Release of Radioactive Steroids from Dried Down Hydrogels of Polyurethane Networks

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SYNOPSIS

The polyurethane networks were based on poly(ethylene glycol) 6000 (PEG), crosslinked with 1,1,1-tris(hydroxy methyl)ethane and with the stoichiometric equivalence of hexamethylene diisocyanate. Radioactive steroids were incorporated into cylindrical hydrogels over a wide range of compositions. The release profiles were drawn from dried down hydrogel of polyurethane networks. The scintillation counter was used for the release study of steroids after different intervals. *In vivo,* the loaded hydrogels were implanted into rats. The results for constant release studies were recorded. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Studies of drug release rates from polymeric materials have been made by a number of investigators.[1-4] In a polymeric controlled release system the drugs are released by diffusion, chemicals, swelling, and magnetic processes. The most common mechanism is diffusion through hydrogels, whereby the drug migrates from its initial position in the plastic to the outer surface. In earlier publications,[5,6] it has been proposed that under certain conditions the rate of diffusion from the surface of the matrix to the surrounding bulk solution makes a significant contribution to the total diffusion process. Haleblian et al.[7] also suggested the possibility that the rate of solute transfer across the matrix solution may control the release.

In the matrix or monolithic system where the drug is distributed uniformly throughout the polymeric matrix, the drug release does not follow zero-order.[8] Lee[9] has described an approach to zero-order drug delivery by immobilizing non-uniform drug distribution in hydrogels. Hydrogels can absorb a significant amount of water to form an elastic gel and at the same time release the dissolved drug by diffusion through the swollen region.[10,11]

In the present work the influence of hydrogel composition on the resulting release characteristics has been studied. *In vivo,* the delivery rates of the drug were determined by surgically implanting or hypodermically injecting the polymeric materials into animals and measuring the radioactivity of steroids at fixed intervals of time. In-vitro, the delivery rates were determined by releasing the steroids in buffer solution at fixed intervals of time.

EXPERIMENTAL

Materials

All general chemicals used in the drug release study were analytical grade, and were obtained from Sigma Chemical Co. USA and E. Merck. The tritium-labelled ^{3}H-steroids (testosterone, progesterone, and estradiol) were obtained from Amersham UK. The scintillation fuel permablend II was obtained from Packard USA.

Preparation of Hydrogels

Poly(ethylene oxide) (PEO) crosslinked hydrogels, based on poly(ethylene glycol), 1,1,1-tris(hydroxy methyl)ethane, and hexamethylene diisocyanate, were prepared according to the procedure described in an earlier publication.[12]

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Purification of Hydrogels

Hydrogels of four varying molar concentrations—namely, 1M-PEG 6000, 1.5M-PEG 6000, 2M-PEG 6000, and 3M-PEG 6000—were used for studying the release profile of the ^{3}H-steroids hormones. The cylindrical pieces of equal length (1 cm) and volume were cut from each block of polymer and soaked separately for 24 hours in a substantial amount of distilled water. The blocks were vacuum-dried at 40°C for 7–8 hours and used in subsequent studies.

Loading of Hydrogels

The vacuum-dried hydrogels were used for loading the hormones onto the gels. A specific amount of radioactive ^{3}H-steroid in 100 μL volume was used for loading. Initially the steroid was dissolved in ethanol and toluene mixture (1 : 9 v/v). After evaporation, the ^{3}H-steroids were reconstituted in a 10mL solution of ethanol : chloroform (1 : 1 v/v). The blocks of polymer (cylindrical) were placed in this solution for 24 hours at 37°C. Before starting the experiment, an aliquot of 100 μL of this solution was drawn for calculating the total amount of radioactivity used for loading on the gel. At the end of the 24-hour period, another aliquot of 100 μL was withdrawn and radioactivity bound to the gel was calculated. The swollen, drug-loaded hydrogels were wiped carefully with tissue paper and then dried at 40°C in a vacuum oven. The dried hydrogels attaining their original shape and dimensions were used for the release studies. All the hydrogels were loaded, using an identical procedure.

In-vitro Release Studies

The *in vitro* release of steroids from the dry, drug-loaded hydrogels were studied by using the releasing phosphate buffer pH 7.2 in 250 mL volume. Release studies were conducted at constant temperature (37°C) and continuous shaking. The release characteristics of each steroid were studied at various time intervals up to 31 hours. Aliquots of 500 μL each were drawn after an hour and were tested for activity. All hydrogels were studied for release characteristics under identical conditions. The scintillation counter (LS-180) was used for measuring the activity after different intervals.

In-Vivo Release

For this purpose male and female rats (three months old) were used. The polymer block (1M-PEG 6000 hydrogel) previously loaded with the ^{3}H-steroid was implanted under the skin on dorsal side. The ^{3}H-steroid used was testosterone. The animals were killed at the desired time interval; blood was collected and radioactivity counted in 100 μL of plasma.

RESULTS AND DISCUSSION

In this study the crosslinked phase separated hydrogels were evaluated for characteristics of *in vitro* and *in vivo* drug release. The dry, drug-loaded hydrogel, when imbibed with water, absorbs a considerable amount of water to become an elastic gel and, simultaneously, release the dissolved drug by diffusion through the swollen region.[10,11] Figure 1 shows the release profile of radioactive steroids from the phase separated hydrogel of 1M-PEG 6000. The conditions of loading and releasing the steroid from the gel were the same as those used for non-phase separated hydrogel.[13] The release was studied for a period of 31 hours.

Generally the release of steroids from the phase separated gel was much slower compared with the non-phase separated gel. The release of activity in the non-phase separated gel was almost complete (70%) within 2 hours, whereas only 45% of the activity was released after 5 hours in the phase separated gels. Also, the extent of total release in the phase separated gels was of the order of 85% by 30 hours. The release profile of the phase-separated

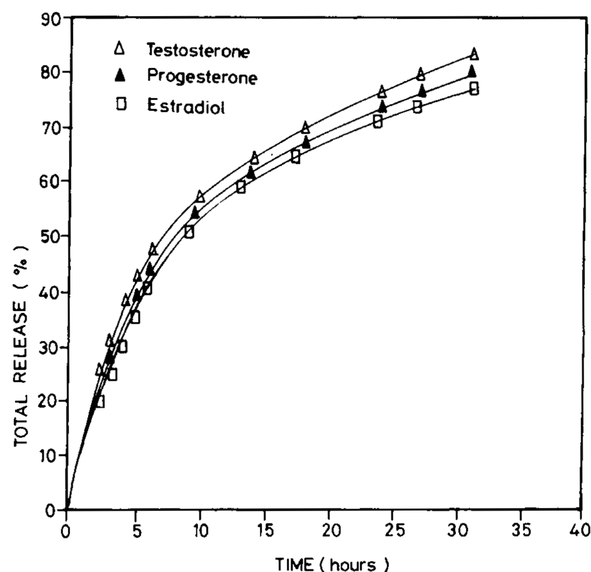

Figure 1 Total drug release of 1M-PEG 6000 at 37°C: (△) Testosterone; (▲) Progesterone; (□) Estradiol.

hydrogels showed a pattern of sustained and prolonged release for each steroid.

The rate of release of the steroids was observed at 37°C, as shown in Figure 2. The release of steroids from 1.5M-PEG 6000 hydrogel was comparatively slower than that of 1M-PEG 6000 hydrogel (Fig. 3). This is evident from the $t^{0.5}$ value of testosterone, which is 6 hours in the case of 1M-PEG 6000 and 7.5 hours for 1.5M-PEG 6000. The release profile of this hydrogel showed a pattern of sustained and prolonged release for each steroid. The extent of total release was of the order of 80% by 30 hours. Figure 3 also indicates that by increasing the cross-linking agent, release of steroids hormones from the polymeric material has been prolonged.

In vivo studies also show the release profile of a steroid hormone bound to a phase-separated hydrogel, which had been subcutaneously implanted in mature rats. For this purpose the hormone steroid used was testosterone, which was loaded onto the hydrogel 3M-PEG 6000 as described under Experimental. The loaded hydrogel was subcutaneously implanted in the animals and release rates were recorded after appropriate time intervals. The results obtained are recorded in Figure 4. The release profile was studied for a time period of 72 hours. The release of activity followed the pattern of sustained prolonged release, but was considerably slower than the *in vitro* release of the same steroid. Only 2.2% of the total activity was released after a period of 72 hours as compared to 70% from the same hydrogel in *in vitro* studies. The rate of steroid release from 3M-PEG 6000 was observed as shown in Figure 5. Several previous studies have shown that hydrogels can be used effectively for the slow release of drugs.[10] However the release characteristics vary with the nature of the drug and the polymer matrix used in the preparation of the gel. More recently, gels based on poly(ethylene oxide) (PEO) have been shown to fulfill a number of criteria needed for the *in vitro* and *in vivo* release of a number of drugs.[14] We have used phase separated PEO hydrogels and studied

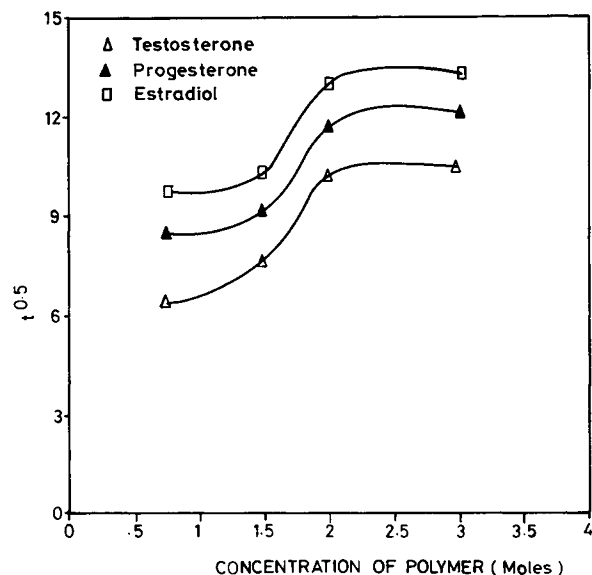

Figure 3 Concentration of polymer VS $t^{0.5}$ relationship: (△) Testosterone; (▲) Progesterone; (□) Estradiol.

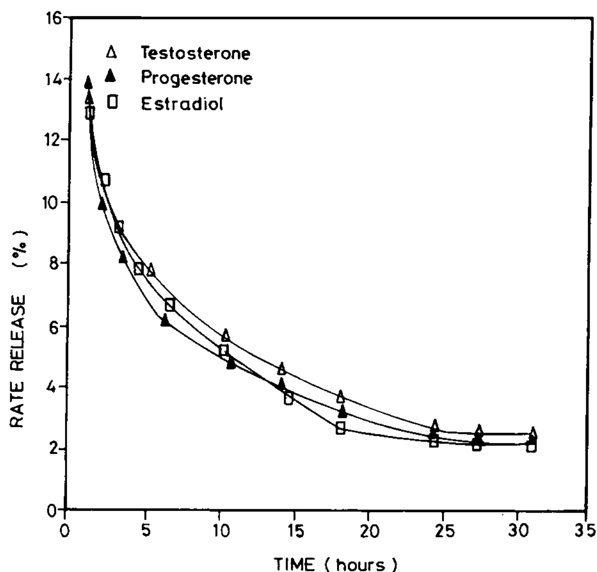

Figure 2 Rate of release of 1M-PEG 6000 at 37°C: (△) Testosterone; (▲) Progesterone; (□) Estradiol.

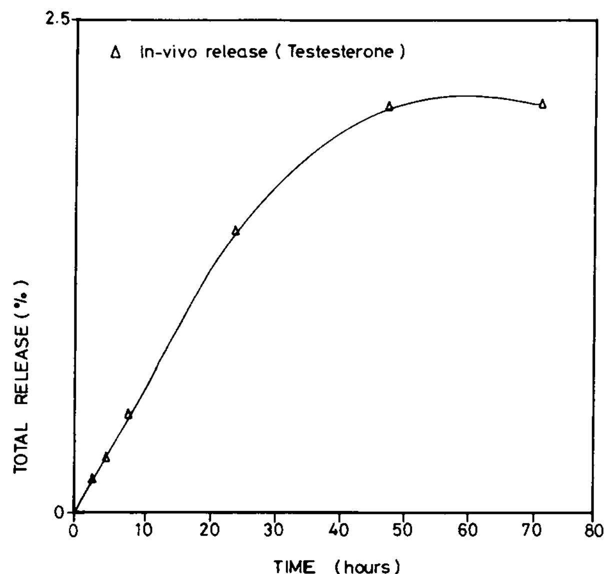

Figure 4 Total drug release *in vivo* from 3M-PEG 6000: (△) Testosterone.

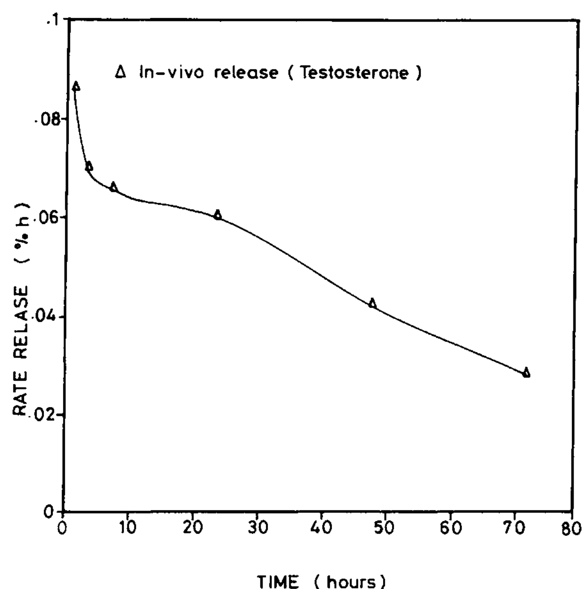

Figure 5 Rate of release *in vivo* from 3M-PEG 6000: (Δ) Testosterone.

the *in vitro* and *in vivo* release profiles of the steroids testosterone, progesterone, and estradiol. Non-phase separated gels have a poor rate of release compared to those in our studies of phase-separated hydrogels. A dramatic change in release pattern was obtained with each steroid when phase separated hydrogel 1.00M-PEG 6000 was used. There was delayed release of each steroid; 85% of the steroid was released after 30 hours of *in vitro* incubation and only 2% was released after the *in vivo* implantation of the dried cylinders of the hydrogels. No attempt has been made to calculate the diffusion coefficients of the steroids from these gels, because drug release from initially dry matrix devices is difficult to characterize due to the constantly changing rate controlling parameters during drug release.

It is generally agreed that water in these gels is in two forms; the bound form and the free form.[15] It has also been suggested that the bound form of water is associated with the ether group of the PEO gel (3 moles/ether group) in crystalline state, and that the delay in the release of the drug is dictated by the content of the bound form of water in the gels. It appears that phase separation increases the concentration of bound water and thus causes a delay in the release of the steroid. To find out whether such is the case, we have prepared phase separated hydrogels with a varying degree of crosslinking agent for *in vitro* release experiments. The extent of delay in the release of steroids is directly related to the degree of crosslinking.

Two factors seem to operate in causing the delayed release. First, the content of the bound form of water increases with increasing crosslinking; for example, more water molecules are bound to the ether groups in 3.0M-PEG 6000 than to 1.0M-PEG 6000. Second, with increasing crosslinking the pore size of the gel decreases. Both of these factors have obvious implications in the release profiles of the steroids.

It may be noted that whereas 85% of the steroid is released after 30 hours from 1.0M-PEG 6000 gel, the release from the 1.50M, 2.0M, and 3.0M-PEG 6000 gels specifically decreased in the same order during the same time interval. It has been suggested that the PEO hydrogel has three domains: A, B, and C. The °A′ domain relates to PEO, the °B′ domain is contributed by the crosslinker, and the °C′ domain is comprised of free water. In the 3.0M-PEG 6000 gel, the °C′ domain is minimal. For this reason, it can be argued that 3.0M-PEG 6000, with high crosslinking, small pore size, and more bound water content, combines the characteristics which result in comparatively delayed release of the steroid in this series of gels.

CONCLUSIONS

Our data indicate that PEO based hydrogels, crosslinked with 1,1,1,-tris(hydroxy methyl)ethane can be used profitably for the slow *in vitro* release of drugs such as steroids. Although only preliminary data have been presented on *in vivo* release of testosterone steroids which were studied in rats, information obtained from *in vitro* experiments holds high promise for their use in animals and humans. The rate of release showed the constant drug delivery from the polymeric materials. Indeed, a number of polymer-tissue compatibility studies will be required if the hydrogels are to be put to medical use. The marked difference in the *in vivo* and *in vitro* rates of release may be related to alteration in the release properties of the implant when placed in the tissue. Further, percentage release of steroids reflects the amount of the hormone in blood alone.

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