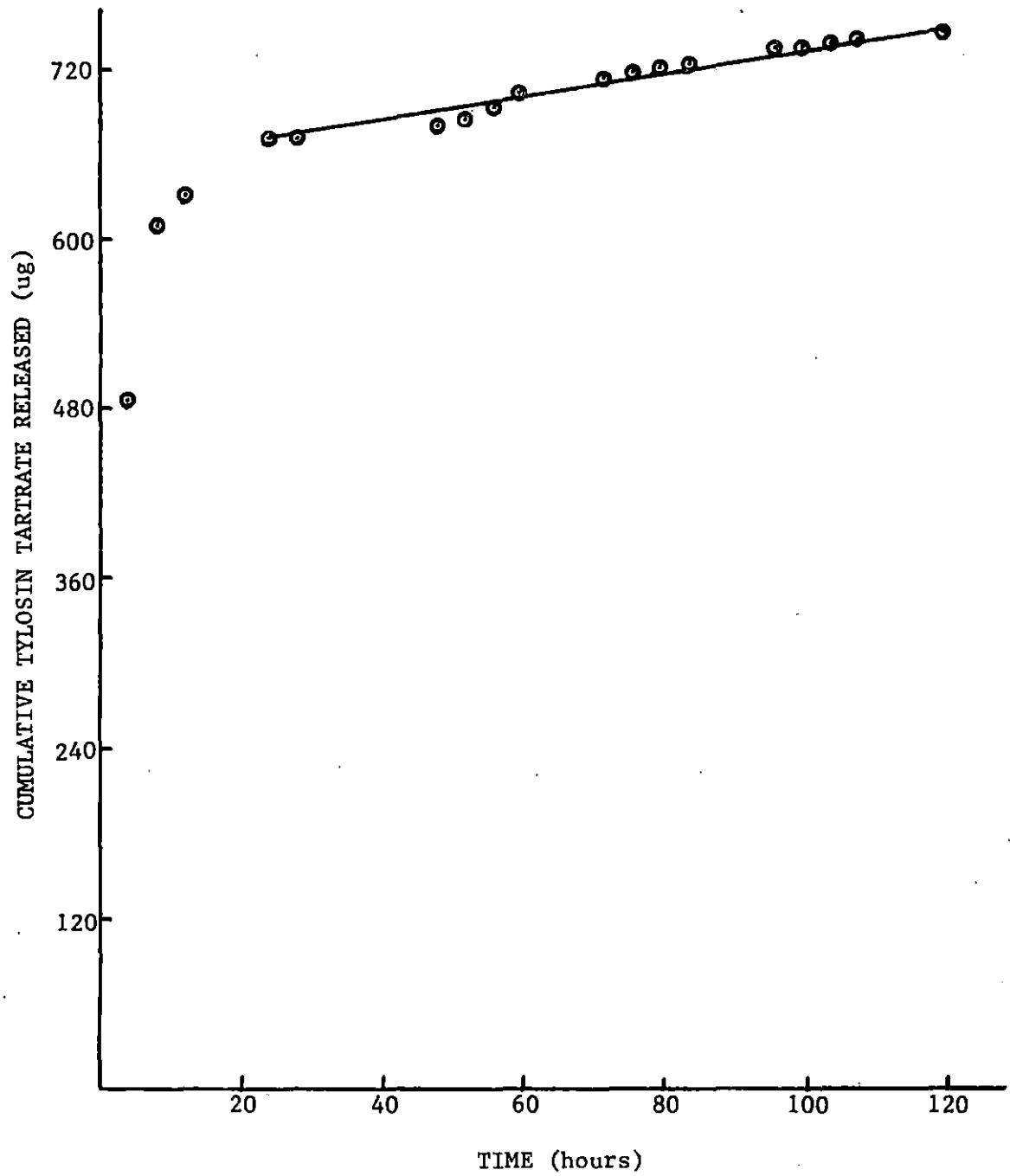

FIGURE 37. Cumulative tylosin tartrate released versus time for system 33E. No spot developed on the TLC plate for the samples collected at 32 and 36 hours

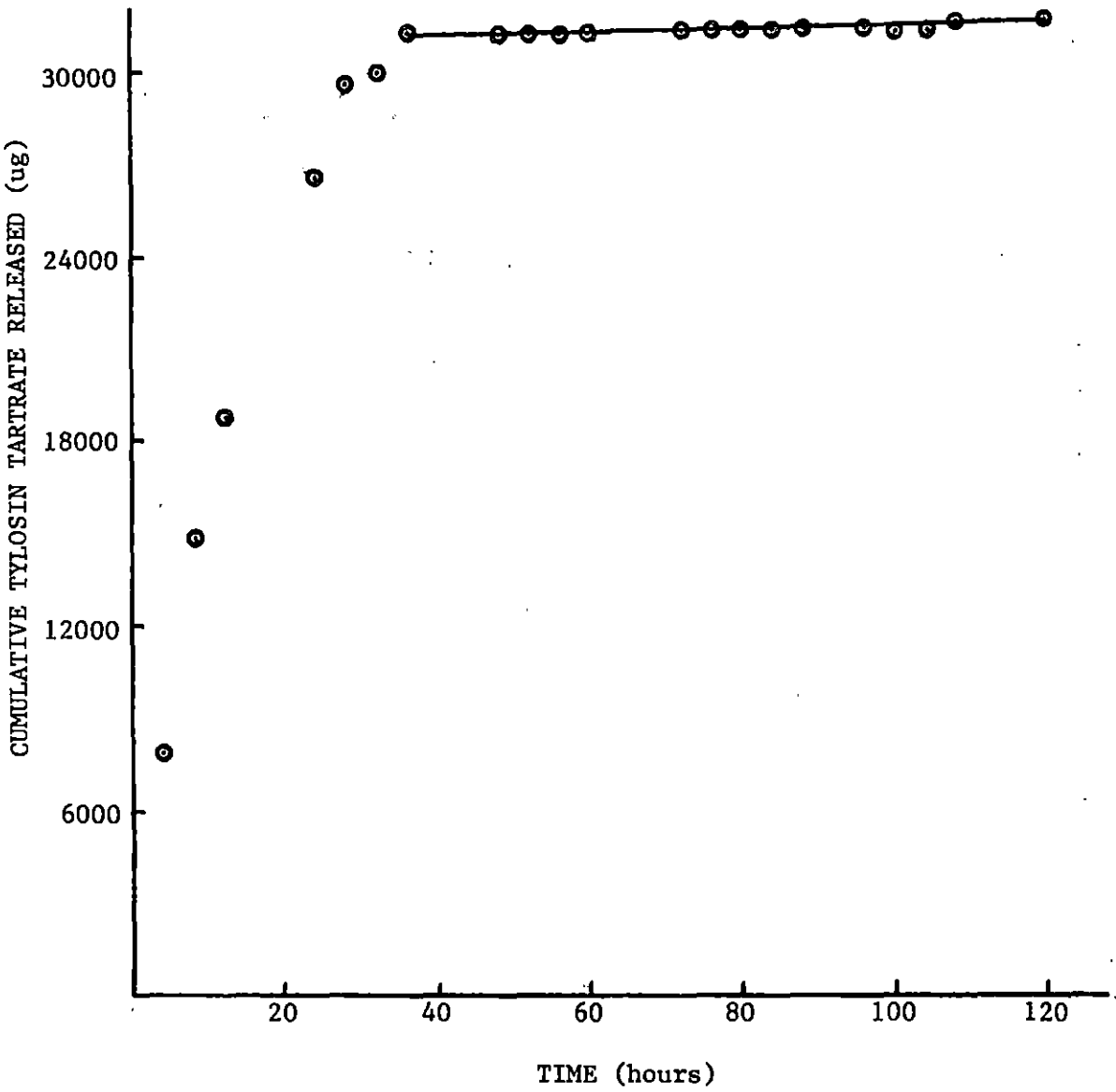

FIGURE 38. Cumulative tylosin tartrate released versus time for system 34A

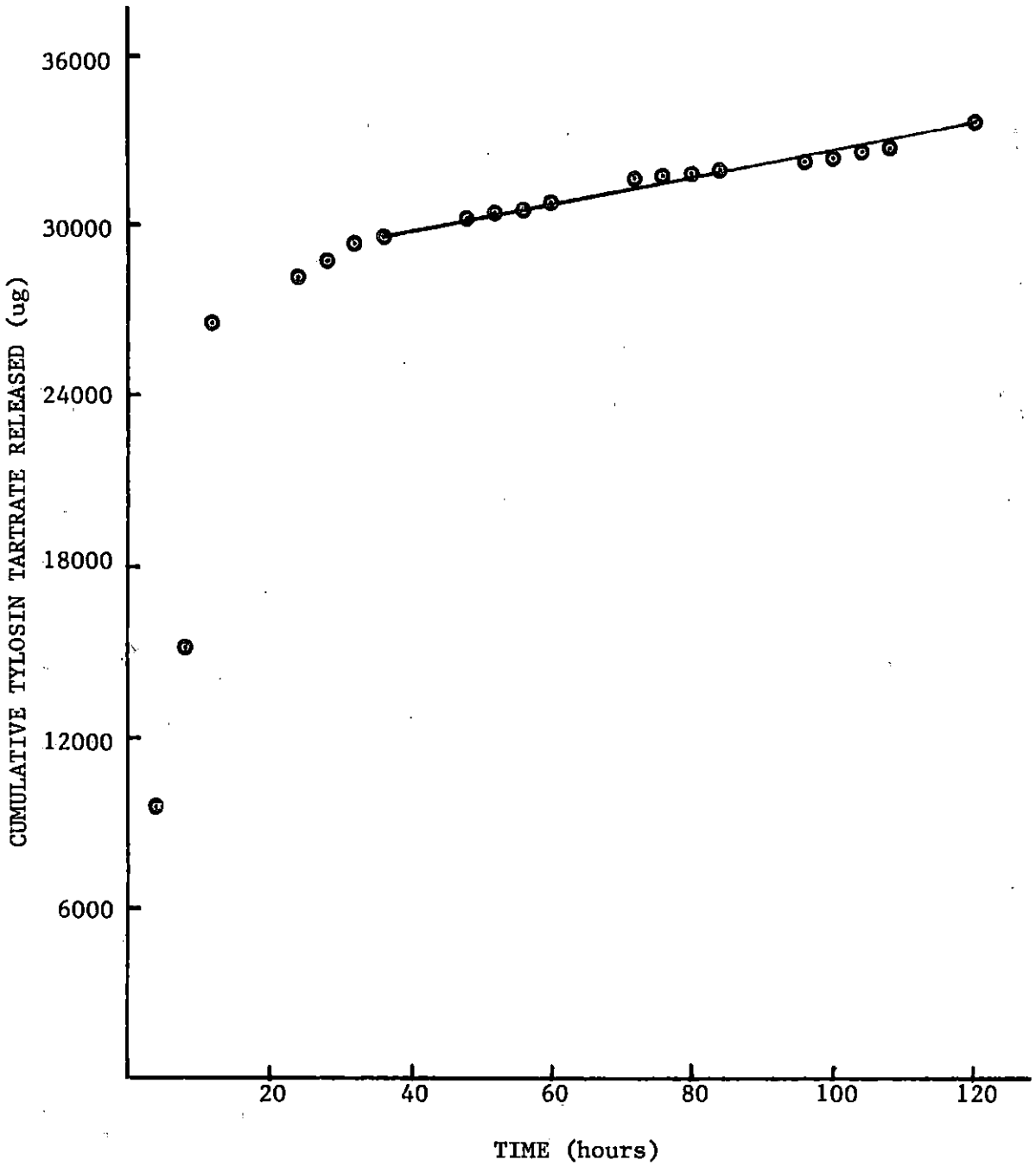

FIGURE 39. Cumulative tylosin tartrate released versus time for system 34B

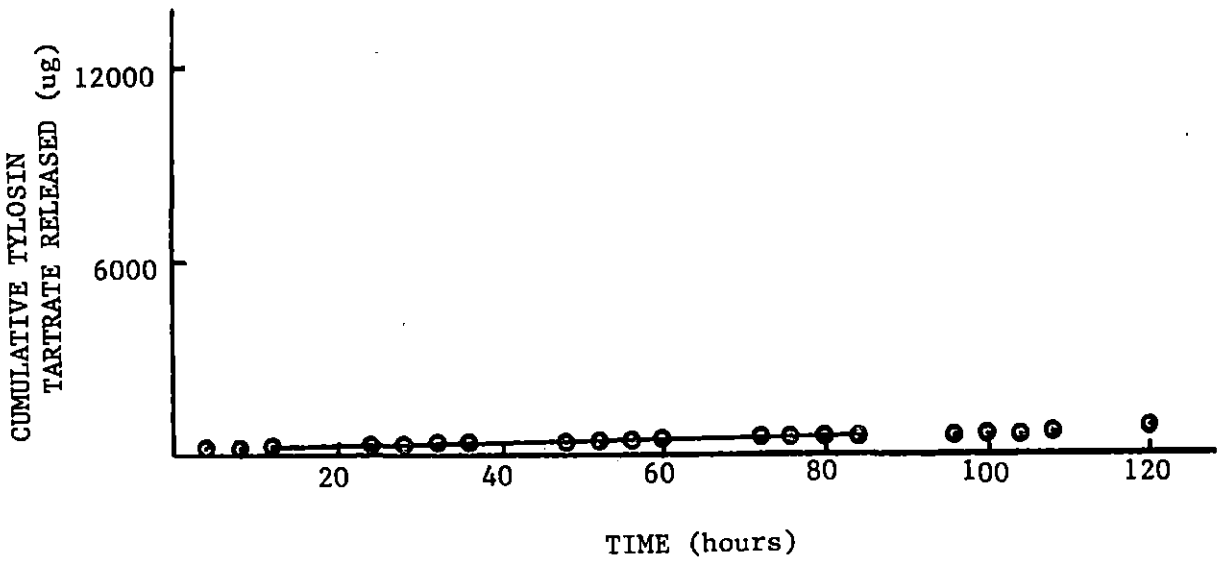

FIGURE 40. Cumulative tylosin tartrate release versus time for system 34C

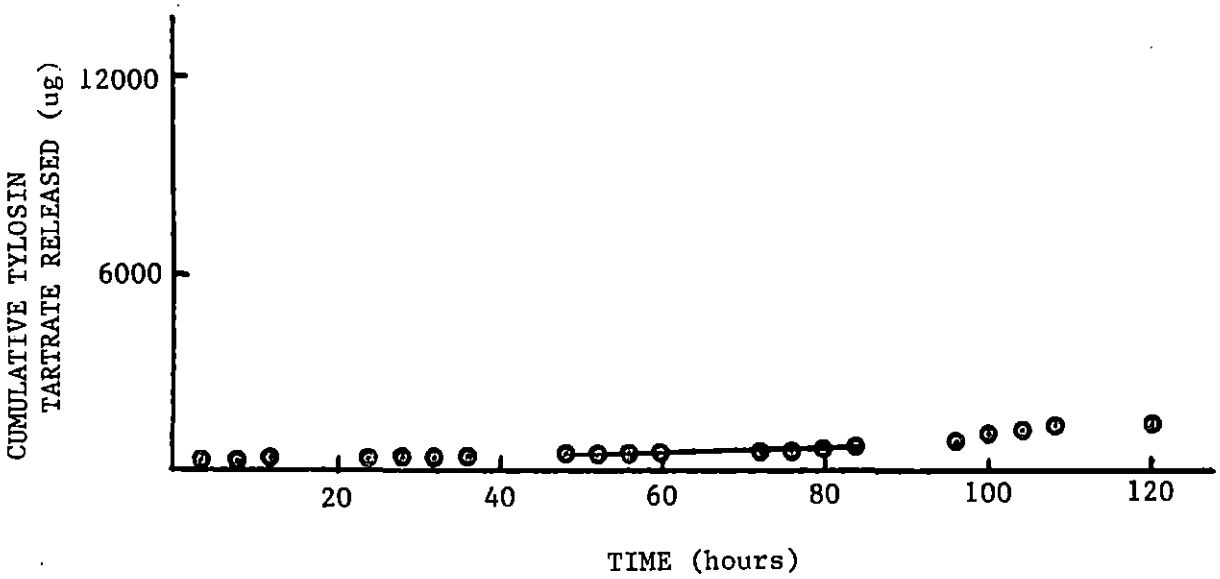

FIGURE 41. Cumulative tylosin tartrate release versus time for system 34D

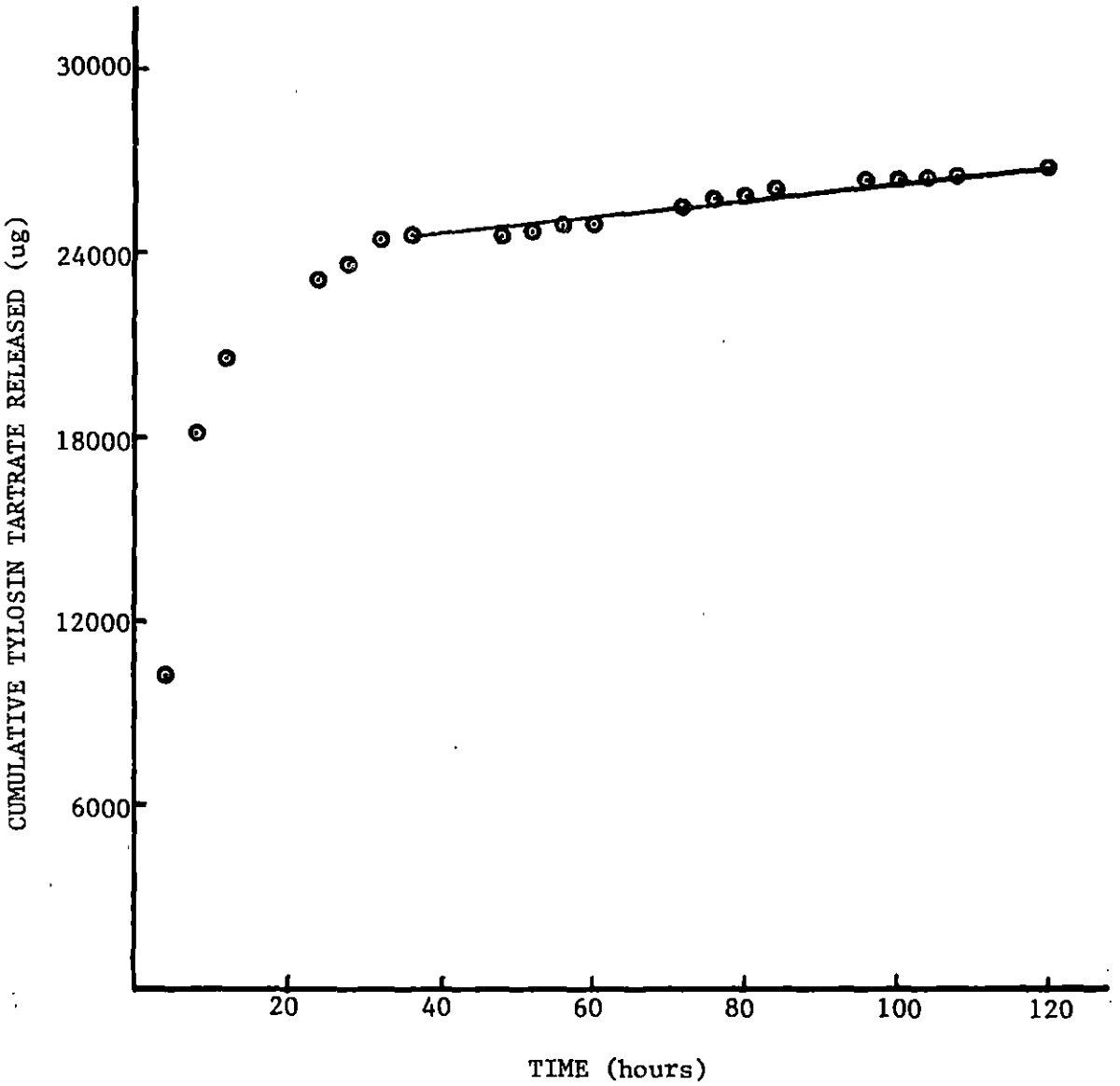

FIGURE 42. Cumulative tylosin tartrate released versus time for system 34E

TABLE 17. Results of the tylosin tartrate release-experiment.

Sample	Number of data points	Linear release rate range (hr)	Time of linear release rate (hr)	Release Rate over linear range (ug/hr)	Release over total time (ug)	Total original drug loading (ug)
32A	11	48-104	56	0.9	611.8	4840
32B	7	48-120	72	0.4	576.1	4840
32C	4	32-96	64	0.1	362.2	4840
32D	2	12-76	64	0.1	495.1	4840
32E	--	---	--	---	870.1	4840
33A	16	28-120	92	0.6	347.0	5000
33B	15	32-120	88	0.7	408.8	5000
33C	12	48-120	72	0.3	283.6	5000
33D	16	24-120	96	0.2	136.8	5000
33E	15	24-120	96	0.8	736.7	5000
34A	14	36-120	84	6.0	31729.8	50000
34B	14	36-120	84	33.3	32619.0	50000
34C	12	12-80	68	1.0	991.8	50000
34D	8	48-84	36	10.0	1316.7	50000
34E	15	32-120	88	20.3	25927.4	50000

to be detected. However, an overall trend in these figures has been indicated. These samples contained less than 2.0 ug of tylosin tartrate, and the collection times are noted in the figure caption. The duration, rate of tylosin tartrate release, and number of data points for the zero-order release period are tabulated in Table 17.

The tylosin tartrate release rate required for an ocular controlled-release system varies in accordance with the lachrymal flow rate. Hoffman and Spadbrow (1978) and Slatter and Edwards (1982) determined bovine lachrymal flow rates, and Table 18 contains the

TABLE 18. Tylosin release rate required with lacrymal flow variation.

Lacrymal flow (ml/hr)	Time required to produce a 0.5 ml tear volume (min)	Tylosin release rate required (ug/hr)
0.1	300.0	0.063
0.3	100.0	0.189
0.5	60.0	0.315
0.7	42.9	0.441
0.9	33.3	0.568
1.1	27.3	0.692
1.3	23.1	0.818
1.5	20.0	0.945
1.7	17.7	1.068
1.9	15.8	1.196
2.1	14.3	1.322
2.3	13.0	1.454
2.5	12.0	1.575
2.7	11.1	1.703
2.9	10.3	1.835
3.1	9.7	1.948
3.3	9.1	2.077
3.5	8.6	2.198
3.7	8.1	2.333
3.9	7.7	2.455

required tylosin tartrate release rate over the range of their reported data. The calculations utilized to produce Table 18 are explained by the following example. A lachrymal flow rate of 0.5 ml/hr will produce the 0.5 ml tear volume in sixty minutes. The minimum inhibitory concentration to suppress bacterial colonization for the bacterial strains of interest when studied for tylosin tartrate is 0.63 ug/ml.

(R. F. Rosenbusch, personal communication.)[1] Therefore, 0.315 ug of tylosin tartrate must be contained in the eye. Since the tear volume is replaced every hour, the release rate must be 0.315 ug/hr.

The required tylosin tartrate release rate ranges from 0.063-2.455 ug/hr (Figure 18). Controlled-release systems 32A-E, 33A-E, and 34C produced release rates ranging from 0.1-0.9 ug/hr, and controlled-release systems 34A, 34B, 34D, and 34E produced tylosin tartrate release rates in excess of 2.455 ug/hr.

There are wide variations in the release rate of similar controlled-release systems. The results of a post-experiment examination (at 40X) of the controlled-release systems (Table 19) obtained by using a stereomicroscope may account for these differences. Each controlled-release system was first examined for external security, and then the device was broken open to examine the interior characteristics.

A comparison of the data in Table 17 and Table 19 indicate that the release rates of the tylosin tartrate release-experiments correlate well with the post-experiment characteristics. This relationship will be examined in the discussion.

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

TABLE 19. Post-experiment characteristics of the controlled-release systems

Controlled-release system	Characteristics
32A 32D	Systems are sealed around the edges, center of the devices are open, and the release area may be approximated as a 10.0 mm diameter circle. No evidence of residual tylosin tartrate.
32B	System is sealed around the edges, center of the device is open, and the release area may be approximated as a 12.0 mm diameter circle. No evidence of residual tylosin tartrate.
32C	System is totally sealed by the joining solution. The inward movement of the joining solution has thickened the release membranes. No evidence of residual tylosin tartrate.
32E	System is sealed around the edges, center of the device is open, and the release area may be approximated as a 14.0 mm diameter circle. No evidence of residual tylosin tartrate.
33A 33B	Systems are totally sealed by the joining solution except for the 5.0 mm diameter bubble that contained tylosin tartrate. No evidence of residual tylosin tartrate.
33C	System is totally sealed by the joining solution. No evidence of residual tylosin tartrate.
33D	System is totally sealed by the joining solution except for the 2.0 mm diameter bubble that contained tylosin tartrate. The bubble is surrounded by the faint yellow color of tylosin tartrate residue.

Table 19 (Continued)

Controlled-release system	Characteristics
33E	System is totally sealed by the joining solution except for the 4.0 mm diameter bubble that contained tylosin tartrate. No evidence of residual tylosin tartrate.
34A 34B	Systems are totally sealed by the joining solution except for the 10.0 mm diameter bubble that contained tylosin tartrate. The bubble is surrounded by the faint yellow color of tylosin tartrate residue.
34C	System is totally sealed by the joining solution except for the 6.0 mm diameter bubble that contained tylosin tartrate. The bubble is surrounded by the faint yellow color of tylosin tartrate, and yellow particulate matter is dispersed in the joining solution.
34D	System is totally sealed by the joining solution except for the 9.0 mm diameter bubble that contained tylosin tartrate. The bubble is surrounded by the faint yellow color of tylosin tartrate, and yellow particulate matter is dispersed in the joining solution.
34E	System is totally sealed by the joining solution except for the 6.0 mm diameter bubble that contained tylosin tartrate. The bubble is surrounded by the faint yellow color of tylosin tartrate, and a small amount of yellow particulate matter is dispersed in the joining solution.

DISCUSSION

TLC Quantitative Analysis of Tylosin Tartrate

Touchstone and Dobbins (1978) reported that most densitometric methods resulted in sensitivities from 0.1 ng to 10.0 ug and coefficients of variance from one to five percent. Only one literature reference was found for a study that utilized TLC to detect tylosin tartrate (a sensitivity in the range of 2.0-4.0 ug was reported); however, direct quantitative analysis was not conducted in that study (Debackere and Baeten, 1971).

In the current study, a sensitivity of 0.2 ug of tylosin tartrate was achieved. Samples in the range of 0.2-0.8 ug produced coefficients of variance from 10.0-28.6 percent when applied as a type-one aqueous solution. Samples from 0.9-10.0 ug had coefficients of variance from 5.6-10.5 percent when applied as a type-one aqueous solution. There is an inverse relationship between spot intensity and variability.

Faint spots produce higher coefficients of variance than intense spots. The spot-intensity produced by a particular amount of tylosin tartrate varies from plate to plate; consequently, the coefficient of variance would be affected accordingly. For example, the 0.3 ug and the 0.9 ug tylosin tartrate samples in Table 13 have similar spot intensities, and their coefficients of variance differ by 0.5 percent.

The application process is potentially a large source of error. This study utilized a single-spot apparatus; however, multi-spot devices are commercially available. With a multi-spot apparatus, all of the

samples on a TLC plate are applied simultaneously under identical environmental conditions.

The saline concentration of the application solution affects its viscosity, and the viscosity of the application solution influences spot diameter and R_f. In this study, the 10.0 ul amounts applied to the TLC plate were applied in approximately 3.0 ul increments. A low concentation saline solution has less viscosity than a high concentration saline solution; therefore, the 3.0 ul amount (approximate) of low saline concentration will spread more when applied to the TLC plate. The low concentration saline solution spreads the tylosin tartrate over a greater area compared with the high concentration saline solution. This results in greater R_f values since the developing solution acts over the larger spot area, facilitating sample movement. Larger spots are more diffuse; consequently, the coefficients of variance are greater. The results of this study indicate that a 500.0 ul amount of type-one water containing the salts from 2.0 ml of mammalian Ringer's solution yielded coefficients of variance twice as large as those for pure type-one water. It was also found that a 100.0 ul amount of type-one water containing the salts from 2.0 ml of mammalian Ringer's solution produced spot diameters and R_f values comparable to those for pure type-one water. The salts in an application solution are retained by the preadsorbent layer of the Whatman $LKC_{18}F$ TLC plate (Sherma, 1982); therefore, aqueous saline solutions may be applied directly to the plate without extraction. To reduce spot diameter and stabalize R_f, the saline concentration of all

application solutions could be increased to the concentration of the 100.0 ul saline solution. This would reduce the saline solution coefficients of variance values to those of the pure type-one water solutions and the extraction process would still be unnecessary.

Thin layer chromatography utilizes physical and chemical properties to separate a sample into components. The TLC process produced a two-spot pattern for 0.2-0.7 ug amounts of tylosin tartrate and a three-spot pattern for 0.8-10.0 ug amounts of tylosin tartrate. The visualization spray (ten percent by volume sulfuric acid in methanol) reacts with organic materials to produce a dark area. In aqueous solution, tylosin tartrate separates into its component ions. Tylosin is amphipathic. The tartrate group is polar. The developing solution is polar relative to the Whatman $LKC_{18}F$ TLC plate; therefore, the tartrate favors the developing solution and moves easily with the developing solution. For this reason, it is hypothesized that the top band of greatest R is due to the tartrate. The dark spot utilized for the densitometric analysis of tylosin appears at an R value indicative of an amphipathic compound. Polar regions permit movement by the developing solution, but nonpolar regions cause tylosin to be retained by the TLC plate. The composition of the light, diffuse spot visible for 0.8-10.0 ug amounts of tylosin tartrate may be due to fragmentation of the tylosin molecule or other organic impurities. (H. M. Stahr, personal communication.)[1] Subsequent controlled-release studies will utilize lachrymal fluid as the medium.

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It is anticipated that the TLC process will separate tylosin from the proteins, enzymes, and other contaminants within lachrymal fluid. This capability is a definite advantage of the TLC process.

The TLC quantitative analysis procedure utilized in this study was time-consuming, but a multi-spotting apparatus would significantly reduce sample application times and an electronic digital integrator would eliminate the "cut and weigh" method of peak-area measurement. Additionally, these two modifications are expected to reduce the coefficient of variance.

TLC quantitative analysis relies upon the linear relationship between average weight of peak and tylosin tartrate amount for the quantitation of unknown amounts of tylosin tartrate. The experimentally determined coefficient of determination values (r^2) were close to 1.0. This indicates that a high degree of linearity was achieved.

Production of the Controlled-Release Systems

The control membrane thickness affects the rate of drug release from a system (Olanoff and Anderson, 1979). A large number of 90:10 MMA/2-HEMA films were produced in order to obtain the thirty disks needed for the controlled-release systems. The 0.223 ± 10% mm thickness utilized for this report was the minimum thickness available that provided thirty disks. The viscosity of the 90:10 MMA/2-HEMA solution limited the production of thinner films; and larger amounts of acetone and dimethylformamide resulted in films with a significant increase in the number and the size of entrapped air bubbles. Olanoff and Anderson

(1979) utilized acetone and dioxane solvents to produce a trilaminar insert, and this choice of solvent might offer somewhat improved fabrication results (a preliminary study indicated that good films can be made).

The post-experiment examination of the controlled-release systems indicated the need for better quality control of the assembly process. The 55.0 gm weight on the top glass-slide ensured that the edges of the two 90:10 MMA/2-HEMA disks remained in contact with the joining solution during fabrication. This was necessary because the disks were not always perfectly flat. The weight also caused the joining solution to flow from its location on the disk perimeter to the interior or exterior of the system. A possible method of producing flat disks is to place water soaked disks between two, weighted, glass slides that hold the disks flat. When the room temperature evaporation process is complete, the disks should be flat and rigid. The 20G-1 needle utilized for joining solution application was the smallest gauge needle that permitted passage of the 90:10 MMA/2-HEMA solution. A less viscous joining solution would pass through a narrower bore needle and permit closer regulation of the joining solution quantity applied to the perimeter of the 90:10 MMA/2-HEMA disk.

The poly (lactic acid)-tylosin tartrate matrix (32A-E) and the 5.0 mg of tylosin tartrate within systems (33A-E) were easily retained by the devices. Controlled-release systems 34A-E contained 50.0 mg of tylosin tartrate. This amount of drug filled the system and resulted in

extensive intermixing of the drug and joining solution in some devices. Therefore, smaller amounts of drug loading are recommended for devices of this size.

Tylosin Tartrate Release-Experiment

The dark spot diameters reported in Table 12 and the diameters of the dark spots from tylosin tartrate mammalian Ringer's solutions in Figure 20 indicate that spot diameter increases with decreased saline concentration between the pure type-one water and 500.0 ul saline solution; however, there is no significant difference in spot diameter due to saline concentration between 500.0 ul saline solutions and mammalian Ringer's solutions. Three hundred samples were collected during the release-experiment and dried. Forty-five samples were resolubilized with 500.0 ul or more of type-one water, 191 samples were resolubilized with 100.0 ul of type-one water, and sixty-four samples were resolubilized with intermediate amounts of type-one water. The maximum expected coefficient of variance occurs for samples resolubilized with 500.0 ul or more of type-one water. The samples diluted with 500.0 ul or more of type-one water produced tylosin tartrate spots in the range of 0.9-5.0 ug. These samples represent dried salts and drug to which type-one water is added. Aqueous solutions in this range produced coefficients of variance of 5.6-10.5 percent (Table 13); therefore, coefficients of variance of 11.2-21.0 percent are expected for these release samples (based on the

coefficients of variance in Table 15 being approximately double those in Table 13). The samples diluted with 100.0 ul of type-one water have coefficients of variance similar to those for the aqueous solutions in Table 13 (0.2-0.8 ug, 10.0-28.6 percent; 0.9-5.0 ug, 5.6-10.5 percent). The samples diluted with intermediate amounts of type-one water have coefficients of variance between these two extremes.

These coefficients of variance are greater than the one to five percent values reported in most densitometic methods (Touchstone and Dobbins, 1978). However, the approximate release rates of the controlled-release systems can be determined. These release rates can be compared with the release rates required to maintain the minimum inhibitory concentration of tylosin tartrate, and this comparison can be utilized as a basis both for improving release systems and for developing prototype units for animal trials.

Olanoff and Anderson (1979) found that the tetracycline release rate from a 63:37 MMA/2-HEMA matrix covered with a 98:2 MMA/2-HEMA coating was a function of general device geometry, control membrane thickness, disk surface area, level of core reservoir drug loading, and copolymer composition of the membrane coating. All of the controlled-release systems in this study have the same general geometry and control membrane composition; therefore, these contributory factors were not examined. The influence of the control membrane thickness, disk surface area, and level of core reservoir drug loading can be examined from the experimental results of this study.

An overall view of the systems indicates that controlled-release systems 32A-E and 33A-E produced zero-order release rates covering the lower portion of the required release rate range, and controlled-release systems 34A-E produced zero-order release rates exceeding the required release rate range. The highest zero-order release rate of 33.3 ug/hr was achieved with system 34B. This amount is far below the 30 mg tylosin tartrate dose currently sprayed into the eye in a single application. Controlled-release systems, that are stored for a period of time before use, exhibit an initially high rate of release called the burst effect. This occurs because the drug has time to saturate the control membrane of the device (Cowsar, 1974). This phenomenon occurred in all of the systems. The largest rate of drug release (2244.2 ug/hr) during the burst effect was from system 34B. The concentration of tylosin tartrate in the eye during the burst effect is greater than the concentration in the eye during the zero-order release period; however, the concentration is still below the single application spray dosage currently utilized. Therefore, the burst effect is not a problem. In fact, the high release rate may be of benefit by eradicating contributory organisms of BIK.

The following discussion combines the results of the post-experiment stereomicroscopic examination and the factors outlined by Olanoff and Anderson (1979) in order to develop an explanation of the range of release rates and release characteristics observed.

Controlled-release systems 32A-E contained a poly (lactic acid)-tylosin tartrate matrix between two 90:10 MMA/2-HEMA control membranes. The thickness of the control membrane and the thickness and weight of the inner layer were carefully regulated so similar release characteristics were expected. However, the release rates were different, and most of the variance may be attributed to the assembly process. After the initial burst effect, system 32A provided zero-order release for fifty-six hours followed by a rapid increase in the release rate and a return to the initial zero-order release rate. It is believed that the rapid increase in the release rate was due to an increase in the concentration of tylosin tartrate within the device. The drug core is a matrix device and uneven distribution of tylosin tartrate within the matrix could cause this phenomenon. System 32B released a similar cumulative amount of tylosin tartrate as 32A; however, the zero-order release occurred at a slower rate for seventy-two hours. Six samples during the zero-order release period did not contain enough tylosin tartrate for detection. The open area of system 32B is approximated by a 12.0 mm diameter circle and that of 32A is approximated by a 10.0 mm diameter circle. Since 32B has the greater effective area of release, it would be expected to have the higher release rate. This apparent discrepancy may be explained by the level of drug loading in the core. If the concentration gradient across the control membrane is less, the release rate will also be less. System 32C was completely sealed with joining solution; therefore, the thickness of the control membrane was greatest for this device. The

thicker control membrane is expected to slow the release rate, and 32C has one of the slowest release rates in the series (0.1 ug/hr). The rate of release was so low that eleven of the samples did not contain a sufficient quantity of tylosin tartrate for detection. The post-experiment characteristics of systems 32A and 32D are identical; thus, similar release characteristics are expected. Unfortunately, the release rate from 32D is nine times slower than the release rate of 32A. Both systems had similar control membrane thickness and effective area of release; thus, the variation must be associated with the level of drug loading in the core. System 32E released 870.1 ug of tylosin tartrate in a forty-eight hour burst, the largest cumulative release in this series. System 32E was the only device that did not produce a period of zero-order release. The 14.0 mm diameter effective release area is the largest area in this series; thus, an increased rate of release would be expected, but that rate should be lower than the experimental values. The release pattern of 32E is indicative of a matrix system, not a reservoir, since there is a rapid decrease in the release rate versus time. Although no perforations were located in the system during the post-experiment analysis, a break in the control membrane would permit this type of release.

Controlled-release systems 33A-E contained 5.0 mg of tylosin tartrate between two 90:10 MMA/2-HEMA control membranes. The thickness of the control membrane and the weight of tylosin were carefully regulated; therefore, similar release characteristics were expected.

However, the release rates were different, and the variance may be attributed to the assembly process. System 33A produced a 0.6 ug/hr zero-order release rate for ninety-two hours and released a total cumulative tylosin tartrate amount of 347.0 ug. The effective release area has a 5.0 mm diameter. Tylosin tartrate remained inside of this area during the assembly process, and did not intermix with the joining solution. The post-experiment characteristics of systems 33A and 33B are identical; thus, similar release characteristics are expected and do occur. System 33B produced a 0.7 ug/hr zero-order release rate for eighty-eight hours and released a total cumulative tylosin tartrate amount of 408.8 ug. System 33C was completely sealed, and the tylosin tartrate was dispersed within the joining solution. After the experiment, there was no evidence of tylosin residue. The thicker control membrane is expected to slow the release rate, and an experimental value of 0.3 ug/hr was obtained. System 33D contained a 2.0 mm diameter effective release area, and the remainder of the device was sealed. The tylosin tartrate intermixed with the joining solution, and the device retained the yellow color of tylosin tartrate after the experiment. The intermixing of the tylosin tartrate and the joining solution coated the drug particles, effectively prohibiting that drug amount from contributing to the concentration gradient. The 0.2 ug/hr zero-order release rate and the 136.8 ug amount of tylosin tartrate were the lowest values in this series. System 33E contained a 4.0 mm diameter effective release area, and the remainder of the device was

sealed. These physical characteristics are similar to those of 33A and 33B; therefore, similar release characteristics are expected. The burst effect of system 33E released more drug than systems 33A or 33B so the 739.0 ug cumulative release is greater. However, the ninety-six hour, 0.8 ug/hr zero-order release rate correlates well with the values obtained for systems 33A and 33B.

Controlled-release systems 34A-E contained 50.0 mg of tylosin tartrate between two 90:10 MMA/2-HEMA control membranes. The thickness of the control membrane and the weight of tylosin were carefully regulated; thus, similar release characteristics were expected. However, the release rates were quite variable, and these differences may be attributed to the assembly process. Due to the physical size limitations of the controlled-release systems it was very difficult to prevent intermixing of the joining solution and tylosin tartrate. This intermixing is the primary cause of variation in the release characteristics for systems 34A-E. System 34A produced a 6.0 ug/hr zero-order release rate for eighty-four hours and released a total cumulative tylosin tartrate amount of 31,729.8 ug. The effective release area has a 6.0 mm diameter. Tylosin tartrate mixed with the joining solution at the interface, and the device retained the faint yellow color of tylosin tartrate after the experiment. The post-experiment characteristics of systems 34A and 34B are identical; thus, similar release characteristics are expected. System 34B produced a 33.3 ug/hr zero-order release rate for eighty-four hours and released a total cumulative tylosin tartrate amount of 32,619.0 ug. Therefore, the

cumulative tylosin tartrate release for systems 34A and 34B are similar, but the release rate for system 34B is 5.6 times higher than that of 34A. This difference cannot be explained by the post-experiment characteristics since the devices appeared identical. However, it is possible that the joining solution may have mixed with the tylosin tartrate to a greater extent in system 34A than in system 34B. Thus, the tylosin tartrate concentration gradient in system 34A would be lower and the release rate smaller. System 34C contained a 6.0 mm diameter effective release area and the remainder of the device was sealed. Extensive intermixing of the joining solution and tylosin tartrate occurred, and tylosin tartrate particles remained in the device after the experiment. This intermixing dramatically reduced the drug concentration gradient across the membrane. System 34C produced a 1.0 ug/hr zero-order release rate for sixty-eight hours and released a total cumulative tylosin tartrate amount of 991.8 ug. System 34D had a 9.0 mm diameter effective release area; otherwise, its post-experiment characteristics were identical to those of system 34C. The intermixing of tylosin tartrate and joining solution is the primary factor in the 10.0 ug/hr, thirty-six hour zero-order release rate and total cumulative tylosin release of 1,316.7 ug for system 34D. System 34E produced a 20.3 ug/hr zero-order release rate for eighty-eight hours and released a total cumulative tylosin tartrate amount of 25,927.4 ug. The effective release area has a 6.0 mm diameter. Some intermixing of the joining solution and tylosin tartrate occurred, and a few tylosin tartrate particles remained in the device after the experiment. This intermixing

was more extensive than systems 34A and 34B and less extensive than systems 34C and 34D; therefore, intermediate release rates were expected and did occur.

The release-experiment data indicate that with improved quality control it is possible to achieve a predictable zero-order release of tylosin tartrate powder from 90:10 MMA/2-HEMA reservoir devices at the rate needed to treat BIK. The release rates from the 33 series were not sufficient to cover the entire lachrymal flow range, and the release rates from the 34 series exceeded the required rates. Therefore, the optimum drug loading is between 5.0-50.0 mg. Although the devices have an exterior diameter of 17.9 mm, the effective release area diameters were on the order of 4.0 mm for the 33 series and 7.0 mm for the 34 series. Zero-order release is achieved by this configuration because the hydrophobic 90:10 MMA/2-HEMA control membrane restricts the flow of water to the interior of the device. Thus, a saturated solution is maintained within the system for the duration of the experiment.

RECOMMENDATIONS FOR FURTHER RESEARCH

Hughes and Pugh (1975) found that 42.9-44.5 mm diameter rings constructed of poly (vinyl chloride) tubing (with a tube outside diameter of 2.82, 1.65, or 0.914 mm) could be retained in the bovine eye for up to nineteen days. Reaction to the devices was minimal and consisted of increased tearing initially and increased mucous secretion after prolonged retention. A ring of this type could serve as the retaining device for a 90:10 MMA/2-HEMA-tylosin tartrate ocular controlled-release system.

Figure 43 illustrates the configuration of a suggested prototype. The poly (vinyl chloride) tubing is first formed into a ring by inserting a short piece of tube with a smaller diameter into the two ends and joining the outer tube edges utilizing a tetrahydrofuran solvent. Next, the 90:10 MMA/2-HEMA films are cut into crescents so the curved edge conforms to the shape of the poly (vinyl chloride) ring (See Figure 43). The crescents are the control membranes and should be totally flat for the assembly process as suggested earlier. Tylosin tartrate powder is weighed onto a crescent, the joining solution is carefully applied, and the top crescent is placed on the joining solution to seal the device. The results of this study indicate the importance of not intermixing the tylosin tartrate and joining solution, and controlling the size of the effective release area. When both crescents are dry, they are attached to the poly (vinyl chloride) ring with 90:10 MMA/2-HEMA joining solution. This device is designed to fit

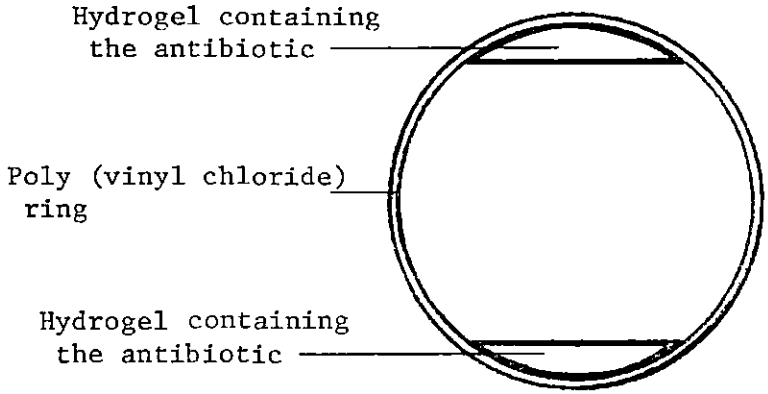

FIGURE 43. Prototype, ocular controlled-release system

into the conjunctival sac. Insertion is accomplished by holding the eyelids open and directing one side of the ring into the lateral fornix. The remainder of the ring is guided under the lids and onto the outer surface of the nictitating membrane (Hughes and Pugh, 1975). The two crescents would be in the upper and lower conjunctival sac. This configuration does not impede oxygen flow to the cornea, and the system would release tylosin tartrate into the region where drugs are currently applied (Blood and Henderson, 1979; Hughes, 1981).

The 90:10 MMA/2-HEMA film is a fairly rigid, hydrophobic material and may cause ocular irritation. To reduce ocular irritation, a more hydrophilic hydrogel may be needed. Since tylosin tartrate is very soluble in water, a controlled-release system composed of hydrophilic hydrogel may not provide long-term release because too much water may flow through the hydrogel. Should this adaptation be required, tylosin (water solubility 5 mg/ml at 25°C) (Windholz et al., 1976) is available

from Elanco Products Co. (T. Matsuoka, personal communication)[1], and could possibly be utilized in place of tylosin tartrate.

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[1] Elanco Products Co., Indianapolis, Indiana.

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APPENDIX A: AVERAGE WEIGHT OF PEAK DATA BASED ON SEVEN IDENTICAL TYLOSIN TARTRATE AMOUNTS IN TYPE-ONE AQUEOUS SOLUTION ON A TLC PLATE

Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
0.2	0.0080	0.0078	0.0079
0.2	0.0038	0.0038	0.0038
0.2	0.0106	0.0108	0.0107
0.2	0.0072	0.0078	0.0075
0.2	0.0062	0.0062	0.0062
0.2	0.0066	0.0064	0.0065
0.2	0.0064	0.0062	0.0063
0.3	0.0096	0.0096	0.0096
0.3	0.0102	0.0102	0.0102
0.3	0.0084	0.0080	0.0082
0.3	0.0110	0.0120	0.0115
0.3	0.0096	0.0104	0.0100
0.3	0.0106	0.0108	0.0107
0.3	0.0092	0.0084	0.0088
0.4	0.0228	0.0206	0.0217
0.4	0.0158	0.0152	0.0155
0.4	0.0135	0.0118	0.0126
0.4	0.0238	0.0253	0.0245
0.4	0.0158	0.0158	0.0158
0.4	0.0148	0.0164	0.0156
0.4	0.0174	0.0178	0.0176
0.5	0.0232	0.0242	0.0237
0.5	0.0207	0.0207	0.0207
0.5	0.0224	0.0228	0.0226
0.5	0.0181	0.0165	0.0173
0.5	0.0174	0.0174	0.0174
0.5	0.0230	0.0240	0.0235
0.5	0.0200	0.0206	0.0203
0.6	0.0188	0.0202	0.0195
0.6	0.0287	0.0271	0.0279
0.6	0.0230	0.0222	0.0226
0.6	0.0284	0.0286	0.0285
0.6	0.0241	0.0241	0.0241
0.6	0.0326	0.0334	0.0330
0.6	0.0291	0.0315	0.0303

Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
0.7	0.0222	0.0234	0.0228
0.7	0.0168	0.0168	0.0168
0.7	0.0164	0.0162	0.0163
0.7	0.0128	0.0126	0.0127
0.7	0.0166	0.0166	0.0166
0.7	0.0170	0.0172	0.0171
0.7	0.0184	0.0186	0.0185
0.8	0.0120	0.0120	0.0120
0.8	0.0140	0.0132	0.0136
0.8	0.0118	0.0128	0.0123
0.8	0.0100	0.0100	0.0100
0.8	0.0088	0.0092	0.0090
0.8	0.0130	0.0132	0.0131
0.8	0.0100	0.0100	0.0100
0.9	0.0224	0.0212	0.0218
0.9	0.0179	0.0179	0.0179
0.9	0.0204	0.0200	0.0202
0.9	0.0228	0.0226	0.0227
0.9	0.0176	0.0176	0.0176
0.9	0.0196	0.0188	0.0192
0.9	0.0163	0.0177	0.0170
1.0	0.0132	0.0134	0.0133
1.0	0.0126	0.0120	0.0123
1.0	0.0149	0.0149	0.0149
1.0	0.0100	0.0110	0.0105
1.0	0.0118	0.0114	0.0116
1.0	0.0121	0.0121	0.0121
1.0	0.0144	0.0140	0.0142
2.0	0.0188	0.0188	0.0188
2.0	0.0181	0.0181	0.0181
2.0	0.0169	0.0169	0.0169
2.0	0.0186	0.0186	0.0186
2.0	0.0168	0.0172	0.0170
2.0	0.0185	0.0177	0.0181
2.0	0.0166	0.0166	0.0166

Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
3.0	0.0518	0.0596	0.0557
3.0	0.0537	0.0537	0.0537
3.0	0.0494	0.0478	0.0486
3.0	0.0522	0.0524	0.0523
3.0	0.0538	0.0536	0.0537
3.0	0.0508	0.0500	0.0504
3.0	0.0500	0.0498	0.0499
4.0	0.0139	0.0137	0.0138
4.0	0.0150	0.0142	0.0146
4.0	0.0134	0.0130	0.0132
4.0	0.0156	0.0158	0.0157
4.0	0.0121	0.0125	0.0123
4.0	0.0153	0.0155	0.0154
4.0	0.0141	0.0143	0.0142
5.0	0.0294	0.0290	0.0292
5.0	0.0288	0.0288	0.0288
5.0	0.0271	0.0271	0.0271
5.0	0.0305	0.0311	0.0308
5.0	0.0300	0.0304	0.0302
5.0	0.0261	0.0269	0.0265
5.0	0.0227	0.0221	0.0224
6.0	0.0331	0.0331	0.0331
6.0	0.0337	0.0349	0.0343
6.0	0.0319	0.0315	0.0317
6.0	0.0346	0.0344	0.0345
6.0	0.0300	0.0308	0.0304
6.0	0.0324	0.0322	0.0323
6.0	0.0355	0.0359	0.0357
7.0	0.0346	0.0352	0.0349
7.0	0.0377	0.0373	0.0375
7.0	0.0326	0.0354	0.0340
7.0	0.0368	0.0364	0.0366
7.0	0.0335	0.0331	0.0333
7.0	0.0375	0.0375	0.0375
7.0	0.0313	0.0297	0.0305

Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
8.0	0.0455	0.0453	0.0454
8.0	0.0470	0.0470	0.0470
8.0	0.0491	0.0483	0.0487
8.0	0.0415	0.0397	0.0406
8.0	0.0459	0.0457	0.0458
8.0	0.0452	0.0442	0.0447
8.0	0.0372	0.0372	0.0372
9.0	0.0712	0.0712	0.0712
9.0	0.0602	0.0600	0.0601
9.0	0.0668	0.0668	0.0668
9.0	0.0625	0.0653	0.0639
9.0	0.0703	0.0707	0.0705
9.0	0.0686	0.0686	0.0686
9.0	0.0660	0.0660	0.0660
10.0	0.0850	0.0838	0.0844
10.0	0.0937	0.0955	0.0946
10.0	0.0904	0.0904	0.0904
10.0	0.0754	0.0714	0.0734
10.0	0.0920	0.0896	0.0908
10.0	0.0889	0.0891	0.0890
10.0	0.0834	0.0836	0.0835

APPENDIX B: AVERAGE WEIGHT OF PEAK DATA FOR THE THREE GROUPS OF TYLOSIN TARTRATE TYPE-ONE AQUEOUS SOLUTION STANDARDS

TLC Plate	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
1	0.1	----	----	----
1	0.2	0.0049	0.0051	0.0050
1	0.3	0.0069	0.0069	0.0069
1	0.4	0.0104	0.0092	0.0098
1	0.5	0.0082	0.0082	0.0082
1	0.6	0.0092	0.0082	0.0087
1	0.7	0.0155	0.0155	0.0155
2	0.1	----	----	----
2	0.2	0.0058	0.0056	0.0057
2	0.3	0.0066	0.0070	0.0068
2	0.4	0.0082	0.0082	0.0082
2	0.5	0.0087	0.0059	0.0073
2	0.6	0.0105	0.0105	0.0105
2	0.7	0.0170	0.0188	0.0179
3	0.1	----	----	----
3	0.2	0.0013	0.0013	0.0013
3	0.3	0.0030	0.0030	0.0030
3	0.4	0.0058	0.0066	0.0062
3	0.5	0.0068	0.0072	0.0070
3	0.6	0.0066	0.0068	0.0067
3	0.7	0.0094	0.0084	0.0089
4	0.1	----	----	----
4	0.2	0.0042	0.0038	0.0040
4	0.3	0.0060	0.0060	0.0060
4	0.4	0.0058	0.0066	0.0062
4	0.5	0.0068	0.0072	0.0070
4	0.6	0.0066	0.0068	0.0067
4	0.7	0.0094	0.0084	0.0089
5	0.1	----	----	----
5	0.2	0.0042	0.0038	0.0040
5	0.3	0.0060	0.0060	0.0060
5	0.4	0.0101	0.0095	0.0098
5	0.5	0.0118	0.0110	0.0114
5	0.6	0.0087	0.0075	0.0081
5	0.7	0.0118	0.0124	0.0121

TLC Plate	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
6	0.8	0.0134	0.0114	0.0124
6	0.9	0.0096	0.0098	0.0097
6	1.0	0.0157	0.0149	0.0153
6	2.0	0.0328	0.0330	0.0329
6	3.0	0.0514	0.0482	0.0498
6	4.0	0.0673	0.0689	0.0681
6	5.0	0.1118	0.1078	0.1098
7	0.8	0.0063	0.0063	0.0063
7	0.9	0.0081	0.0083	0.0082
7	1.0	0.0146	0.0154	0.0150
7	2.0	0.0269	0.0285	0.0277
7	3.0	0.0504	0.0476	0.0490
7	4.0	0.0716	0.0708	0.0712
7	5.0	0.0938	0.0906	0.0922
8	0.8	0.0035	0.0029	0.0032
8	0.9	0.0055	0.0055	0.0055
8	1.0	0.0072	0.0082	0.0077
8	2.0	0.0218	0.0228	0.0223
8	3.0	0.0309	0.0313	0.0311
8	4.0	0.0391	0.0423	0.0407
8	5.0	0.5360	0.0614	0.0575
9	0.8	0.0031	0.0039	0.0035
9	0.9	0.0072	0.0086	0.0079
9	1.0	0.0117	0.0107	0.0112
9	2.0	0.0221	0.0215	0.0218
9	3.0	0.0373	0.0367	0.0370
9	4.0	0.0504	0.0536	0.0520
9	5.0	0.0773	0.0771	0.0772
10	0.8	0.0168	0.0184	0.0176
10	0.9	0.0156	0.0154	0.0155
10	1.0	0.0236	0.0248	0.0242
10	2.0	0.0419	0.0401	0.0410
10	3.0	0.0751	0.0787	0.0769
10	4.0	0.0941	0.0965	0.0953
10	5.0	0.1359	0.1413	0.1386

TLC Plate	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
11	4.0	0.0167	0.0155	0.0161
11	5.0	0.0212	0.0196	0.0204
11	6.0	0.0268	0.0280	0.0274
11	7.0	0.0348	0.0336	0.0342
11	8.0	0.0381	0.0395	0.0388
11	9.0	0.0436	0.0428	0.0432
11	10.0	0.0482	0.0474	0.0478
12	4.0	0.0155	0.0153	0.0154
12	5.0	0.0250	0.0224	0.0237
12	6.0	0.0309	0.0305	0.0307
12	7.0	0.0407	0.0383	0.0395
12	8.0	0.0447	0.0435	0.0441
12	9.0	0.0473	0.0437	0.0455
12	10.0	0.0481	0.0507	0.0494
13	4.0	0.0104	0.0086	0.0095
13	5.0	0.0144	0.0138	0.0141
13	6.0	0.0184	0.0176	0.0180
13	7.0	0.0248	0.0250	0.0249
13	8.0	0.0287	0.0281	0.0284
13	9.0	0.0328	0.0308	0.0318
13	10.0	0.0342	0.0350	0.0346
14	4.0	0.0169	0.0183	0.0176
14	5.0	0.0266	0.0266	0.0266
14	6.0	0.0319	0.0277	0.0298
14	7.0	0.0354	0.0330	0.0342
14	8.0	0.0382	0.0378	0.0380
14	9.0	0.0365	0.0373	0.0369
14	10.0	0.0450	0.0466	0.0458
15	4.0	0.0106	0.0114	0.0110
15	5.0	0.0170	0.0178	0.0174
15	6.0	0.0214	0.0196	0.0205
15	7.0	0.0252	0.0266	0.0259
15	8.0	0.0307	0.0299	0.0303
15	9.0	0.0342	0.0352	0.0347
15	10.0	0.0381	0.0409	0.0395

APPENDIX C: AVERAGE WEIGHT OF PEAK DATA FOR VARIOUS SALINE CONCENTRATIONS WITH IDENTICAL TYLOSIN TARTRATE AMOUNTS

TLC plate	Amount of tylosin tartrate (ug)	Amount of type-one water added to the salts of 2 ml of Ringer's solution (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
1	2.0	100% type-one water	0.0112	0.0112	0.0112
1	2.0	100% type-one water	0.0116	0.0110	0.0113
1	2.0	100.0 ul	0.0080	0.0090	0.0085
1	2.0	100.0 ul	0.0089	0.0091	0.0090
1	2.0	300.0 ul	0.0108	0.0104	0.0106
1	2.0	300.0 ul	0.0087	0.0089	0.0088
1	2.0	500.0 ul	0.0145	0.0141	0.0143
1	2.0	500.0 ul	0.0127	0.0123	0.0125
2	3.0	100% type-one water	0.0167	0.0177	0.0172
2	3.0	100% type-one water	0.0171	0.0177	0.0174
2	3.0	100.0 ul	0.0158	0.0160	0.0159
2	3.0	100.0 ul	0.0149	0.0141	0.0145
2	3.0	300.0 ul	0.0152	0.0160	0.0156
2	3.0	300.0 ul	0.0142	0.0146	0.0144
2	3.0	500.0 ul	0.0191	0.0189	0.0190
2	3.0	500.0 ul	0.0197	0.0181	0.0189
3	4.0	100% type-one water	0.0163	0.0165	0.0164
3	4.0	100% type-one water	0.0181	0.0171	0.0176
3	4.0	100.0 ul	0.0192	0.0196	0.0194
3	4.0	100.0 ul	0.0179	0.0179	0.0179
3	4.0	300.0 ul	0.0200	0.0196	0.0198
3	4.0	300.0 ul	0.0180	0.0196	0.0188
3	4.0	500.0 ul	0.0239	0.0235	0.0237
3	4.0	500.0 ul	0.0226	0.0226	0.0226

APPENDIX D: TYLOSIN TARTRATE RELEASE-EXPERIMENT 32A

Sample number	Regression line data[a]	Total volume of sample[b] (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	1	1090.0	0.0194	0.0180	0.0187	1.1	119.9
2	1	590.0	0.0506	0.0502	0.0504	2.9	171.1
3	1	190.0	0.0438	0.0474	0.0456	2.6	49.4
4	1	100.0	0.0321	0.0321	0.0321	1.8	18.0
5	2	100.0	0.0034	0.0032	0.0033	2.4	24.0
6	-	100.0	No spot developed on the TLC plate				
7	-	100.0	No spot developed on the TLC plate				
8	2	100.0	0.0478	0.0480	0.0479	3.5	35.0
9	3	100.0	0.0137	0.0145	0.0141	0.7	7.0
10	3	100.0	0.0035	0.0035	0.0035	0.2	2.0
11	3	100.0	0.0023	0.0017	0.0020	0.2	2.0
12	3	100.0	0.0026	0.0026	0.0026	0.2	2.0
13	4	100.0	0.0041	0.0039	0.0040	0.4	4.0
14	4	100.0	0.0039	0.0029	0.0034	0.4	4.0
15	4	100.0	0.0050	0.0042	0.0046	0.4	4.0
16	5	190.0	0.0270	0.0284	0.0277	1.6	30.4
17	4	100.0	0.0033	0.0031	0.0032	0.3	3.0
18	6	100.0	0.0065	0.0055	0.0060	0.6	6.0
19	5	590.0	0.0327	0.0355	0.0341	2.0	118.0
20	5	100.0	0.0203	0.0207	0.0205	1.2	12.0

[a] See Appendix S.

[b] Amount of type-one water added to the dried sample; this amount of liquid provides the drug level in 10 ul for the spotting to be in the range 0.2-5.0 ug of tylosin tartrate. For example, in sample number one, there are 119.9 ug in 1090.0 ul to give a detected level of 1.1 ug in 10.0 ul.

APPENDIX E: TYLOSIN TARTRATE RELEASE-EXPERIMENT 32B

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	5	1090.0	0.0435	0.0469	0.0452	2.8	305.2
2	7	590.0	0.0435	0.0421	0.0428	2.3	135.7
3	7	190.0	0.0341	0.0333	0.0337	1.8	34.2
4	7	190.0	0.0236	0.0256	0.0246	1.2	22.8
5	6	100.0	0.0118	0.0116	0.0117	1.0	10.0
6	6	100.0	0.0145	0.0125	0.0135	1.1	11.0
7	6	100.0	0.0112	0.0110	0.0106	0.9	9.0
8	7	290.0	0.0172	0.0170	0.0171	0.8	23.2
9	8	100.0	0.0070	0.0080	0.0075	0.4	4.0
10	8	100.0	0.0110	0.0110	0.0110	0.5	5.0
11	8	100.0	0.0102	0.0098	0.0100	0.5	5.0
12	8	100.0	0.0108	0.0098	0.0103	0.5	5.0
13	9	100.0	0.0027	0.0021	0.0024	0.2	2.0
14	-	100.0	No spot developed on the TLC plate				
15	-	100.0	No spot developed on the TLC plate				
16	-	100.0	No spot developed on the TLC plate				
17	-	100.0	No spot developed on the TLC plate				
18	-	100.0	No spot developed on the TLC plate				
19	-	100.0	No spot developed on the TLC plate				
20	10	100.0	0.0013	0.0015	0.0015	0.4	4.0

[a]See Appendix S.

APPENDIX F: TYLOSIN TARTRATE RELEASE-EXPERIMENT 32C

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	11	1090.0	0.0300	0.0296	0.0298	1.5	163.5
2	11	590.0	0.0371	0.0389	0.0380	2.0	118.0
3	11	190.0	0.0256	0.0258	0.0257	1.3	24.7
4	11	100.0	0.0557	0.0529	0.0543	2.9	29.0
5	12	100.0	0.0290	0.0332	0.0311	1.5	15.0
6	12	100.0	0.0138	0.0140	0.0390	0.6	6.0
7	12	100.0	0.0033	0.0033	0.0033	0.2	2.0
8	12	100.0	0.0037	0.0043	0.0040	0.2	2.0
9	--	100.0	No spot developed on the TLC plate				
10	--	100.0	No spot developed on the TLC plate				
11	--	100.0	No spot developed on the TLC plate				
12	--	100.0	No spot developed on the TLC plate				
13	--	100.0	No spot developed on the TLC plate				
14	--	100.0	No spot developed on the TLC plate				
15	--	100.0	No spot developed on the TLC plate				
16	13	100.0	0.0024	0.0022	0.0023	0.2	2.0
17	--	100.0	No spot developed on the TLC plate				
18	--	100.0	No spot developed on the TLC plate				
19	--	100.0	No spot developed on the TLC plate				
20	--	100.0	No spot developed on the TLC plate				

[a]See Appendix S.

APPENDIX G: TYLOSIN TARTRATE RELEASE-EXPERIMENT 32D

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	14	1090.0	0.0615	0.0649	0.0632	4.1	446.9
2	14	100.0	0.0168	0.0164	0.0166	1.0	10.0
3	14	190.0	0.0292	0.0270	0.0281	1.8	34.2
4	14	100.0	No spot developed on the TLC plate				
5	--	100.0	No spot developed on the TLC plate				
6	--	100.0	No spot developed on the TLC plate				
7	--	100.0	No spot developed on the TLC plate				
8	--	100.0	No spot developed on the TLC plate				
9	--	100.0	No spot developed on the TLC plate				
10	--	100.0	No spot developed on the TLC plate				
11	--	100.0	No spot developed on the TLC plate				
12	--	100.0	No spot developed on the TLC plate				
13	15	100.0	0.0052	0.0040	0.0046	0.4	4.0
14	--	100.0	No spot developed on the TLC plate				
15	--	100.0	No spot developed on the TLC plate				
16	--	100.0	No spot developed on the TLC plate				
17	--	100.0	No spot developed on the TLC plate				
18	--	100.0	No spot developed on the TLC plate				
19	--	100.0	No spot developed on the TLC plate				
20	--	100.0	No spot developed on the TLC plate				

[a]See Appendix S.

APPENDIX H: TYLOSIN TARTRATE RELEASE-EXPERIMENT 32E

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	17	1090.0	0.0437	0.0435	0.0436	2.9	316.1
2	17	590.0	0.0424	0.0450	0.0437	2.9	171.1
3	17	190.0	0.0478	0.0472	0.0475	3.1	58.9
4	17	190.0	0.0430	0.0428	0.0429	2.9	55.1
5	16	100.0	0.0292	0.0280	0.0286	1.7	17.0
6	16	100.0	0.0177	0.0157	0.0167	1.0	10.0
7	--	100.0	No spot developed on the TLC plate				
8	16	590.0	0.0671	0.0695	0.0683	4.1	241.9
9	--	100.0	No spot developed on the TLC plate				
10	--	100.0	No spot developed on the TLC plate				
11	--	100.0	No spot developed on the TLC plate				
12	--	100.0	No spot developed on the TLC plate				
13	--	100.0	No spot developed on the TLC plate				
14	--	100.0	No spot developed on the TLC plate				
15	--	100.0	No spot developed on the TLC plate				
16	--	100.0	No spot developed on the TLC plate				
17	--	100.0	No spot developed on the TLC plate				
18	--	100.0	No spot developed on the TLC plate				
19	--	100.0	No spot developed on the TLC plate				
20	--	100.0	No spot developed on the TLC plate				

[a]See Appendix S.

APPENDIX I: TYLOSIN TARTRATE RELEASE-EXPERIMENT 33A

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	1	490.0	0.0726	0.0706	0.0716	4.2	205.8
2	2	180.0	0.0336	0.0330	0.0333	2.4	43.2
3	2	100.0	0.0286	0.0296	0.0291	2.1	21.0
4	2	100.0	0.0194	0.0190	0.0192	1.3	13.0
5	3	100.0	0.0198	0.0190	0.0194	1.0	10.0
6	4	100.0	0.0058	0.0060	0.0059	0.5	5.0
7	5	100.0	0.0097	0.0109	0.0103	0.5	5.0
8	6	100.0	0.0031	0.0031	0.0031	0.4	4.0
9	7	100.0	0.0115	0.0111	0.0113	0.5	5.0
10	8	100.0	0.0040	0.0044	0.0042	0.2	2.0
11	9	100.0	0.0022	0.0022	0.0022	0.2	2.0
12	9	100.0	0.0038	0.0036	0.0035	0.4	4.0
13	9	100.0	0.0017	0.0017	0.0017	0.2	2.0
14	9	100.0	0.0018	0.0018	0.0018	0.2	2.0
15	13	100.0	0.0010	0.0010	0.0010	0.2	2.0
16	10	100.0	0.0077	0.0083	0.0080	1.1	11.0
17	10	100.0	0.0011	0.0011	0.0011	0.3	3.0
18	13	100.0	0.0015	0.0015	0.0015	0.2	2.0
19	11	100.0	0.0061	0.0063	0.0062	0.3	3.0
20	12	100.0	0.0066	0.0070	0.0068	0.2	2.0

[a] See Appendix S.

APPENDIX J: TYLOSIN TARTRATE RELEASE-EXPERIMENT 33B

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	19	480.0	0.0745	0.0723	0.0734	5.0	240.0
2	--	No spot developed on the TLC plate					
3	19	180.0	0.0284	0.0302	0.0293	2.1	37.8
4	18	100.0	0.0591	0.0593	0.0592	2.9	29.0
5	20	100.0	0.0204	0.0200	0.0202	1.0	10.0
6	20	100.0	0.0502	0.0492	0.0497	2.7	27.0
7	20	100.0	0.0105	0.0085	0.0095	0.4	4.0
8	20	100.0	0.0268	0.0256	0.0262	1.3	13.0
9	20	100.0	0.0010	0.0010	0.0010	0.2	2.0
10	21	100.0	0.0008	0.0008	0.0008	0.2	2.0
11	21	100.0	0.0012	0.0014	0.0013	0.2	2.0
12	21	100.0	0.0031	0.0039	0.0035	0.3	3.0
13	21	100.0	0.0013	0.0011	0.0012	0.2	2.0
14	21	100.0	0.0016	0.0014	0.0015	0.2	2.0
15	22	100.0	0.0010	0.0008	0.0009	0.2	2.0
16	18	100.0	0.0435	0.0431	0.0433	2.1	21.0
17	22	100.0	0.0039	0.0041	0.0040	0.5	5.0
18	22	100.0	0.0022	0.0020	0.0021	0.3	3.0
19	22	100.0	0.0018	0.0022	0.0020	0.2	2.0
20	22	100.0	0.0013	0.0013	0.0013	0.2	2.0

[a] See Appendix S.

APPENDIX K: TYLOSIN TARTRATE RELEASE-EXPERIMENT 33C

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	20	580.0	0.0052	0.0052	0.0052	2.8	162.4
2	19	180.0	0.0359	0.0341	0.0350	2.4	43.2
3	22	100.0	0.0144	0.0134	0.0139	1.5	15.0
4	13	100.0	0.1111	0.0105	0.0108	0.9	9.0
5	23	100.0	0.0085	0.0087	0.0086	0.8	8.0
6	23	100.0	0.0051	0.0053	0.0052	0.5	5.0
7	23	100.0	0.0045	0.0049	0.0047	0.5	5.0
8	23	100.0	0.0094	0.0082	0.0088	0.8	8.0
9	23	100.0	0.0029	0.0029	0.0029	0.4	4.0
10	24	100.0	0.0026	0.0024	0.0025	0.2	2.0
11	24	100.0	0.0033	0.0031	0.0032	0.2	2.0
12	24	100.0	0.0061	0.0067	0.0064	0.4	4.0
13	24	100.0	0.0028	0.0028	0.0028	0.2	2.0
14	--	100.0	No spot developed on the TLC plate				
15	24	100.0	0.0010	0.0012	0.0011	0.2	2.0
16	25	100.0	0.0062	0.0060	0.0061	0.3	3.0
17	25	100.0	0.0018	0.0018	0.0018	0.2	2.0
18	25	100.0	0.0016	0.0012	0.0014	0.2	2.0
19	25	100.0	0.0015	0.0013	0.0014	0.2	2.0
20	25	100.0	0.0055	0.0055	0.0055	0.3	3.0

[a] See Appendix S.

APPENDIX L: TYLOSIN TARTRATE RELEASE-EXPERIMENT 33D

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	19	180.0	0.0334	0.0358	0.0346	2.4	43.2
2	27	180.0	0.0297	0.0303	0.0300	1.7	30.6
3	27	100.0	0.0171	0.0147	0.0159	0.9	9.0
4	27	100.0	0.0210	0.0196	0.0203	1.2	12.0
5	25	100.0	0.0046	0.0058	0.0052	0.3	3.0
6	26	100.0	0.0022	0.0022	0.0022	0.2	2.0
7	26	100.0	0.0030	0.0032	0.0031	0.2	2.0
8	26	100.0	0.0084	0.0098	0.0091	0.5	5.0
9	26	100.0	0.0044	0.0044	0.0044	0.3	3.0
10	26	100.0	0.0014	0.0014	0.0014	0.2	2.0
11	--	100.0	No spot developed on the TLC plate				
12	19	100.0	0.0048	0.0044	0.0046	0.4	4.0
13	29	100.0	0.0055	0.0055	0.0055	0.2	2.0
14	29	100.0	0.0072	0.0068	0.0070	0.3	3.0
15	29	100.0	0.0043	0.0039	0.0041	0.2	2.0
16	29	100.0	0.0118	0.0122	0.0120	0.5	5.0
17	28	100.0	0.0027	0.0027	0.0027	0.2	2.0
18	28	100.0	0.0008	0.0008	0.0008	0.2	2.0
19	28	100.0	0.0016	0.0016	0.0016	0.2	2.0
20	28	100.0	0.0055	0.0055	0.0055	0.3	3.0

[a]See Appendix S.

APPENDIX M: TYLOSIN TARTRATE RELEASE-EXPERIMENT 33E

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	30	1180.0	0.0392	0.0412	0.0402	4.1	483.8
2	30	290.0	0.0426	0.0446	0.0436	4.3	124.7
3	30	190.0	0.0137	0.0133	0.0135	1.2	22.8
4	30	190.0	0.0180	0.0168	0.0174	1.6	30.4
5	28	100.0	0.0138	0.0144	0.0141	0.7	7.0
6	--	100.0	No spot developed on the TLC plate				
7	--	100.0	No spot developed on the TLC plate				
8	31	100.0	0.0046	0.0052	0.0049	0.6	6.0
9	31	100.0	0.0021	0.0021	0.0021	0.4	4.0
10	31	100.0	0.0102	0.0100	0.0101	1.1	11.0
11	32	100.0	0.0087	0.0089	0.0088	0.7	7.0
12	32	100.0	0.0160	0.0150	0.0155	1.2	12.0
13	32	100.0	0.0069	0.0061	0.0065	0.6	6.0
14	32	100.0	0.0024	0.0026	0.0025	0.3	3.0
15	32	100.0	0.0032	0.0032	0.0032	0.4	4.0
16	33	100.0	0.0091	0.0093	0.0092	0.7	7.0
17	33	100.0	0.0012	0.0018	0.0016	0.2	2.0
18	33	100.0	0.0010	0.0010	0.0010	0.2	2.0
19	33	100.0	0.0011	0.0011	0.0011	0.2	2.0
20	33	100.0	0.0007	0.0009	0.0008	0.2	2.0

[a]See Appendix S.

APPENDIX N: TYLOSIN TARTRATE RELEASE-EXPERIMENT 34A

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	34	48090.0	0.0297	0.0283	0.0290	1.7	8175.3
2	35	48090.0	0.0157	0.0149	0.0153	1.5	7213.5
3	36	16030.0	0.0182	0.0192	0.0187	2.4	3847.2
4	37	48090.0	0.0205	0.0197	0.0201	1.6	7694.4
5	38	16030.0	0.0046	0.0036	0.0041	1.8	2885.4
6	39	1290.0	0.0575	0.0585	0.0580	2.7	348.3
7	39	2580.0	0.0881	0.0869	0.0875	4.1	1057.8
8	39	290.0	0.0664	0.0622	0.0643	3.0	87.0
9	39	190.0	0.0530	0.0516	0.0523	2.4	45.6
10	39	290.0	0.0700	0.0672	0.0686	3.2	92.8
11	40	290.0	0.0244	0.0254	0.0249	1.9	55.1
12	40	100.0	0.0219	0.0223	0.0221	1.7	17.0
13	40	100.0	0.0199	0.0197	0.0198	1.5	15.0
14	40	100.0	0.0214	0.0224	0.0219	1.7	17.0
15	40	100.0	0.0206	0.0206	0.0206	1.6	16.0
16	41	290.0	0.0400	0.0394	0.0397	3.1	89.9
17	41	100.0	0.0084	0.0074	0.0079	0.7	7.0
18	41	100.0	0.0040	0.0042	0.0041	0.4	4.0
19	41	190.0	0.0136	0.0128	0.0132	1.1	20.9
20	41	290.0	0.0170	0.0178	0.0174	1.4	40.6

[a]See Appendix S.

APPENDIX O: TYLOSIN TARTRATE RELEASE-EXPERIMENT 34B

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	42	48090.0	0.0169	0.0165	0.0167	2.0	9618.0
2	43	48090.0	0.0241	0.0233	0.0237	1.2	5770.8
3	44	48090.0	0.0261	0.0263	0.0262	2.4	11541.6
4	45	4360.0	0.0227	0.0333	0.0330	3.4	1482.4
5	47	2360.0	0.0280	0.0272	0.0286	3.1	731.6
6	34	1090.0	0.0902	0.0918	0.0910	4.8	523.2
7	34	1090.0	0.0433	0.0459	0.0446	2.4	261.6
8	34	1090.0	0.0884	0.0912	0.0898	4.7	512.3
9	34	380.0	0.0603	0.0617	0.0610	3.2	121.6
10	34	380.0	0.0508	0.0490	0.0499	2.7	102.6
11	46	1160.0	0.0191	0.0197	0.0194	4.7	545.2
12	46	3270.0	0.0183	0.0179	0.0181	2.5	817.5
13	46	290.0	0.0225	0.0235	0.0230	3.2	92.8
14	46	190.0	0.0191	0.0185	0.0188	2.6	49.4
15	46	190.0	0.0128	0.0124	0.0126	1.8	34.2
16	49	380.0	0.0373	0.0383	0.0378	3.1	117.8
17	49	380.0	0.0360	0.0344	0.0352	2.7	102.6
18	49	190.0	0.0604	0.0588	0.0596	4.8	91.2
19	49	190.0	0.0480	0.0498	0.0489	4.0	76.0
20	49	190.0	0.0158	0.0166	0.0162	1.4	26.6

[a]See Appendix S.

APPENDIX P: TYLOSIN TARTRATE RELEASE-EXPERIMENT 34C

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	38	300.0	0.0043	0.0047	0.0045	0.7	21.0
2	38	300.0	0.0202	0.0188	0.0195	2.9	87.0
3	38	100.0	0.0025	0.0025	0.0025	1.2	12.0
4	38	100.0	0.0020	0.0020	0.0020	0.6	6.0
5	38	100.0	0.0039	0.0017	0.0028	0.8	8.0
6	44	100.0	0.0035	0.0033	0.0034	0.4	4.0
7	44	100.0	0.0014	0.0014	0.0014	0.2	2.0
8	44	100.0	0.0065	0.0063	0.0064	0.7	7.0
9	44	100.0	0.0049	0.0043	0.0046	0.5	5.0
10	44	100.0	0.0025	0.0021	0.0023	0.5	5.0
11	36	100.0	0.0072	0.0076	0.0074	0.3	3.0
12	36	100.0	0.0187	0.0185	0.0186	0.8	8.0
13	36	100.0	0.0097	0.0087	0.0092	0.4	4.0
14	36	100.0	0.0412	0.0412	0.0412	1.9	19.0
15	36	100.0	0.0352	0.0334	0.0343	1.5	15.0
16	48	190.0	0.0538	0.0526	0.0532	1.2	22.8
17	48	190.0	0.0378	0.0360	0.0369	5.0	95.0
18	48	380.0	0.0253	0.0241	0.0247	3.3	125.4
19	48	380.0	0.0231	0.0243	0.0237	3.1	117.8
20	37	1180.0	0.0412	0.0402	0.0407	3.6	424.8

[a] See Appendix S.

APPENDIX Q: TYLOSIN TARTRATE RELEASE-EXPERIMENT 34D

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	42	300.0	0.0034	0.0024	0.0029	3.9	117.0
2	50	100.0	0.0060	0.0064	0.0062	0.3	3.0
3	51	100.0	0.0154	0.0134	0.0144	1.0	10.0
4	42	100.0	0.0066	0.0062	0.0064	1.6	16.0
5	42	100.0	0.0026	0.0026	0.0026	0.6	6.0
6	43	100.0	0.0329	0.0337	0.0333	1.6	16.0
7	43	100.0	0.0343	0.0367	0.0355	1.8	18.0
8	43	200.0	0.0573	0.0567	0.0570	2.9	58.0
9	43	100.0	0.0764	0.0768	0.0766	3.9	39.0
10	43	190.0	0.0541	0.0549	0.0545	2.7	51.3
11	45	100.0	0.0259	0.0245	0.0252	2.6	26.0
12	45	290.0	0.0387	0.0373	0.0380	3.9	113.1
13	45	290.0	0.0129	0.0121	0.0125	1.4	40.6
14	45	290.0	0.0110	0.0108	0.0109	1.2	34.8
15	45	290.0	0.0188	0.0182	0.0185	2.0	58.0
16	48	1180.0	0.0206	0.0190	0.0198	2.6	306.8
17	47	290.0	0.0327	0.0339	0.0333	3.5	101.5
18	47	290.0	0.0252	0.0260	0.0256	2.7	78.3
19	47	290.0	0.0148	0.0164	0.0156	1.7	49.3
20	47	580.0	0.0286	0.0280	0.0283	3.0	174.0

[a]See Appendix S.

APPENDIX R: TYLOSIN TARTRATE RELEASE-EXPERIMENT 34E

Sample number	Regression line data[a]	Total volume of sample (ul)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)	Amount of tylosin tartrate in 10 ul (ug)	Amount of tylosin tartrate in total sample volume (ug)
1	50	48090.0	0.0255	0.0297	0.0276	2.1	10098.9
2	50	48090.0	0.0212	0.0224	0.0218	1.6	7694.4
3	50	7160.0	0.0438	0.0458	0.0448	3.6	2577.6
4	50	16030.0	0.0087	0.0091	0.0089	1.5	2404.5
5	50	1690.0	0.0438	0.0452	0.0445	3.6	608.4
6	51	1690.0	0.0703	0.0657	0.0680	4.4	743.6
7	51	590.0	0.0344	0.0364	0.0354	2.3	135.7
8	51	100.0	0.0076	0.0060	0.0068	0.5	5.0
9	51	590.0	0.0593	0.0617	0.0605	3.9	230.1
10	51	190.0	0.0474	0.0482	0.0478	3.1	58.9
11	53	290.0	0.0070	0.0080	0.0075	1.4	40.6
12	53	1080.0	0.0371	0.0359	0.0365	5.0	540.0
13	53	1080.0	0.0256	0.0250	0.0253	3.7	399.6
14	53	290.0	0.0073	0.0073	0.0073	1.3	37.7
15	53	590.0	0.0091	0.0091	0.0091	1.9	112.1
16	52	590.0	0.0386	0.0366	0.0376	2.5	147.5
17	52	290.0	0.0032	0.0034	0.0033	0.6	17.4
18	52	290.0	0.0034	0.0034	0.0034	0.6	17.4
19	52	290.0	0.0092	0.0088	0.0090	1.2	34.8
20	52	290.0	0.0054	0.0062	0.0058	0.8	23.2

[a] See Appendix S.

APPENDIX S: REGRESSION LINE DATA FOR THE TYLOSIN TARTRATE RELEASE EXPERIMENT

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
1	0.4	0.0072	0.0066	0.0069
1	0.8	0.0133	0.0137	0.0135
1	1.0	0.0188	0.0204	0.0196
1	2.0	0.0362	0.0376	0.0369
1	3.0	0.0485	0.0523	0.0504
2	0.4	0.0048	0.0044	0.0046
2	0.8	0.0110	0.0122	0.0166
2	1.0	0.0170	0.0160	0.0165
2	2.0	0.0296	0.0306	0.0301
2	3.0	0.0380	0.0404	0.0392
3	0.2	0.0041	0.0039	0.0040
3	0.5	0.0089	0.0093	0.0091
3	0.8	0.0149	0.0147	0.0148
3	1.0	0.0230	0.0234	0.0232
3	2.0	0.0380	0.0382	0.0381
4	0.2	0.0024	0.0016	0.0020
4	0.5	0.0046	0.0046	0.0046
4	0.8	0.0088	0.0090	0.0089
4	1.0	0.0128	0.0122	0.0125
4	2.0	0.0249	0.0241	0.0245
5	0.4	0.0070	0.0068	0.0069
5	0.8	0.0150	0.0146	0.0148
5	1.0	0.0195	0.0197	0.0196
5	2.0	0.0372	0.0352	0.0362
5	3.0	0.0483	0.0453	0.0468
6	0.2	0.0019	0.0019	0.0019
6	0.5	0.0046	0.0054	0.0050
6	0.8	0.0068	0.0074	0.0071
6	1.0	0.0125	0.0125	0.0125
6	2.0	0.0264	0.0260	0.0262

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
7	0.4	0.0100	0.0086	0.0093
7	0.8	0.0162	0.0158	0.0160
7	1.0	0.0209	0.0225	0.0217
7	2.0	0.0377	0.0389	0.0383
7	3.0	0.0539	0.0539	0.0539
8	0.2	0.0043	0.0037	0.0040
8	0.5	0.0123	0.0095	0.0109
8	0.8	0.0172	0.0162	0.0167
8	1.0	0.0205	0.0209	0.0207
8	2.0	0.0434	0.0426	0.0430
9	0.2	0.0024	0.0024	0.0024
9	0.6	0.0046	0.0048	0.0047
9	0.8	0.0075	0.0073	0.0074
9	1.0	0.0104	0.0094	0.0099
9	2.0	0.0181	0.0181	0.0181
10	0.2	0.0010	0.0010	0.0010
10	0.6	0.0027	0.0027	0.0027
10	0.8	0.0054	0.0054	0.0054
10	1.0	0.0071	0.0073	0.0072
10	2.0	0.0163	0.0163	0.0163
11	0.4	0.0083	0.0083	0.0083
11	0.8	0.0158	0.0148	0.0153
11	1.0	0.0196	0.0214	0.0205
11	2.0	0.0419	0.0403	0.0411
11	3.0	0.0569	0.0531	0.0550
12	0.2	0.0055	0.0057	0.0056
12	0.6	0.0126	0.0144	0.0135
12	1.0	0.0232	0.0234	0.0233
12	2.0	0.0425	0.0445	0.0435
12	3.0	0.0619	0.0539	0.0579
13	0.2	0.0034	0.0030	0.0032
13	0.6	0.0078	0.0074	0.0076
13	0.8	0.0128	0.0124	0.0126
13	1.0	0.0176	0.0164	0.0170
13	2.0	0.0300	0.0290	0.0295

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
14	0.2	0.0037	0.0027	0.0032
14	0.6	0.0100	0.0108	0.0104
14	1.0	0.0180	0.0188	0.0184
14	2.0	0.0337	0.0325	0.0331
14	3.0	0.0455	0.0447	0.0451
15	0.2	0.0023	0.0023	0.0023
15	0.6	0.0096	0.0086	0.0091
15	0.8	0.0136	0.0126	0.0131
15	1.0	0.0174	0.0180	0.0177
15	2.0	0.0310	0.0294	0.0302
16	0.2	0.0026	0.0028	0.0027
16	0.6	0.0087	0.0083	0.0085
16	1.0	0.0172	0.0180	0.0176
16	2.0	0.0339	0.0343	0.0341
16	3.0	0.0526	0.0456	0.0491
17	0.2	0.0022	0.0028	0.0025
17	0.6	0.0072	0.0070	0.0071
17	1.0	0.0139	0.0147	0.0143
17	2.0	0.0308	0.0290	0.0299
17	3.0	0.0476	0.0450	0.0463
18	0.2	0.0045	0.0049	0.0047
18	0.6	0.0102	0.0110	0.0106
18	1.0	0.0221	0.0217	0.0219
18	2.0	0.0418	0.0410	0.0414
18	3.0	0.0611	0.0605	0.0608
19	0.2	0.0023	0.0023	0.0023
19	0.6	0.0045	0.0057	0.0051
19	1.0	0.0136	0.0138	0.0137
19	2.0	0.0287	0.0285	0.0286
19	3.0	0.0443	0.0425	0.0434

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
20	0.2	0.0045	0.0041	0.0043
20	0.6	0.0122	0.0128	0.0125
20	1.0	0.0216	0.0214	0.0215
20	2.0	0.0398	0.0390	0.0394
20	3.0	0.0548	0.0532	0.0540
21	0.2	0.0012	0.0012	0.0012
21	0.6	0.0078	0.0070	0.0074
21	1.0	0.0134	0.0134	0.0134
21	2.0	0.0261	0.0247	0.0254
21	3.0	0.0413	0.0395	0.0404
22	0.2	0.0013	0.0015	0.0014
22	0.6	0.0067	0.0059	0.0063
22	0.8	0.0073	0.0061	0.0067
22	1.0	0.0079	0.0085	0.0082
22	2.0	0.0186	0.0178	0.0182
23	0.2	0.0022	0.0018	0.0020
23	0.6	0.0053	0.0045	0.0049
23	0.8	0.0083	0.0081	0.0082
23	1.0	0.0115	0.0155	0.0115
23	2.0	0.0285	0.0271	0.0278
24	0.2	0.0042	0.0042	0.0042
24	0.6	0.0112	0.0100	0.0106
24	0.8	0.0162	0.0160	0.0161
24	1.0	0.0225	0.0229	0.0227
24	2.0	0.0448	0.0448	0.0448
25	0.2	0.0037	0.0035	0.0036
25	0.6	0.0114	0.0118	0.0116
25	0.8	0.0156	0.0160	0.0158
25	1.0	0.0201	0.0203	0.0202
25	2.0	0.0400	0.0428	0.0414

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
26	0.2	0.0032	0.0036	0.0034
26	0.6	0.0106	0.0100	0.0103
26	0.8	0.0156	0.0160	0.0158
26	1.0	0.0204	0.0220	0.0212
26	2.0	0.0408	0.0394	0.0401
27	0.2	0.0029	0.0027	0.0028
27	0.6	0.0076	0.0074	0.0075
27	1.0	0.0174	0.0170	0.0172
27	2.0	0.0430	0.0400	0.0415
27	3.0	0.0495	0.0527	0.0511
28	0.2	0.0032	0.0034	0.0033
28	0.6	0.0093	0.0095	0.0094
28	1.0	0.0222	0.0210	0.0216
28	2.0	0.0454	0.0462	0.0458
28	3.0	0.0639	0.0631	0.0635
29	0.2	0.0041	0.0041	0.0041
29	0.6	0.0113	0.0113	0.0113
29	1.0	0.0248	0.0236	0.0242
29	2.0	0.0480	0.0476	0.0478
29	3.0	0.0656	0.0588	0.0622
30	0.6	0.0096	0.0088	0.0092
30	1.0	0.0138	0.0148	0.0143
30	2.0	0.0212	0.0212	0.0212
30	3.0	0.0298	0.0298	0.0298
30	4.0	0.0419	0.0413	0.0416
31	0.2	0.0012	0.0012	0.0012
31	0.6	0.0042	0.0046	0.0044
31	1.0	0.0087	0.0087	0.0087
31	2.0	0.0189	0.0191	0.0190
31	3.0	0.0318	0.0288	0.0303

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
32	0.2	0.0021	0.0021	0.0021
32	0.6	0.0055	0.0055	0.0055
32	1.0	0.0120	0.0120	0.0120
32	2.0	0.0288	0.0304	0.0296
32	3.0	0.0456	0.0438	0.0447
33	0.2	0.0020	0.0020	0.0020
33	0.6	0.0077	0.0073	0.0075
33	1.0	0.0146	0.0136	0.0141
33	2.0	0.0315	0.0293	0.0304
33	3.0	0.0482	0.0492	0.0487
34	1.0	0.0172	00170	0.0171
34	2.0	0.0368	0.0374	0.0371
34	3.0	0.0528	0.0546	0.0537
34	4.0	0.0720	0.0744	0.0732
34	5.0	0.0994	0.0976	0.0985
35	1.0	0.0106	0.0116	0.0111
35	2.0	0.0213	0.0235	0.0224
35	3.0	0.0346	0.0332	0.0339
35	4.0	0.0442	0.0440	0.0441
35	5.0	0.0656	0.0654	0.0655
36	0.2	0.0044	0.0040	0.0042
36	0.6	0.0118	0.0118	0.0118
36	1.0	0.0250	0.0250	0.0250
36	2.0	0.0478	0.0466	0.0472
36	3.0	0.0642	0.0646	0.0644
37	0.6	0.0072	0.0064	0.0068
37	1.0	0.0134	0.0140	0.0137
37	2.0	0.0282	0.0290	0.0286
37	3.0	0.0350	0.0364	0.0357
37	4.0	0.0428	0.0430	0.0429

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
38	0.2	0.0025	0.0021	0.0023
38	0.6	0.0035	0.0033	0.0034
38	1.0	0.0081	0.0087	0.0084
38	2.0	0.0164	0.0182	0.0173
38	3.0	0.0230	0.0232	0.0231
39	1.0	0.0241	0.0245	0.0243
39	2.0	0.0456	0.0464	0.0460
39	3.0	0.0634	0.0660	0.0641
39	4.0	0.0820	0.0832	0.0826
39	5.0	0.1078	0.1088	0.1083
40	0.2	0.0028	0.0024	0.0026
40	0.6	0.0063	0.0055	0.0059
40	1.0	0.0144	0.0134	0.0139
40	2.0	0.0274	0.0282	0.0278
40	3.0	0.0399	0.0387	0.0393
41	0.2	0.0027	0.0025	0.0026
41	0.6	0.0058	0.0062	0.0060
41	1.0	0.0129	0.0121	0.0125
41	2.0	0.0243	0.0273	0.0258
41	3.0	0.0394	0.0388	0.0391
42	0.2	0.0016	0.0016	0.0016
42	0.6	0.0050	0.0046	0.0048
42	1.0	0.0079	0.0075	0.0077
42	2.0	0.0171	0.0165	0.0168
42	3.0	0.0265	0.0237	0.0251
43	0.2	0.0041	0.0049	0.0045
43	0.6	0.0133	0.0109	0.0111
43	1.0	0.0230	0.0220	0.0225
43	2.0	0.0436	0.0444	0.0440
43	3.0	0.0570	0.0576	0.0573

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
44	0.2	0.0012	0.0012	0.0012
44	0.4	0.0039	0.0041	0.0040
44	1.0	0.0106	0.0114	0.0110
44	2.0	0.0255	0.0245	0.0250
44	3.0	0.0300	0.0324	0.0312
45	0.4	0.0024	0.0026	0.0025
45	1.0	0.0100	0.0088	0.0094
45	2.0	0.0190	0.0202	0.0196
45	3.0	0.0268	0.0282	0.0275
45	4.0	0.0384	0.0402	0.0393
46	0.2	0.0012	0.0012	0.0012
46	0.6	0.0034	0.0032	0.0033
46	1.0	0.0066	0.0064	0.0065
46	2.0	0.0137	0.0139	0.0138
46	3.0	0.0214	0.0224	0.0219
47	1.0	0.0083	0.0087	0.0085
47	2.0	0.0208	0.0202	0.0205
47	3.0	0.0265	0.0279	0.0272
47	4.0	0.0371	0.0365	0.0368
47	5.0	0.0472	0.0478	0.0475
48	0.4	0.0030	0.0030	0.0030
48	1.0	0.0090	0.0090	0.0090
48	2.0	0.0174	0.0172	0.0173
48	3.0	0.0202	0.0228	0.0215
48	4.0	0.0309	0.0289	0.0299
49	0.2	0.0036	0.0030	0.0033
49	0.6	0.0042	0.0050	0.0046
49	1.0	0.0102	0.0108	0.0105
49	2.0	0.0249	0.0249	0.0249
49	3.0	0.0366	0.0364	0.0365

Regression line data	Amount of tylosin tartrate (ug)	Weight of peak #1 (gm)	Weight of peak #2 (gm)	Average weight of peak (gm)
50	0.4	0.0071	0.0059	0.0065
50	1.0	0.0140	0.0152	0.0146
50	2.0	0.0284	0.0274	0.0279
50	3.0	0.0361	0.0375	0.0368
50	4.0	0.0494	0.0464	0.0479
51	0.4	0.0050	0.0046	0.0048
51	1.0	0.0143	0.0155	0.0149
51	2.0	0.0312	0.0328	0.0320
51	3.0	0.0468	0.0454	0.0461
51	4.0	0.0609	0.0617	0.0613
52	0.4	0.0079	0.0065	0.0072
52	1.0	0.0130	0.0132	0.0131
52	2.0	0.0308	0.0300	0.0304
52	3.0	0.0434	0.0410	0.0422
52	4.0	0.0618	0.0628	0.0623
53	0.4	0.0041	0.0043	0.0042
53	1.0	0.0067	0.0071	0.0069
53	2.0	0.0096	0.0092	0.0094
53	3.0	0.0115	0.0115	0.0115
53	4.0	0.0180	0.0168	0.0174