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DEVELOPMENT OF OCULAR INSERTS FOR CATTLE

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Abstract

Ring shaped ocular inserts have been developed to administer a therapeutic level of tylosin tartrate throughout a five day period to treat pinkeye in cattle. The inserts are based on polyvinyl chloride rings which are dip coated with a copolymer containing the antibiotic (tylosin tartrate). Scanning electron microscope (SEM) characterization of surfaces has been of value to evaluate the presence and extent of surface flaws in the hydrogel coating, and to contribute to improvement in fabrication of the rings to insure the establishment of satisfactory seals at joints, uniformity of microporosity and cross sections, and the absence of significant cracking or flaking. In vitro release rates were determined using thin layer chromatography techniques, and rates were seen to be above a few micrograms of antibiotic per hour for experiments as long as nine days at simulated tear rates as high as 2 milliliters per hour.

KEY WORDS: Scanning Electron Microscopy, Controlled Drug Release, Pinkeye, Bovine Keratoconjunctivitis, Tylosin, Methylmethacrylate, Hydroxyethyl Methacrylate, Antibiotic, Thin Layer Chromatography, Hydrogel, Cattle, Eye Diseases, Ocular Insert

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Introduction

The treatment of certain eye diseases can be significantly improved over that of conventional treatments, such as eyedrops, by using controlled drug release ocular inserts. An example of a disease of potential interest where this type of treatment method could be advantageous is pinkeye in cattle. To be effective such inserts must release a therapeutic level of drug continuously, and must be non-irritating to the eye. Other factors, such as cost, are also important. Ideally, a bioerodable device might be preferred, but a device that will remain in place throughout an appropriate time period, and that can be easily removed, would certainly be satisfactory.

The characteristics of pinkeye in cattle (Bovine Infectious Keratoconjunctivitis, BIK, or IBK) are lacrimation, photophobia, corneal ulcers, and corneal opacities. It is a contagious disease, and can result in significant economic losses associated with weight loss and decrease in milk production. In the State of Iowa, six to ten percent of weaned beef calves suffer significant enough damage from pinkeye to reduce their economic value by 1/3 to 1/2. This represents a loss of tens of millions of dollars in Iowa alone.

A virulent form of Moraxella bovis, a common agricultural bacterium, is the primary infectious agent of BIK (Pugh and Hughes, 1968; Hughes and Pugh, 1970). Current medication application techniques exhibit severe drawbacks such as rapid removal of ointments or drops by tear fluids. The primary method for ocular delivery of antibiotic solutions is the use of eyedrops. A considerable fraction of an eyedrop is lost immediately after instillation (about 80 percent in humans; Gelatt et al., 1979); the primary disadvantage of usual aqueous eyedrop preparations is the short duration of drug action. For comparable time periods, sprays produce drug action results similar to those achieved by using eyedrops (Chiou and Watanabe, 1982). For example, a 30 mg dose of tylosin tartrate [$C_{45}H_{77}NO_{17}$-$C_4H_6O_6$] applied to the eyes of cattle twice daily as a 50 milligrams/ml aqueous spray eliminated clinical signs of BIK and Moraxella bovis after five days (Ellis and Barnes, 1961); an in vitro test on a

Moraxella bovis isolate demonstrated inhibition by tylosin at a level of 0.4 micrograms per ml. This antibiotic also possesses inhibitory action on several members of the genus Mycoplasma, for example. Recent work has shown that Mycoplasma bovoculi enhances the colonization of cattle eyes by Moraxella bovis (Rosenbusch, 1983, 1985; Rosenbusch and Knudtson, 1980).

Improvements may be made in the administration of therapeutic levels of such an antibiotic by use of specialized ocular inserts for controlled release. Several studies have contributed supplemental information in support of this concept. For example, Hughes and Pugh (1975) found that 42.9 to 44.5 millimeter diameter rings constructed of polyvinyl chloride tubing (outside diameters of 2.82, 1.65, or 0.914 millimeters) could be retained in the bovine eye. Olanoff and co-workers (1979) demonstrated that certain hydrogel copolymers were capable of releasing microgram/hour quantities of tetracycline (using copolymer areas and thicknesses roughly comparable to those of interest for potential ring-shaped ocular insert applications as will be described in the current work), a relatively large molecule, from a trilaminate delivery disk for periods of the order of months in in vitro diffusion studies using Ringer's solution [8.60 g NaCl, 0.30 g KCl, 0.33 g $CaCl_2$ in 1.00 l aqueous solution] at 37° C. By varying the relative percentage of 2-hydroxyethyl methacrylate (hydrophilic monomer component) and methyl methacrylate (hydrophobic monomer component) of a copolymer, the diffusivity of the tetracycline through the copolymer could be varied over a range of values. Thus, this copolymer system appeared to offer general characteristics of interest for use in developing ring-shaped ocular controlled drug release devices in which an antibiotic such as tylosin tartrate could be of value.

Hoffmann and Spadbrow (1978) reported mean lachrymal flow rates in cattle of 0.18-1.86 ml/h ; Slatter and Edwards (1982) measured mean flow rates of 1.96+/-1.84 ml/h (+/-s.d.). Disease or other conditions will affect flow rates; a relatively high flow rate of 2 ml/h was chosen as representative of a condition of irritation. The instantaneous volume of lachrymal fluid in the bovine eye may be approximated as 0.5 ml (R. Rosenbusch, personal communication). As the minimum inhibitory concentration for Moraxella bovis isolates is low, and of the order of 0.5 microgram/ml (MIC; taken as the lowest concentration of antimicrobial agent at which the tested organism did not show growth), desired drug release rates from the insert system would be anticipated to be at least of the order of a few micrograms/h.

Materials and Methods

Materials Fabrication

Room temperature copolymerization according to the method of Olanoff and co-workers (1979) is followed to make the copolymer used for dip coating. This is a batch process in which the copolymer forms throughout a ten day period. The following components are added together to obtain the desired 90 mole percent methyl methacrylate - 10 mole percent hydroxyethyl methacrylate bulk copolymer; 570 ml ethanol, 380 ml water (type-one), 6.1 ml 2-hydroxyethyl methacrylate (Polysciences Inc., Warrington, PA; opthalmic grade), 46.6 ml methyl methacrylate, 0.25 g sodium persulfate, and 0.13 g potassium persulfate. Throughout a ten day period, the solution is bubbled with nitrogen. At the end of the ten day period, the 1 liter solution and white copolymer precipitate is added to 3 liters of water, and the precipitate copolymer is filtered and dried (50° C for five days, 635 mm Hg vacuum).

Quantities of the copolymer are then dissolved in a suitable solvent and the drug is then mixed into the solution. This forms the dip coating material. For example, a representative batch would consist of 1 g of copolymer and 20 ml of dimethyl formamide (stirred at 40-45° C for 6 hours). The solution is then cooled to room temperature before the addition of 0.5 g of tylosin tartrate.

Vinyl tubing (polyvinyl chloride, medical grade, both regular (i.d. 0.112cm x o.d. 0.165cm) and radiopaque (i.d. 0.086cm x o.d. 0.117cm) varieties) is cut into suitable lengths (e.g., 126 mm long to permit making a ring 40 mm in diameter). This tubing is threaded onto a wire which is subsequently bent into a ring shape prior to dipping. The dipping procedure is done in an inert atmosphere (nitrogen in a glove bag; Drierite desiccant) to insure uniformity of microstructure. To build up a sufficient amount of polymer and dissolved drug, ten to fifteen dips are made, and the surface is allowed to dry between subsequent dips. The weight is monitored so that the amount of drug in a ring device is approximately 50 milligrams. (Note that this total amount of drug loading per device is of the order of a typical single eyedrop application of antibiotic.) A thinner (i.d. 0.051cm x o.d. 0.091cm), 1cm length of radiopaque polyvinyl chloride tubing is then wetted with either a polyvinyl chloride - tetrahydrofuran solution (5 weight percent PVC in tetrahydrofuran) or a solution of the copolymer in dimethyl formamide and inserted into the ends of the dipped ring shaped tubing (removing the wire mandrel first); this results in a strong joint, and completes the ring.

Drug Release Characterization

In vitro drug release experiments were conducted using Ringer's solution as an elution medium. The quantitative method for measuring drug release is based on thin layer chromatography (TLC) (Ryoo (1986)). The presence of salt broadens the spots used in the TLC method; however, the amounts of the drug are determined at the microgram level.

For in vitro drug release experiments, a continuous flow apparatus was used and the salt solution flowed over the ring at a rate of 2

ml/hr. A 2 ml sample was collected on an hourly basis. A sample was dried, then redissolved in water to a suitable concentration level for spotting on 20 cm x 20 cm TLC plates. Several tests were done to insure that the concentration level was within a range for standards spotted on the same plate. Whatman $LKC_{18}F$ TLC plates were analyzed using a Kontes Fiber Optics Scanner (model 800) and a chart recorder. This type of TLC plate (linear-K plate with a preabsorbent area) uses a silica gel medium with hydrocarbonaceous functional groups covalently bonded to surface hydroxyls of the silica gel (related to the C_{18} part of the plate designation). Also, octadecylsilane is bonded to the silica gel. The material is fluorescent (absorbs 254 nanometer light), giving dark spots on a green background. To provide a more uniform visualization, the silicone coating is first removed from a clean plate by treating the plate with methanol and drying. After this plate pretreatment, 2.5 microliter droplets of the samples and standards are spotted onto the preadsorbent area of a plate (10 spots/plate), and dried at room temperature. The plate is developed in an 85% methanol - 15% water solution causing a spot migration of the order of 10 centimeters. The plates are then air dried for a 1 h period; ultraviolet light is then used to check the spots to insure that the drug release samples are within the concentration range represented by the standards on the same plate. Then the TLC plate is sprayed with a 10% H_2SO_4 - 90% methanol solution (at a rate of 15 ml/min for 15 sec). The plate is then placed in a preheated oven at 100° C for 5 min. The spots will now be visible without the UV light. The plate is analyzed on the Kontes system within 15 min of removal of a plate from the oven (fading occurs within 3 h). Each spot is analyzed twice; the peaks are cut from the chart paper, and are weighed. The quantity of drug in a release sample is determined by linear interpolation in reference to the peak weights for the standards (Touchstone and Dobbins (1978); Leytem (1984)).

Scanning Electron Microscopy

Before and after release experiments, representative hydrogel coated ring specimens are cut into 1 cm lengths. In vitro release experiment samples are usually air dried, and mounted on carbon stubs. Some samples are critical point dried in a Polaron Model E3000 drier using CO_2. The specimens receive a 300 Å coating of gold (Polaron E5100 sputter coater), and then are examined using an SEM (JEOL U3 equipped with an energy dispersive X-ray analysis system and JSM 840-A; the photos which are shown were obtained from the JSM 840-A). Operating conditions of relatively low probe current ($\sim 3 \times 10^{-11}$ A) and relatively low accelerating voltages (5-10 kV) are generally preferred to avoid beam damage to sample surfaces. An energy dispersive X-ray analysis (Tracor Northern - 2000 energy dispersive analysis system; Kevex detector) is also done on the PVC source material to monitor the presence or absence of certain elements or impurities.

Results

Microstructure

The surface of the uncoated polyvinyl chloride (PVC) tubing is smooth (Figure 1a-1b). By comparison, the surface of the uncoated radiopaque PVC tubing is rough (Figure 2). The hydrogel coating that results from the dip processing is also smooth. Only minor surface blemishes are seen on such surfaces. Typically the hydrogel-antibiotic mixture is built up as a result of several consecutive coatings, and as a result the hydrogel surfaces on the radiopaque PVC tubing appear smooth. The exposure to the elution solution for the nine day test periods does not appear to alter the surface appearance (Figure 3). Since the elution solution was Ringer's, a number of small sodium chloride crystals are seen on the hydrogel surface after a test. Ring device joints should be monitored to avoid forming rough portions of a ring such as that seen in Figure 4. Also, attempts to smooth dip coated surfaces with a brush before the coating hardens can result in forming a variety of surface grooves. Although such surface features are small, and probably would cause no major problems in using a ring with such features as an insert in the eye of a cow, such handling is unnecessary, and should be avoided.

Microchemistry

The use of energy dispersive X-ray analysis shows the presence of a chemical difference between the two types of PVC tubing. An analysis performed on a gold coated segment of plain PVC tubing shows the presence of the gold (300 angstrom coating thickness) and the chlorine of the underlying PVC tubing (Figure 5). By comparison, in addition to these peaks, the presence of iodine is seen in the radiopaque PVC tubing (Figure 6). Also note that the hydrogel coating completely covers these surfaces to a thickness of approximately 0.025cm (dry state; spectrum shown in Figure 7). These chemical elements, as well as other components such as plasticizers, which are in the medical grade PVC tubings, are not expected to cause eye irritation during the typical insertion periods of several days (based on observations reported in the literature by Hughes and Pugh).

Release Rates

Within the first 8 h of contact with the elution solution, the maximum release rate is seen (Figure 8a-8c). For typical ring devices, such as those listed in Table 1, (hydrogel coated radiopaque PVC for these cases) the maximum rate is of the order of several hundred micrograms per hour. After the release rate peak is reached, the release rate falls off rapidly. During the first three days of a release experiment, the release rate of antibiotic is above 50 micrograms per hour. At

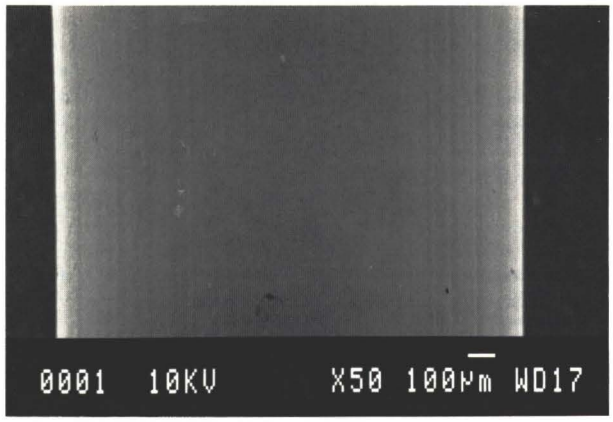

Figure 1a. Plain PVC tubing surface (before dip coating).

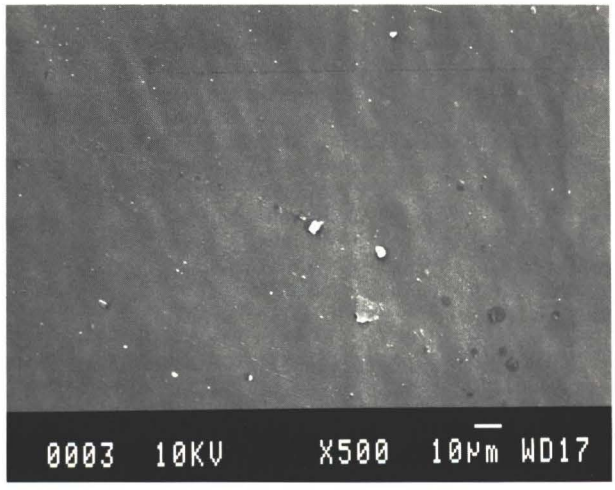

Figure 1b. Higher magnification view of plain PVC sample of Figure 1a.

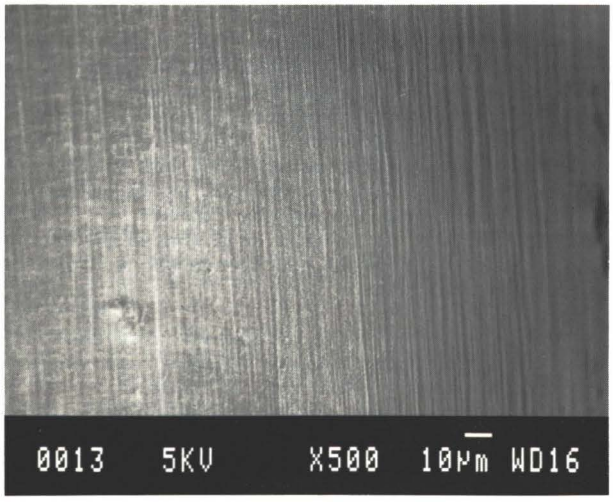

Figure 2. Radiopaque PVC tubing surface (before dip coating). Compare with Figure 1b, and note that the surface of this type of PVC tubing is relatively rough (extrusion marks).

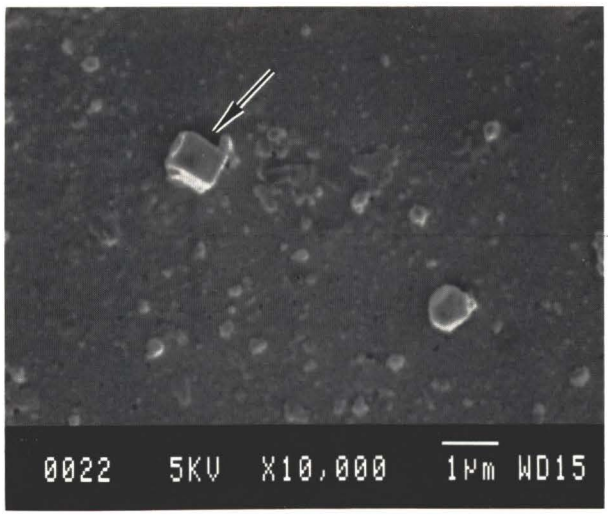

Figure 3. Hydrogel surface coating on radiopaque PVC tubing after 9-day in vitro antibiotic release test. Arrow indicates sodium chloride residue from the Ringer's solution. Note that the hydrogel surface is smooth and similar to that for a sample prior to a drug release test (i.e., similar smoothness and no salt crystals).

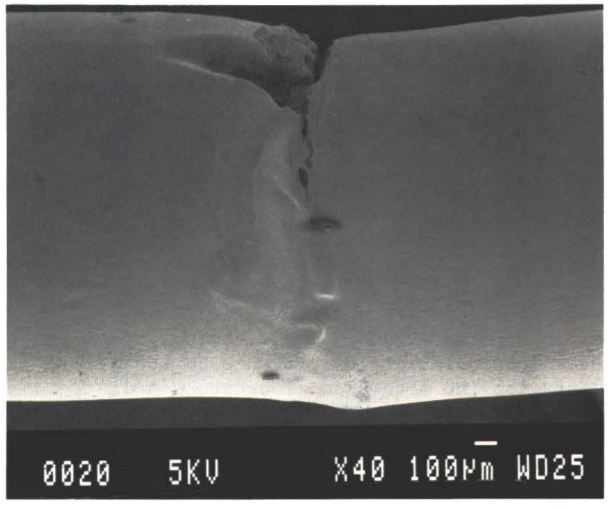

Figure 4. Incomplete seal of a PVC ring joint; hydrogel coated radiopaque tubing.

nine days, the release rate is seen to be at least 3 micrograms per hour.

Conclusion

The physical and chemical characteristics of the ring device appear to be satisfactory for use as an ocular insert for cattle. The in vitro tests were performed on rings of sizes of interest for use as ocular inserts for cattle (results were reported above for a typical diameter and circumference that might be needed; for best retention, Hughes and Pugh (1975) recommend that such devices meet

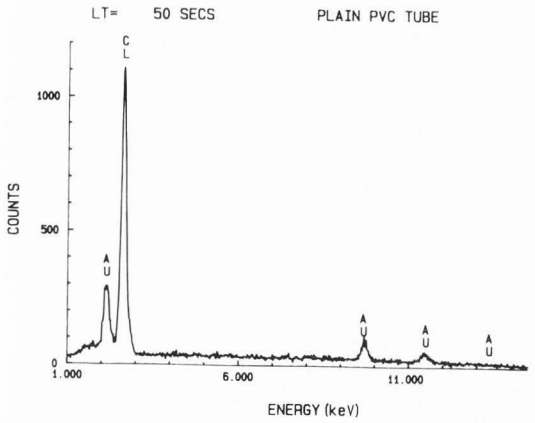

Figure 5. Energy dispersive X-ray analysis spectrum obtained from gold coated PVC tubing. Au peaks are due to the gold coating, and the Cl peak is due to the chlorine in the PVC molecules.

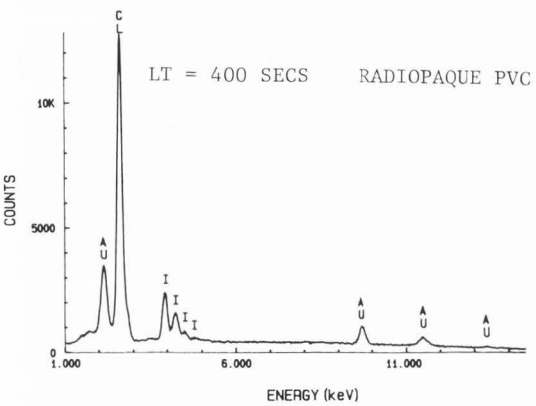

Figure 6. Energy dispersive X-ray analysis spectrum obtained from gold coated radiopaque PVC tubing. Au peaks are due to the gold coating, and the Cl and I peaks are due to the chlorine and iodine in the PVC.

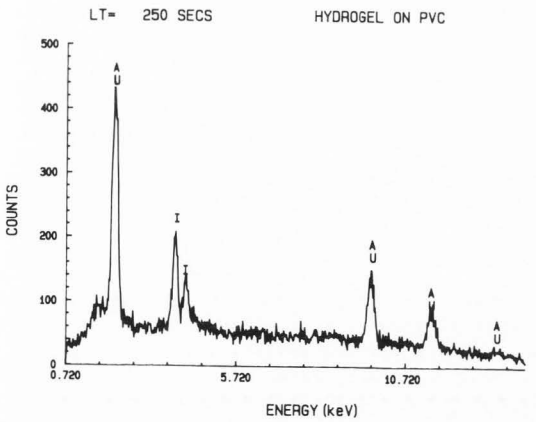

Figure 7. Hydrogel coated radiopaque PVC tubing. The presence of the coating suppresses the Cl signal from the PVC.

the following criterion: rings should have a circumference smaller than that of the conjunctival sac, but larger than that of the globe of the eye). The hydrogel coating appears to release a sufficient amount of antibiotic to remain above the minimal inhibitory concentration necessary to suppress bacterial growth. It is anticipated that the inserts would be effective for therapeutic administration of the antibiotic throughout a 5 to 7 day period at a rate at least of the order of a few micrograms per hour. For the tearing rate ranges reported for cattle, the ocular insert prototype has potential application in treating pinkeye in cattle.

Acknowledgements

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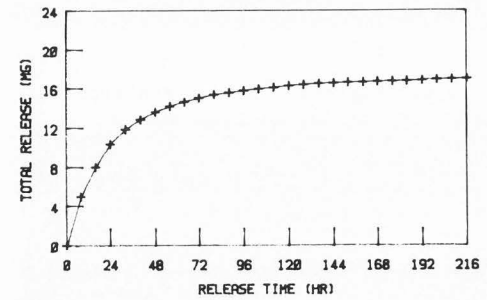
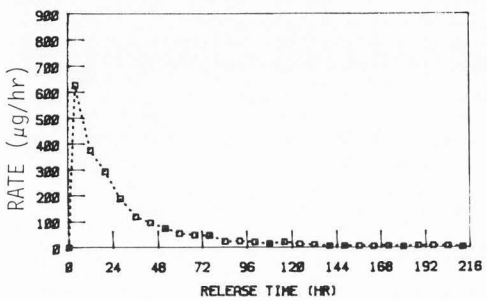
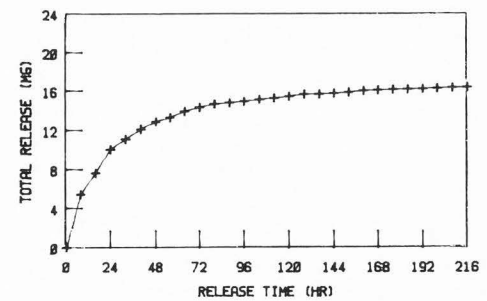
Table 1. Release Rate Characteristics for 40 Millimeter Diameter Ring Devices (50 mg loading).

Device Number	Release Rate Maximum Achieved During First Two Days (µg/hr)	Release Rate After 6 Days (µg/hr)	Total Release Rate (µg)	Total Released / Total Loading (%)
061	682	3 to 22	16363	32.2
062	626	3 to 8	16978	35.4
063	632	4 to 12	18273	34.6
064	925	3 to 7	18181	36.8
065	629	6 to 13	15851	28.7



(8a)





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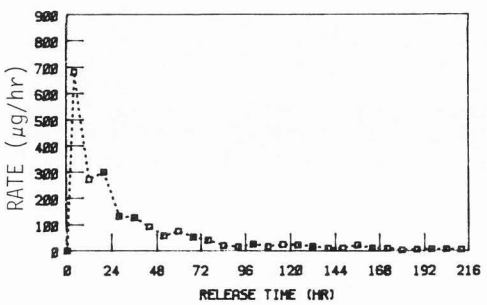

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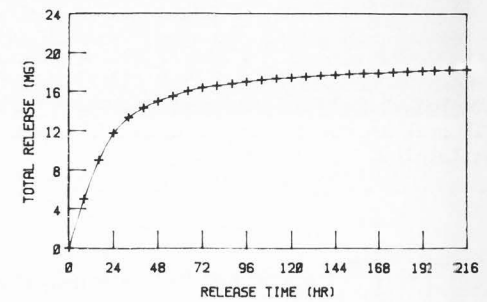

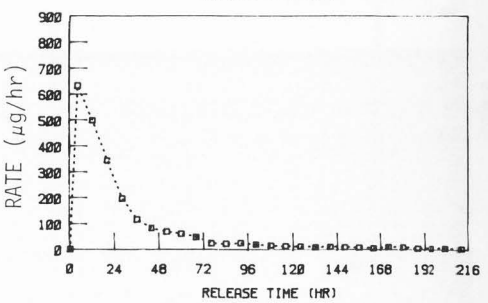

Figure 8. Curves of the release rate of the antibiotic (µg/hr) versus time (hr) and of the total amount of antibiotic released (mg) as a function of time (hr) obtained from hydrogel coated PVC rings (50 mg antibiotic loading; 40 mm diameter). (a) Ring device 061, (b) ring device 062, and (c) ring device 063.

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Discussion with Reviewers

Cristopher D. Batich and Moshe Yalon: Has any monomer or oligomer extraction been studied for potential toxic effects on the eye?
Authors: We have not done such studies.

Alan H. Brightman: Was there another co-polymer precipitated and studied?
Authors: A relatively hydrophilic formulation of 75 mole percent hydroxyethyl methacrylate - 25 mole percent methyl methacrylate (25 MMA: 75 HEMA) was also studied. The release rate profiles for ring devices utilizing a coating of this formulation were similar to that of the 90:10 copolymer coatings reported above. The 25 MMA:75 HEMA formulation was studied as a type of matrix for the antibiotic that would be expected to exhibit minimal eye irritation. No overt inflammation was observed in limited preliminary in vivo testing of the 90:10 copolymer coating on a ring device (1 week). No in vivo tests have been done using the 25 MMA:75 HEMA formulation.

Cristopher D. Batich and Moshe Yalon: Why are different release rates seen for the three samples? Are the same thickness coatings used?
Authors: The PVC tubing shrinks (shortens) in response to the solvent of the dipping treatment. The coating thicknesses are about the same in each case; however, the tube lengths vary. The longer a tube, the more surface area, and the greater the amount of drug release during the sampling periods.

Alan H. Brightman: Do the authors feel that other antibiotics could be incorporated into this coating?
Authors: Yes. Other antibiotics such as lincomycin are of interest. However, you need a sensitive method to quantify the release of particular antibiotics for the time period of interest.

Cristopher D. Batich and Moshe Yalon: What is the source of the PVC tubing? Many varieties are commercially available.
Authors: The source of the PVC for the experiments reported above is Becton, Dickinson and Co., Rutherford, NJ. The PVC material with iodine is the transparent/radiopaque type (Clearex #15654 and #15652, sizes 0.086cm i.d. x 0.117cm o.d. and 0.051cm i.d. x 0.091cm o.d., respectively). The other variety is plain medical grade vinyl tubing (B-D #6179; 0.112cm i.d. x 0.165 cm o.d.; discontinued).

Alan H. Brightman: If the devices are used as intended, does the coating withstand physical handling necessary to insert a ring into a cow's eye?
Authors: Yes. A ring is placed in physiological saline for 8-12 h prior to insertion. This is a preconditioning step to soften the hydrogel coating.

Cristopher D. Batich and Moshe Yalon: After testing release rates, had the hydrogel been damaged? Mechanical properties of hydrogels are very poor and one would expect significant abrasion in vivo.
Authors: No. Please refer to Figure 3 in the paper for an in vitro example, and to Figure 9 (below) for an in vivo example. Preliminary in vivo experiments are being conducted in collaboration with Dr. R. Rosenbusch (Veterinary Medical Research Institute, Iowa State University). Problems of insertion and retention of rings have been identified, and associated studies are in progress.

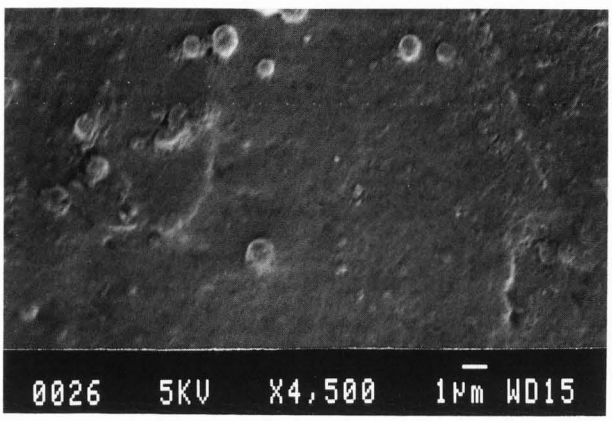

Figure 9. Surface of an ocular insert after a 7-day in vivo antibiotic release experiment in the eye of a healthy cow. Notice that the hydrogel surface is intact. (Sample was fixed in a 3% glutaraldehyde solution, critical point dried using CO_2, and gold coated prior to SEM examination).

R. Holm: In figure 7, why is iodine detected while chlorine is not? Did iodine migrate into the hydrogel?
Authors: Chlorine X-rays are absorbed by the thickness of the hydrogel coating, whereas those from iodine are less affected and can be detected by energy dispersive X-ray analysis in this case.

R. Holm: Does the tylosin tartrate exhibit cathodoluminescence?
Authors: We do not have cathodoluminescence information. However, optical fluorescence of the powder is excited by short wavelength (254 nm) ultraviolet light (brown fluorescence emission), by long wavelength (365 nm) ultraviolet light (bright yellow fluorescence emission), or by both short and long wavelength ultraviolet light (dark brown fluorescence emission). This is of interest in a general way in the TLC method. In spotting the antibiotic on the TLC plates, ultraviolet light can be used to excite a green luminescence for the plate, whereas the antibiotic spots appear black (for both long and short wavelength ultraviolet and combined ultraviolet).