An Implantable Dosage Form for the Treatment of Bone Infections

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The object of this investigation was the development of an implantable sustained-release dosage form, for the treatment of bone infections. Cross-linked polydimethylsiloxane (PDMS) was used as the matrix material. The drug delivery system was prepared by incorporating tobramycin, as a free base ($C_{18}H_{37}N_5O_9 \cdot H_2O$) or as a sulfate salt [$(C_{18}H_{37}N_5O_9)_2 \cdot 5H_2SO_4$], into the matrix and molding into spherical beads. Following *in vitro* studies, the cumulative amount of drug released when plotted as a function of the square root of time was linear for both the base and the salt. The addition of glycerol to the matrix substantially accelerated the rate of drug release and the plots of cumulative amount of drug released continued to be linear as a function of the square root of time. The glycerol-incorporated beads swelled in contact with the aqueous medium but a negligible amount of glycerol was released even after exposure to the medium for 20 days. ^{13}C solid-state and high-resolution NMR studies indicated that a fraction of the added glycerol participated in the cross-linking reaction of the polymer. The effect of the initial molecular weight of PDMS and the effect of the concentration of the cross-linker on the kinetics of drug release were investigated.

KEY WORDS: tobramycin; polydimethylsiloxane; *in vitro* release; osteomyelitis.

INTRODUCTION

Bone infections (osteomyelitis) can be of three types (1). In hematogenous osteomyelitis, the infection is conveyed to the bone tissue through the blood stream. Direct infection from a source outside the body or the spread of infection from tissue adjacent to the bone is referred to as osteomyelitis secondary to a contiguous focus of infection. Some examples of direct infection from an exogenous source are penetrating wound from a gunshot, open fractures and orthopedic procedures. Finally, osteomyelitis associated with peripheral vascular disease occurs in patients with vascular insufficiency.

Depending on the duration of the infection or the histopathologic findings, osteomyelitis can be either acute or chronic. Acute hematogenous osteomyelitis can be successfully treated with antibiotics provided the disease is diagnosed early (2). In the treatment of chronic osteomyelitis, antibiotic therapy alone has not always yielded satisfactory results (3). Though the dose of antibiotic administered systemically was high, therapeutically effective drug concentration was not always achieved at the site of infection. This is because bones are moderately perfused organs, and furthermore, there is reduced blood supply and diffusional barriers in the infected bone tissues (4,5). Klemm (6) developed a novel approach to treat this problem. The aminoglycoside antibiotic gentamicin was incorporated into polymethylmethacrylate and hand rolled into spherical beads, and these beads were surgically placed close to the site of infection. Sustained release of the antibiotic from the polymer occurred. The advantage of this approach was that an effective drug concentration was attained at the site of infection, while the systemic drug concentration was very low. The main disadvantage was that the beads had to be removed, usually 2 to 3 weeks after implantation. Several other workers have successfully used this approach for the treatment of bone infections (7).

So far, only polymethylmethacrylate has been extensively used as a matrix material for drug incorporation (7,8). The major disadvantage with this matrix was that only a small fraction of the incorporated drug was released. For example, during *in vitro* studies, only 13% of the incorporated tobramycin was released from polymethylmethacrylate beads in 2 weeks (7). Moreover, the kinetics of drug release was amenable to very little control. Finally, the polymer is known to be unsuitable for the incorporation of some antibiotics including chloramphenicol and tetracycline (9).

Implantable biodegradable polymer microcapsules containing gentamicin have been prepared and evaluated in the treatment of osteomyelitis (10,11). The unique advantage of this approach is that the dosage form need not be removed from the body. Resorption of the implant was observed to occur in 6 to 8 weeks. However, subtherapeutic concentrations of antibiotic in the bone for extended time period could lead to the development of resistant organisms (12). Moreover, prolonged exposure to the antibiotic could lead to hypersensitivity reactions (13,14).

Polydimethylsiloxane (PDMS) is an inorganic, synthetic, and biocompatible polymer. The medical-grade silicones are prepared from PDMS and have been successfully used for the preparation of sustained- and controlled-release drug delivery systems (15,16). Silicones are hydrophobic, nonbiodegradable polymers. The release rate of lipophilic drugs, such as progesterone and testosterone, from silicone polymers has been reported to be several orders of magnitude higher than from organic polymers (17). However, the release rate of hydrophilic drugs incorporated into silicone matrix was very low. The addition of hydrophilic compounds, such as glycerol, to the polymer matrix greatly enhanced the release rate of several hydrophilic drugs (18–21). This was thought to be due to the creation of aqueous pores in the matrix, which provided a preferential pathway for drug release (21). When placed in contact with water, glycerol-incorporated PDMS absorbed water and swelled. Interestingly, a negligible amount of glycerol was released from the drug delivery system (22).

The object of this investigation was to develop an implantable tobramycin-containing PDMS drug delivery system to be used in the treatment of bone infections.

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EXPERIMENTAL

Materials

Hydroxy end-blocked polydimethylsiloxane with number average molecular weights of 36,000, 77,000, and 110,000 (Scientific Polymer Products, Ontario, NY), propyl orthosilicate (Aldrich Chemicals, Milwaukee, WI), stannous octoate (Dow Corning, Midland, MI), glycerol (Fisher Scientific, Fair Lawn, NJ), monobasic potassium phosphate, dibasic sodium phosphate (Mallinckrodt, Paris, KY), tobramycin ($C_{18}H_{37}N_5O_9 \cdot H_2O$) and tobramycin sulfate [$(C_{18}H_{37}N_5O_9)_2 \cdot 5H_2SO_4$, Nebcin, Eli Lilly, Indianapolis, IN] were used as received. The heat vulcanization PDMS kit (E 6380-89) was a gift from Dow Corning, Midland, MI. This did not contain any filler.

Fabrication of the Drug Delivery System

The medical-grade silicones were classified into two groups by Braley (23). These are the room-temperature vulcanizing (RTV) type and the heat vulcanizing (HV) type. The RTV-type PDMS was used for the preparation of the drug delivery systems. Hydroxy end-blocked PDMS was the polymer base material and propyl orthosilicate was the cross-linker. Stannous octoate was used to catalyze the cross-linking reaction. Two types of drug delivery systems were prepared. In the first type, the matrix did not contain any additive. In the second type, varying amounts of a hydrophilic compound was added to the matrix.

Without Hydrophilic Additive. To 4.8 g of the hydroxy end-blocked polydimethylsiloxane (polymer base), 0.17 g of propyl orthosilicate (cross-linker) was added. The cross-linker constituted 3.4% (w/w) of the matrix. The relative amounts of the above two components were maintained constant unless otherwise stated. A weighed amount of the drug (tobramycin base or tobramycin sulfate) was then added and mixed well. Finally, 65 mg of stannous octoate (catalyst) was added and mixed. The mixture was degassed under reduced pressure (2.54 mm of Hg) for 5 min, transferred into a 12-ml polypropylene syringe (Monoject, St. Louis, MO), and then forced into a Teflon-coated mold. The mold was assembled with a surgical steel wire (Type 316L, Ethicon, Somerville, NJ) placed through the center. After the completion of the vulcanization reaction, spherical beads 3.2 mm in diameter, were obtained. The beads were held together by the wire.

With Hydrophilic Additive. Weighed amounts of tobramycin, the polymer base, the cross-linker, and glycerol (the hydrophilic additive) were intimately mixed. The catalyst was added and the mixture was degassed under reduced pressure. The rest of the fabrication procedure was exactly the same as described above. Drug delivery systems with three additive concentrations were prepared. These were 5, 10, and 20% (w/w) of the polymeric matrix. In all these systems, the (polymer base + additive) constituted 96.6% (w/w) of the matrix and the cross-linker concentration was 3.4% (w/w).

The calculations of the ingredient concentration in the drug delivery systems were based on the assumption that the catalyst concentration was negligible.

Fabrication of the HV-Type Beads

In this system, the curing reaction required heating at 80°C for 30 min. The effect of such a reaction condition on the stability of the drug has not been studied. Therefore, these beads were fabricated without any drug. These beads were prepared to study the interaction, if any, between the polymer matrix components and the hydrophilic additive. This is discussed further under Results and Discussion.

Without Hydrophilic Additive. To 6 g of the base material (a copolymer primarily consisting of dimethyl type but with small amounts of methylvinyl siloxy units), 1 g of the curing agent (dichlorobenzoyl peroxide) was added, mixed, and degassed under reduced pressure. The mixture was forced into a Teflon-coated mold and allowed to cure at 80°C for 30 min.

With Hydrophilic Additive. The polymer base, the curing agent, and the glycerol were weighed, mixed, degassed, and forced into a Teflon-coated mold and cured at 80°C for 30 min. The additive concentrations were 5, 10, and 20% (w/w) of the matrix.

In Vitro Release Studies

The release of tobramycin from the beads was evaluated using the USP Dissolution Apparatus 2 (24) (Hanson Research Corporation, Chatsworth, CA). Nine hundred milliliters of Sorensen's phosphate buffer (pH 7.4), maintained at 37 ± 1°C, was used as the release medium (25). The number of beads used varied from one experiment to another. The paddle speed was 50 rpm. At definite time intervals, 200 μl of the release medium was collected and the tobramycin content determined by a stability-indicating liquid chromatographic method (26).

Swelling in Contact with Water

Both the HV- and RTV-type PDMS beads containing glycerol were immersed in water at 37°C and weighed periodically until they attained a constant weight.

Thermal Analysis

The thermal analysis system consisted of a differential scanning calorimeter (Model 910), a thermogravimetric analyzer (Model 951), and a thermal analysis operating system (Thermal analyst 2000), all from Du Pont. The differential scanning calorimeter (DSC) was calibrated with indium (Du Pont). Typically, about 3 mg of sample was weighed into an aluminum pan and the pan was crimped nonhermetically. It was heated in the DSC from 30 to 250°C under a stream of nitrogen. For the thermogravimetric analysis (TGA), about 10 mg of sample was weighed into a platinum pan and heated from 30 to 250°C under a stream of nitrogen. In both the DSC and the TGA, the heating rate was 10°C/min.

NMR Spectroscopy

The NMR studies were carried out on the vulcanized polymers without any added drug.

^{13}C Solid-State NMR. The polymer matrix components were mixed and transferred to glass pipettes (5.6-mm internal diameter). After completion of the vulcanization reac-

tion, the polymer was removed and the cylindrical rods were cut to the appropriate length and loaded into the NMR rotor. The spectra were obtained with a 100-MHz (for proton) superconducting magnet on a Bruker (AC-100) solid-state spectrometer and IBM (NR/100 AF) console. The techniques of cross-polarization, magic-angle spinning, and dipolar decoupling were used. Spin-locked cross-polarization was used to establish the single contact Hartman–Hahn condition. The samples were spun at the magic angle (54.7°) at a frequency of 1.7 ± 0.2 kHz. The delay time between the pulses was 8 sec and the contact time was 6 msec. The chemical shift was measured relative to tetramethysilane using 1,4-di-t-butyl benzene as an external standard.

^{13}C High-Resolution NMR. The polymer components were mixed and transferred to NMR tubes (17 mm long), where the vulcanization reaction was allowed to proceed to completion. Spectra were obtained with a 200-MHz spectrometer (Bruker AC-200) using a 5-mm broad-band dual probe ($^{1}H/^{13}C$) at 50 MHz.

Scanning Electron Microscopy (SEM)

Cylindrical polymer rods (as prepared for solid state NMR) were cut into thin slices with a razor blade. The samples were mounted on SEM sample stubs using colloidal graphite (Tepella, Redding, CA) and coated with platinum plasma (100-Å thickness) in a minideposition system (MED010, Balzer, Switzerland) and examined under a scanning electron microscope (Jeol 840 II, Tokyo) operated at 10 kV.

Preparation of PDMS Beads with ^{14}C-Glycerol

The radioactive glycerol (NEC-441X, Du Pont, NEN Research Products, Boston, MA) and the cold glycerol were mixed at a ratio of 1:99 (w/w). The ^{14}C-glycerol used was labeled at each carbon atom and the specific activity was 165.8 mCi/mmol. A weighed amount of this mixture was added to the RTV-type PDMS elastomer before curing. The procedure used for the fabrication of these beads was exactly the same as described earlier. HV-type PDMS beads containing glycerol were also prepared. The glycerol content in both cases was 20% (w/w) of the polymer matrix. These beads did not contain any drug.

Release of ^{14}C-Glycerol from PDMS Beads

Five beads were immersed in 20 ml of Sorensen's phosphate buffer and placed in a water bath with a shaking arrangement. The release medium was maintained at 37 ± 1°C and was sampled periodically. It was replaced every 24 hr. The amount of glycerol released was determined by measuring the radioactivity in the release medium. To 100 μl of the medium, 10 ml of the scintillation cocktail (Ecolume, ICN Biomedicals, CA) was added, and the radioactivity measured in a liquid scintillation counter (LS 3801, Beckman, CA).

Quantification of Glycerol Remaining in the Beads

In order to determine the amount of glycerol remaining in the beads at the end of the above experiment, the beads were immersed in liquid nitrogen for 5 min and immediately ground in a glass pestle and mortar. The glycerol was extracted from a weighed amount of the crushed beads with 5 ml of Sorensen's phosphate buffer. The extraction procedure was repeated three times. The buffer solutions were pooled and the radioactivity was measured.

Lyophilization of Tobramycin Base

An aqueous solution (10%, w/v) of tobramycin base was frozen by immersing in a mixture of dry ice and acetone and freeze-dried in a lyophilizer (LYPH · LOCK18, Labconco, MO). The powder X-ray diffraction pattern of the lyophilized base was obtained. It was also subjected to differential scanning calorimetry.

Content Uniformity

Both the content uniformity and the stability studies (described in the next paragraph) were performed in the beads wherein 20% (w/w) of the polymer base had been replaced with glycerol. The content uniformity was evaluated by determining the amount of tobramycin base present in several beads from three different batches immediately after fabrication. The beads were immersed in liquid nitrogen for 5 min and ground in a glass pestle and mortar. Tobramycin was extracted from the crushed beads four times each with 5 ml of Sorensen's phosphate buffer. The tobramycin content in the extracted buffer solution was determined.

Stability of the Drug in the Dosage Form

The dosage forms were stored at room temperature (~23°C) in a tightly closed screw-capped container and the content of tobramycin base was determined 0, 15, and 60 days after the fabrication of the beads. The procedure followed was the same as described under the content uniformity study.

RESULTS AND DISCUSSION

We had earlier characterized in detail the solid-state properties of tobramycin base and tobramycin sulfate (27). The tobramycin base was crystalline and occurred as a monohydrate ($C_{18}H_{37}N_5O_9 \cdot H_2O$), while the tobramycin sulfate [$(C_{18}H_{37}N_5O_9)_2 \cdot 5H_2SO_4$] was X-ray amorphous. The chemical stability of aqueous solutions of both tobramycin base and tobramycin sulfate has been studied (28). Solutions of tobramycin base ranging in pH from 1 to 11 have been observed to be stable for several weeks. Tobramycin sulfate solution is stable to autoclaving.

Drug Delivery Systems Without Hydrophilic Additive

The theoretical analysis of the kinetics of drug release from a spherical pellet having a homogeneous matrix was developed by Higuchi (29). In this system, Higuchi assumed that (a) the release of the drug occurred by a simple diffusional process, (b) a pseudo-steady-state condition existed during the release process, (c) the drug particles were uniformly distributed in the matrix, (d) the drug particle size was quite small relative to the average distance of diffusion, (e) the amount of drug present in the matrix per unit volume was substantially greater than the solubility of the drug in the

matrix, and (f) the release medium acted as a perfect sink. During the initial stage of drug release (release of less than 50% of the incorporated drug), a similar extent of release from both spherical and planar matrices was expected since a plane of the same area was a good approximation for the sphere. During this stage, a plot of the cumulative amount of drug released as a function of square root of time will be linear.

We were able to verify experimentally the validity of several of these assumptions. In all the drug delivery systems prepared, a major fraction of the incorporated drug had not dissolved in the polymer. This was apparent from visual as well as microscopic observation of the sliced beads. Tobramycin base is reported to be freely soluble in water (24). The tobramycin sulfate was observed to be very soluble in water at room temperature (30). Therefore sink conditions were maintained during the release studies. The content uniformity studies (results presented later) strongly suggested that the drug particles were uniformly distributed in the matrix.

PDMS of different molecular weights was used for the preparation of the drug delivery systems and the *in vitro* release profiles were obtained (Fig. 1). The plots of the cumulative amount of tobramycin base released as a function of the square root of time were linear. The release was consistent with a diffusion-controlled mechanism (29). The percentage of incorporated drug released in 72 hr following the use of PDMS with molecular weights of 36,000, 77,000, and 110,000 were 13.2, 6.2, and 5.7, respectively. From these preliminary release studies, it was clear that only a small fraction of the incorporated drug was released from these drug delivery systems in 72 hr. An increase in the molecular weight of the polymer decreases the diffusion coefficient of the drug in the polymer (31). However, the above observation was made in a non-cross-linked system. From among the PDMS bases of different molecular weights, the highest rate and greatest extent of drug release occurred from the base with a molecular weight of 36,000 (Fig. 1). This polymer base was used for the fabrication of all the drug delivery systems.

Tobramycin is available both as a crystalline free base and as a noncrystalline (lyophilized) sulfate salt. It was of interest to compare the release rates of tobramycin base and tobramycin sulfate from the polymer matrix. Beads were prepared with a tobramycin sulfate concentration of 7.7% (w/w) (equivalent to 5.0%, w/w, of the base). Figure 2 and Table I contain the results.

Depending on the drug load in the matrix, three types of monolithic dispersion systems have been described by Baker (16). In these systems, as the name implies, a fraction of the incorporated drug is dispersed in the matrix. In the first case, the drug load is typically $\leq 5\%$ (v/v). The mechanism of release in this system involves the dissolution of the drug into the matrix, followed by its diffusion into the release medium. The release from such systems can be described by Higuchi's square root equation (29). This type of matrix system is called the simple monolithic dispersion. When the loading level is 5 to 10% (v/v), the release mechanism becomes more complex. The pores arising from loss of the drug near the surface are filled with the release medium and provide preferential paths for the remaining drug to diffuse. At these loading levels, the pores do not form continuous pathways. These are referred to as complex monolithic dispersions. When the loading level is greater than 20% (v/v), the large number of pores formed due to the loss of the drug results in the formation of continuous channels and release can occur by a leaching mechanism. These are called monolithic matrix systems.

The density of the hydroxy end-blocked polydimethylsiloxane is 0.97 g/cm^3 (32). Since it forms the major component of the polymer matrix, it is reasonable to assume that the density of the polymer matrix will be close to that of PDMS. The density of the tobramycin base and tobramycin sulfate determined by the flotation method were approximately 1.4 and 1.57 g/cm^3, respectively (33). The drug loading of 5.0% (w/w) (in the case of the tobramycin base) and 7.7% (w/w) (in the case of tobramycin sulfate) were calculated to be 3.6 and 4.9% (v/v), respectively. Therefore, these are simple monolithic dispersions, and in both cases, plots of the cumulative amount of drug released as a function of square root of time were linear (Fig. 2). The extent of release of tobramycin sulfate was much higher than that of the tobramycin base and this is due to the initial burst effect. We

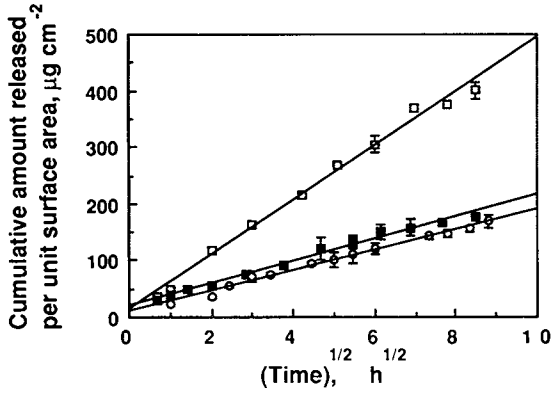


Fig. 1. The effect of the molecular weight of the hydroxy end-blocked polydimethylsiloxane on the kinetics of release of tobramycin base. The cross-linker concentration was 3.4% (w/w) of the polymeric matrix. The drug load was 5.0% (w/w). Error bars represent one standard deviation (n = 3). (□) 36,000; (■) 77,000; (○) 110,000.

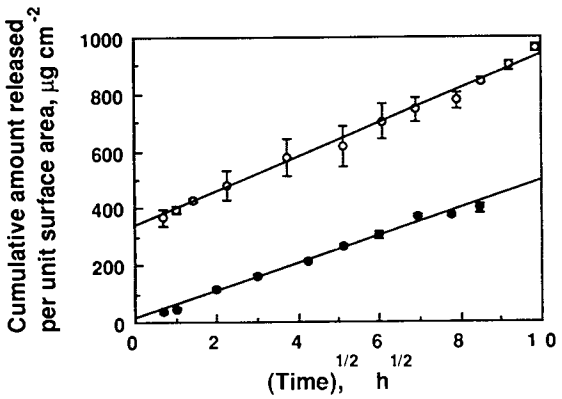


Fig. 2. Comparison of the release profiles of tobramycin base and tobramycin sulfate from PDMS beads. The drug load was 5.0% (w/w) in the case of tobramycin base and 7.7% (w/w) in the case of tobramycin sulfate. (●) Tobramycin base; (○) tobramycin sulfate.

Table I. Rate and Extent of Drug Release from PDMS (RTV-Type) Beads

Drug	Drug load (%, w/w)	Cross-linker concn. (%, w/w)[a]	Release rate ($\mu g\ cm^{-2}\ hr^{-1/2}$)	Percentage of incorporated drug released in 72 hr
Tobramycin base	5.0	1.5	66.8	20.0
Tobramycin base	5.0	3.4	48.6	13.2
Tobramycin base	5.0	12	44.4	12.0
Tobramycin sulfate[b]	7.7[c]	3.4	60.4	28.1

[a] Expressed as percentage (w/w) of the polymeric matrix.
[b] Nebcin (Eli Lilly).
[c] Equivalent to 5% (w/w) of the tobramycin base.

do not know why this is observed only in the case of tobramycin sulfate.

Barrer and co-workers (34,35) have shown that as the degree of cross-linking in a rubber increases, the diffusion of gases through the rubber decreases. The decrease in the diffusion coefficient was pronounced when the penetrant molecule was large. It was of interest to determine the effect of cross-linker concentration on the release of drug from the PDMS matrix. The usual cross-linker concentration in the matrix was 3.4% (w/w). PDMS beads with cross-linker concentrations in the matrix of 1.5, 3.4, and 12% (w/w) were fabricated. The concentration of tobramycin base in all three cases was kept at 5.0% (w/w). Even at the lowest cross-linker concentration, the beads maintained their integrity. The rate of drug release from the beads with the lowest cross-linker concentration (1.5%, w/w) was 1.5 times that from the formulation with the highest (12%, w/w) cross-linker concentration (Fig. 3, Table I). According to the hole theory of diffusion, the rate of diffusion depends on two factors (36): (a) the number and size distribution of the existing holes and (b) the ease with which these holes are formed. Joining molecular segments through cross-links, the polymer flexibility is decreased and the hole formation is rendered more difficult. Therefore an increase in cross-linking will decrease the diffusion coefficient (31). In this system, a change in the cross-linker concentration from 1.5 to 3.4% (w/w) caused a pronounced decrease in the release rate, while a further increase in cross-linker concentration appeared to have a negligible effect on the release rate.

The release data from the different drug delivery systems are summarized in Table I. The rate of release of tobramycin base from this hydrophobic polymer matrix was low. Though tobramycin sulfate was more rapidly released from the matrix, it was not available in a pure form.[4] Therefore, our next objective was to enhance the rate and extent of the release of the tobramycin base from this matrix. One possible approach was to enhance the apparent solubility of the tobramycin base in the polymer matrix. This may be accomplished by converting it into the noncrystalline (amorphous) form, which would have a higher free energy and therefore a higher apparent solubility in the matrix. An aqueous solution of tobramycin base was lyophilized in an effort to prepare the amorphous form. The solid state of the lyophilized material was characterized by powder X-ray diffractometry and differential scanning calorimetry (DSC). The X-ray diffraction patterns of the crystalline free base and the lyophilized base were identical. Their DSC curves were also quite similar. This suggested that either amorphous tobramycin was not formed by lyophilization or the amorphous compound formed was rapidly transformed to crystalline tobramycin (27).

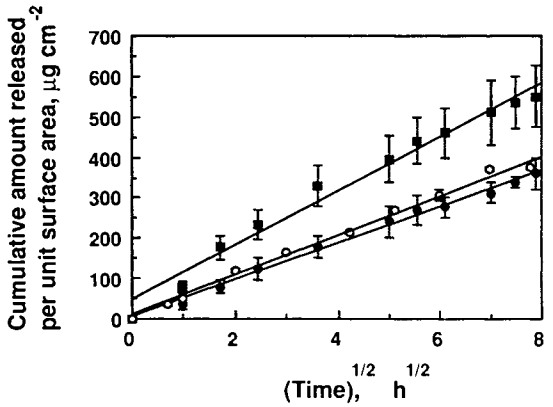


Fig. 3. The effect of cross-linker concentration on the release kinetics of tobramycin base from PDMS beads: (■) 1.5% (w/w); (○) 3.4% (w/w); (●) 12% (w/w). The drug load was 5.0% (w/w).

Drug Delivery Systems with Hydrophilic Additive

The next approach to enhance the release of tobramycin from this polymeric matrix was to modify the polymer matrix by the addition of a hydrophilic additive. The additive used was glycerol. The glycerol loads selected were 5, 10, and 20% (w/w) of the polymeric matrix. The drug load was 5% (w/w) in all cases. The tobramycin release profiles from the glycerol incorporated PDMS matrices are shown in Fig. 4. A linear relationship between the cumulative amount of drug released and the square root of time was observed in all cases. The effect of glycerol load on the rate and extent of drug release is presented in Table II. The glycerol-incorporated beads swelled during the release studies.

Hsieh and Chien (22) had reported earlier that glycerol-incorporated PDMS, when placed in contact with water, absorbed water and swelled. Interestingly, negligible release of glycerol occurred from the matrix to the release medium. According to these authors, "one or more of the three hydroxy groups in the glycerol molecule may be covalently

[4] For all the preliminary studies, a marketed tobramycin sulfate formulation was used. Our attempts to obtain pure tobramycin sulfate from chemical companies and from pharmaceutical manufacturers were unsuccessful. Therefore, we were forced to discontinue our studies with the tobramycin sulfate.

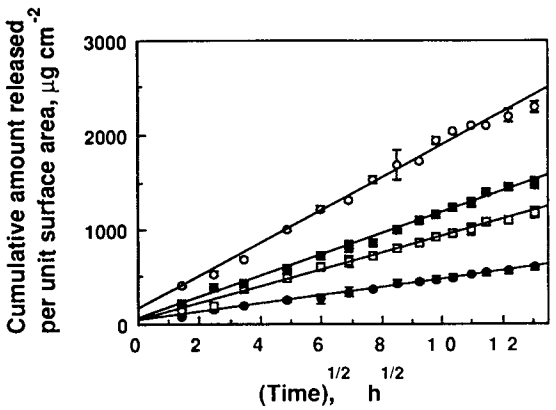


Fig. 4. The effect of glycerol concentration on the kinetics of release of tobramycin base from PDMS beads: (●) 0% (w/w); (□) 5% (w/w); (■) 10% (w/w); (○) 20 (w/w). The drug load was 5.0% (w/w).

bound with the polydimethylsiloxane backbone during the curing process" (22). However, no supportive experimental evidence was provided.

We were interested in determining if any of the polymer components interacted with glycerol. In the absence of glycerol, the cross-linking reaction involves the alkoxy type of condensation, where the hydroxyl group of the polymer reacts with the propyl group of the cross-linker (23) (Scheme I). When glycerol is present, one or more hydroxyl groups of glycerol may interact with the cross-linker. For comparison purposes a second polymer, heat-vulcanized (HV) PDMS, was selected where the cross-linking reaction involves a free radical mechanism and does not involve hydroxyl groups (23).

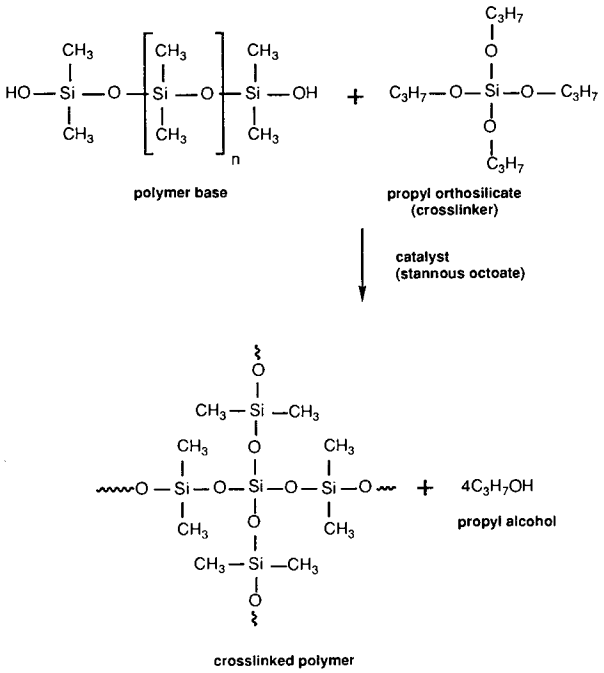
Both the RTV- and the HV-type PDMS beads containing glycerol were placed in contact with water and their weight change was monitored until they attained a constant weight. The beads did not contain any drug. The RTV-type PDMS beads showed a much higher percentage weight gain than the HV-type PDMS beads (Figs. 5a and b). The RTV beads took a much longer time to attain a constant weight than did the HV beads. Both the RTV- and the HV-type PDMS beads were subjected to DSC and TGA after they had attained constant weight. Only one endothermic peak at ~100°C was observed when the swollen beads were subjected to DSC. The weight loss in the TGA occurred in the temperature range of 80 to 105°C and this matched the weight gain of the polymer during its immersion in water.

Table II. Release of the Tobramycin Base from Glycerol-Incorporated PDMS (RTV-Type) Beads[a]

Glycerol load (%, w/w)[b]	Release rate (μg cm^{-2} hr$^{-1/2}$)	Percentage of incorporated drug released in 171 hr
0	44.3	16.4
5	88.7	31.8
10	111	39.7
20	173	57.1

[a] The cross-linker concentration was 3.4% (w/w) of the polymeric matrix. The drug load was 5% (w/w).

[b] Expressed as percentage (w/w) of the polymeric matrix.



Scheme I. Cross-linking reaction in the room-temperature vulcanization-type polydimethylsiloxane (23).

Therefore the weight loss observed during the thermal studies was attributed to loss of water.

It was of interest to determine the amount of glycerol released from these beads. Therefore RTV- and HV-type PDMS beads containing radiolabeled glycerol were fabricated. The glycerol load was 20% (w/w) of the polymeric matrix. From the radioactivity quantified in the release medium, the total amount of glycerol released was calculated.

In the case of the RTV-type PDMS, only 0.17 ± 0.01% (mean ± SD; n = 3) of the incorporated glycerol was released during immersion in water for 18 days, and this was in good agreement with the previously reported results (22). This strongly suggested that the glycerol was tightly bound to the matrix. In the case of HV-type PDMS beads, 84.5 ± 3.2% (n = 3) of the incorporated glycerol was leached out within 2 days. Glycerol is known to have a high affinity for water. The glycerol incorporated into the RTV-type PDMS took up water, resulting in the swelling of the beads. In the case of the HV-type PDMS, since most of the incorporated glycerol was readily leached out, the beads underwent only a small weight change (Fig. 5).

The microstructure of the polymers was examined by scanning electron microscopy. The microstructures of the RTV- and HV-type PDMS (without glycerol) were quite similar (Fig. 6a). The microstructures of the polymers, after glycerol incorporation, showed marked differences. In the RTV-type PDMS, the average diameter of the dispersed glycerol was about 10 μm, while in the HV-type PDMS, the average diameter was much higher (Figs. 6b and c). The vulcanization mechanism is different in the two types of polymers. In the RTV system, the propyl orthosilicate reacts with the hydroxyl end group of the polymer to form the cross-linked polymeric network (Scheme I). When glycerol is mixed with the polymer base, one or more of the hydroxyl

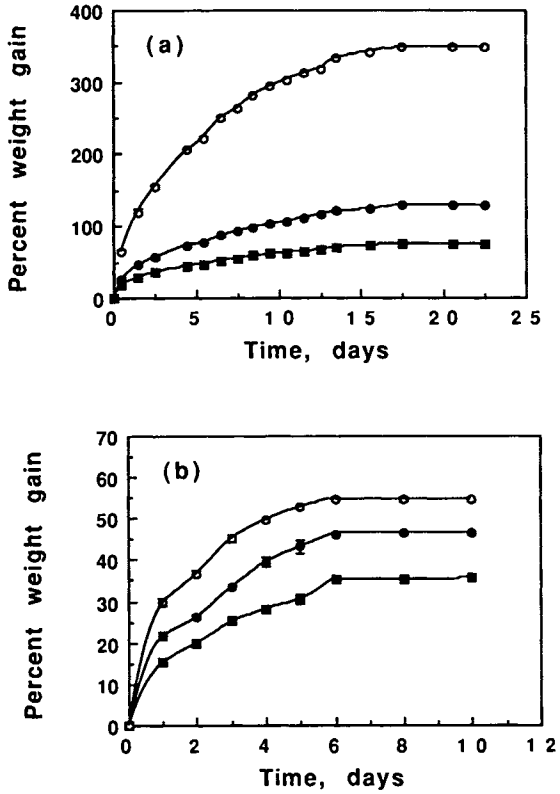


Fig. 5. Water uptake of glycerol incorporated PDMS beads as a function of time (n = 4). (a) PDMS (RTV) type. Glycerol concentrations: (■) 5.2% (w/w); (●) 8.6% (w/w); (○) 20% (w/w). (b) PDMS (HV) type. Glycerol concentrations: (■) 5% (w/w); (●) 10% (w/w); (○) 20% (w/w).

groups in glycerol may participate in the cross-linking reaction. The fraction of the incorporated glycerol that participates in the cross-linking reaction is not likely to be released from the matrix. In the case of the HV system, interaction between the polymer components and the glycerol is unlikely. Therefore, the incorporated glycerol is readily leached out from this matrix.

To obtain direct molecular-level evidence of the possible interaction between glycerol and the polymer, NMR studies of the polymers were carried out. The ^{13}C solid-state NMR spectrum of glycerol-incorporated RTV-type PDMS exhibited peaks at 1.2, 64, and 73 ppm, which were attributed to the $-CH_3$ carbon of the polymer, $-CHOH$ carbon of glycerol, and $-CH_2OH$ carbon of glycerol, respectively (Fig. 7a). Therefore at least a part of the incorporated glycerol was in an "immobile" state, suggesting the participation of glycerol in the cross-linking reaction. In the case of the glycerol-incorporated HV-type PDMS, the peaks due to glycerol were absent, thereby suggesting that all the incorporated glycerol was in the liquid state (Fig. 7b).

When the glycerol-incorporated RTV- and HV-type PDMS were subjected to high-resolution ^{13}C NMR, peaks at 1.2, 64, and 73 ppm were observed and were again attributed to the $-CH_3$ carbon of the polymer, $-CHOH$ carbon of glycerol, and $-CH_2OH$ carbon of glycerol respectively (Figs. 8a and b). Besides the above three peaks, the glycerol-incorporated RTV-type PDMS also exhibited three very

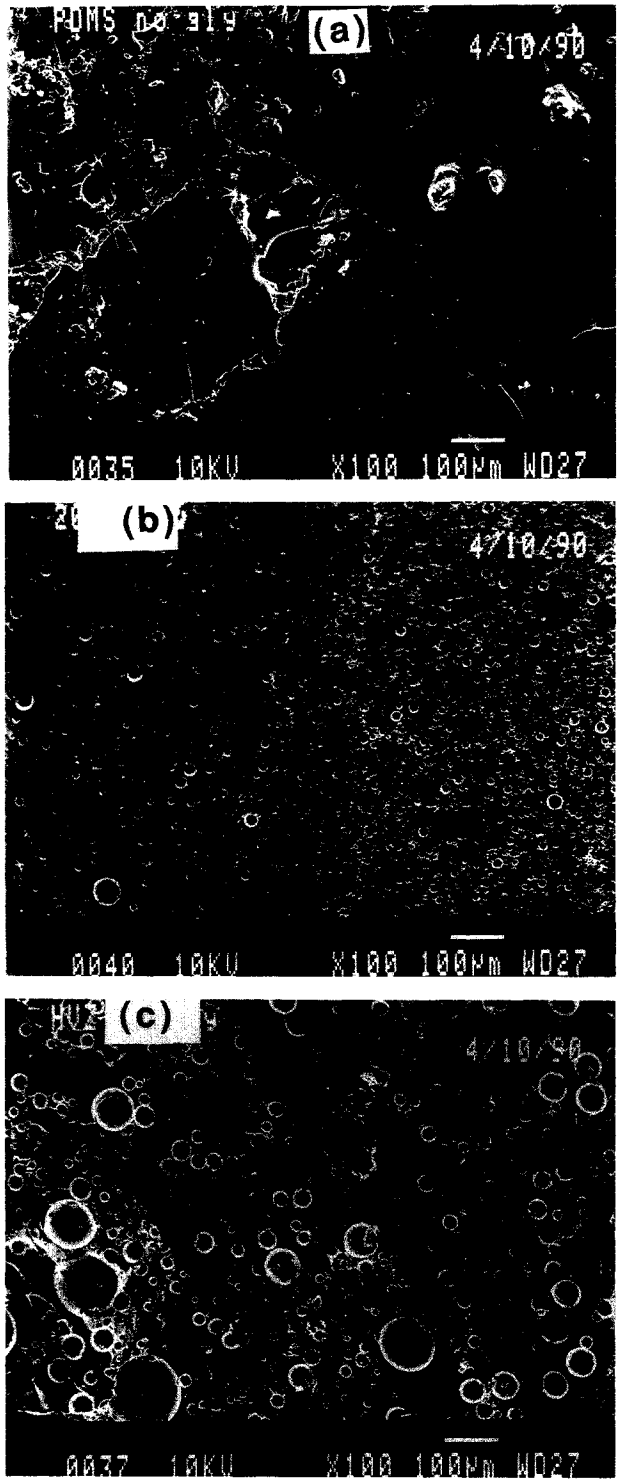


Fig. 6. Scanning electron micrographs of (a) RTV-type PDMS matrix without glycerol; (b) RTV-type PDMS matrix with 20% (w/w) glycerol; (c) HV-type PDMS matrix with 20% (w/w) glycerol.

small peaks at 10.5, 26.3, and 64.0 ppm, which were, respectively, attributed to the $-CH_3$ carbon, $-CH_2$ carbon, and $-CH_2OH$ carbon of the 1-propanol (37). The peak at 64.0 ppm due to the $-CH_2OH$ carbon of the 1-propanol is readily discernible in the case of the RTV-type PDMS without glyc-

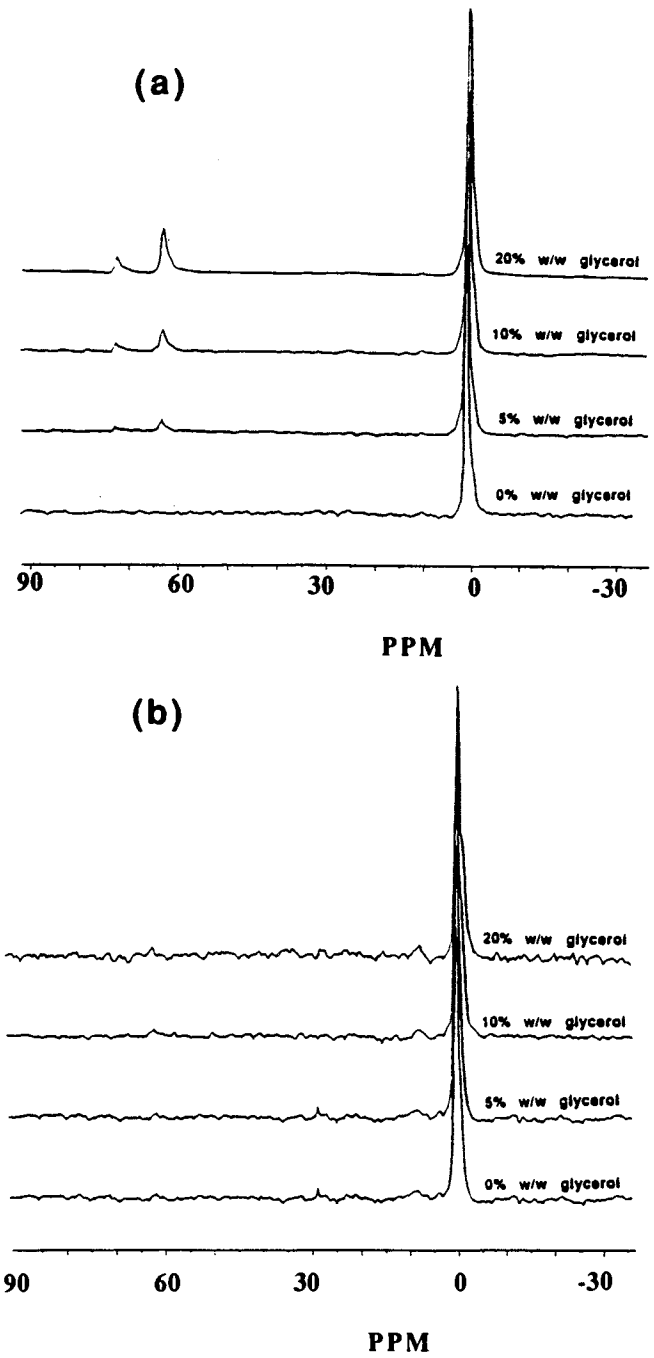


Fig. 7. ^{13}C solid-state NMR spectra of (a) RTV-type PDMS with different glycerol concentrations; (b) HV-type PDMS with different glycerol concentrations.

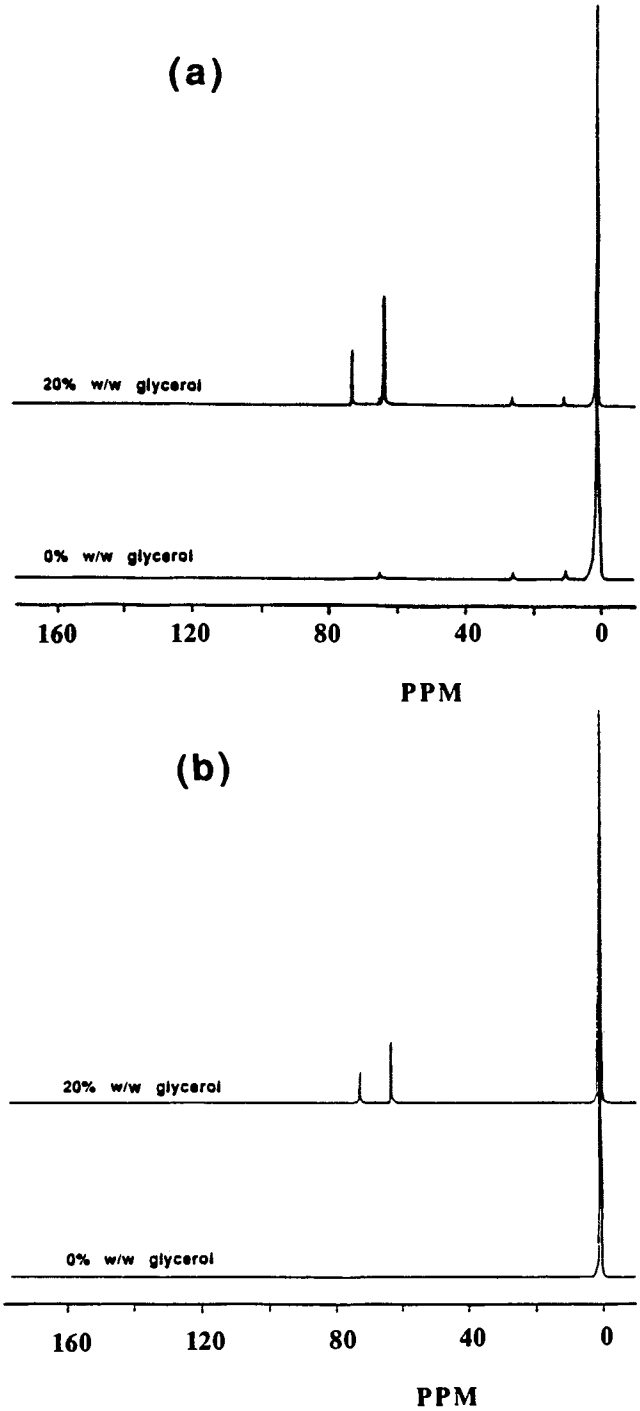


Fig. 8. ^{13}C high-resolution NMR spectra of (a) RTV-type PDMS with and without glycerol; (b) HV-type PDMS with and without glycerol.

erol (Fig. 8a). In the case of the RTV-type PDMS containing glycerol, this peak occurs as a shoulder. These three small peaks are direct evidence of the reaction between the hydroxy end-blocked PDMS base and propyl orthosilicate to form the cross-linked polymer and 1-propanol as a by-product (Scheme I). During the preparation of the drug delivery systems, the drug and the polymer matrix components were mixed and the mixture was degassed under reduced pressure for 5 min. The relevant details are given under Experimental. However, the method used for the preparation of samples for NMR analysis did not permit us to degas the samples. Therefore, the 1-propanol remained in the system. The cross-linking reaction in the case of the HV-type PDMS being different, 1-propanol was not formed as a by-product (23).

The NMR studies suggested that all the incorporated glycerol in the HV-type PDMS was in the liquid state. This conclusion was also supported by the glycerol leaching stud-

ies. In the case of the RTV-type PDMS, the NMR studies suggested that the glycerol existed in the liquid as well as the "immobile" states. However, a negligible amount of glycerol was released during the leaching studies. We are unable to explain why the glycerol in the liquid state is not leaching out. It must be pointed out that the amount of propyl orthosilicate is so small that even if only one hydroxyl group from each of the glycerol molecules was interacting with the propyl orthosilicate, only a fraction of the incorporated glycerol would be participating in the reaction (this argument applies even at the lowest glycerol concentration).

Content Uniformity

The measured drug content was compared with the nominal drug content in the beads. The determined content was 93 ± 1.3% (mean ± SD; $n = 3$) of the nominal drug content. The low coefficient of variation value (1.4%) indicated uniform drug distribution in the beads.

Stability of the Drug in the Dosage Form

The tobramycin content determined 0, 15, and 60 days after the fabrication of the beads was found to be 93, 93, and 91%, respectively, of the labeled amount. It was therefore concluded that the dosage form had a shelf life of at least 2 months.

In conclusion, an implantable dosage form for the treatment of bone infections was prepared by incorporating tobramycin in cross-linked polydimethylsiloxane. The rate and extent of drug release from the polymer matrix were unacceptably low. The addition of glycerol to the matrix substantially accelerated the drug release. When placed in contact with the aqueous medium, the glycerol-incorporated beads absorbed water and swelled but glycerol was not released from the beads. NMR studies indicated that a fraction of the incorporated glycerol had participated in the cross-linking reaction of the polymer. Uniform distribution of the drug in the beads was concluded based on content uniformity studies. Short-term stability studies suggested a shelf life of at least 2 months for the dosage forms when stored under ambient conditions.

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