# Release of Tritium-Labeled Estradiol Steroids from Fully Swollen Hydrogels of Polyurethane Networks

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**SYNOPSIS**

Diffusion of uniformly dispersed tritium-labeled estradiol in water-swollen poly(ethylene oxide)-based hydrogels was studied by assaying the release of solute from cylindrical hydrogels into a finite volume of solution at 37°C. Fractional release followed a $t^{0.5}$ relationship for a range of radii between 0.20 and 0.35 cm and different polymer compositions with equilibrium water uptake of 220–750 parts per hundred dry polymer. Values of the diffusion coefficient were calculated from the fully swollen hydrogels, which represents the swelling and release profile quite accurately. © 1994 John Wiley & Sons, Inc.

## INTRODUCTION

Synthesis, swelling characteristics, and the nature of water in hydrogels have been reported in our earlier publication.[1] It was shown that the hydrogels based on poly(ethylene oxide) are capable of imbibing large quantities of water and the swelling phenomenon involves possible hydrate formation. A number of references in the literature support an association of poly(ethylene oxide) with close to three molecules of water in solution,[2,3] whereas other references interpret the behavior in water by thermodynamics without specific complex formation[4] or in the crystalline state as the result of the formation of a crystalline eutectic that possesses a ratio of three molecules of water to each ether group in the poly(ethylene oxide) backbone and is related to bound water associated with the poly(ethylene oxide).[5]

The high water content and soft rubbery consistency of such hydrogels contribute to their superficial resemblance to human tissues[6–9] and may also contribute to their biocompatibility by minimizing mechanical irritation to surrounding tissue. Such a highly hydrated and water-plasticized polymer network is often mechanically weak, but is still finding ever-increasing use as a component of controlled release drug-delivery systems.[10–13]

Radioisotopes have been used extensively in the testing of various pharmaceutical dosage forms.[14,15] The literature contains several reports of evaluative methodology regarding *in vivo* and *in vitro* uses of radioisotopes with the sustained-release dosage form. A rapid procedure was developed for the study of the *in vitro* release characteristics of radiolabeled drugs in sustained delivery systems. The use of scintillation spectroscopy allowed for simultaneous determinations of percent release of each labeled drug from the single sustained-action hydrogel.

Biologically active molecules can often be permanently or temporarily immobilized within hydrogels.[16] The release of such bioactive agents usually takes place by diffusion of solute (e.g., drug) molecules, and if the diffusion characteristics are known, a device can be designed to release the solute at a predetermined rate. The most important single diffusion parameter desired for this design is the diffusion coefficient.

It was the purpose of this study to show the effect of chemical composition[17–21] (the monomer composition and quantity of cross-linker present) on the rates of diffusion from samples of fully swollen material of various physical dimensions and to test whether the presence of bound water leads to any unusual diffusion patterns in this class of hydrogels.

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## EXPERIMENTAL

### Materials

All general chemicals used in this drug-release study were analytical grade and were obtained from Sigma Chemical Co., USA, and E. Merck. The tritium-labeled $^3$H-estradiol steroid was obtained from Amersham, UK. The scintillation fuel permablend II was obtained from Packard, USA.

### Preparation of Hydrogels

Poly(ethylene oxide) (PEO) cross-linked 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.[1]

### Purification of Hydrogels

Hydrogels of five varying molar concentrations, namely, 0.75*M*, 1*M*, 2*M*, 3*M*, and 4*M* PEG 6000, were used for studying the release profile of the $^3$H-estradiol. The cylindrical pieces of equal length (1 cm) and volume were cut from each block of polymer and soaked separately for 24 h in a substantial amount of distilled water. The cylinder blocks were vacuum-dried at 40°C for 7–8 h and used in subsequent studies.

### Drug Incorporation in Hydrogels

The vacuum-dried hydrogels were used for loading the $^3$H-harmones onto the gels. A specific amount of radioactive steroid in a 100 μL volume was used for loading. Initially, the steroid was dissolved in an ethanol and toluene mixture (1 : 9 v/v). After evaporation, the $^3$H-steroids were reconstituted in a 10 mL solution of ethanol : chloroform (1 : 1 v/v). The blocks of polymer (cylindrical) were placed in this solution for 24 h 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 h period, another aliquot of 100 μL was with drawn and radioactivity bound to the gel was calculated. The swollen drug-loaded hydrogels were used for the release studies. All the hydrogels were loaded, using an identical procedure.

### Release Studies

The *in vitro* release of steroids from the dry drug-loaded hydrogels were studied using the releasing phosphate buffer, pH 7.2, in a 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 33 h. Aliquots of 500 μL each were drawn after 1 h and were tested for activity. All hydrogels were studied for release characteristics under identical conditions. A scintillation counter (LS-180) was used for measuring the activity after different intervals.

## RESULTS AND DISCUSSION

The incorporation of $^3$H-estradiol into cylindrical pieces of hydrogels of varying compositions of water content was achieved by swelling the gels in a solution of the steroids.[22] Since the ether group in PEO hydrogel is capable of hydrogen bonding, the incorporation of polyacids in these gels may reduce the degree of swelling of the networks. Therefore, certain interactions of a drug with the PEO hydrogel, if the degree of swelling is affected, can significantly affect its release profile from the matrix as compared with a similar noninteracting material. Estradiol has a limited solubility in water and displays neither a significant tendency to change the swelling properties of the PEO gels nor any effect on its dimension. It was therefore chosen as a suitable drug/polymer combination for examining the release behavior.

The $^3$H-estradiol load was kept below 1% w/w of the swollen gel to ensure uniform dispersion of the solute in the polymer and to reduce further the possibility of any significant change in polymer swelling caused by the presence of the diffusate. The release profile of $^3$H-estradiol for 1*M* PEG 6000 (Fig. 1) showed that the total amount released in 33 h is about 85%, as would be expected from devices subject to diffusion control. However, the rate of release in Figure 2 shows that a brief transient state occurred during the first hour in which approximately 9% of the solute contained in the matrix was released. This transient state may be due, in part, to the experimental procedure adopted, since the hydrogel (swollen in water/ethanol) carrier may take some time to become compatible with the buffer in which the release was carried out. Later, the constant release was observed.

It is interesting to note that the straight-line relationship holds up to $Qt/Q\alpha$ of 1.0 in the case of 1*M* PEG 6000 (Fig. 3). However, in the cases of 2*M*, 3*M*, and 4*M* PEG 6000, the straight-line relationship between $Qt/Q\alpha$ varies from 0.95 to 0.6.

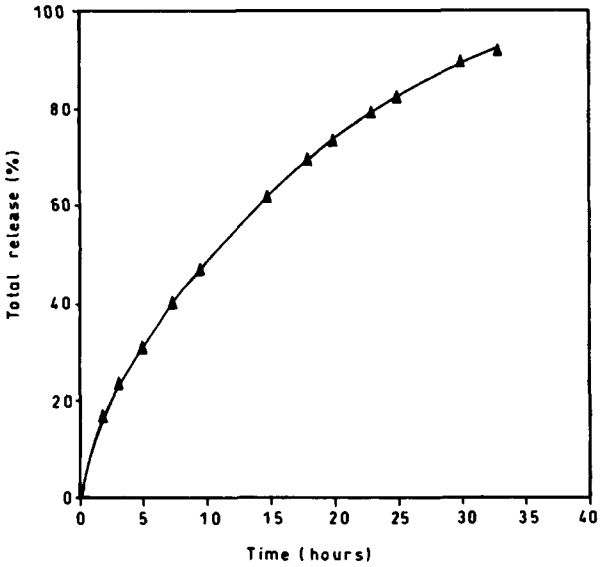


**Figure 1** Total release vs. time: (▲) 1*M* PEG 6000.

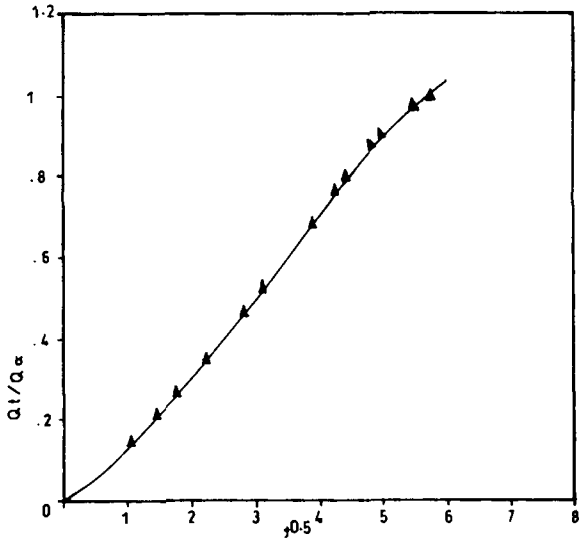


**Figure 3** $Qt/Q\alpha$ vs. $t^{0.5}$: (▲) 1*M* PEG 6000.

Similar studies[23,24] made on hydrogel prepared from PEG, methylene diphenyl diisocyanate (MDI), and 1,2,6-hexane triol (transparent on swelling) yielded a straight-line relationship between $Qt/Q\alpha$ vs. $t^{0.5}$ that varied from 0.5 to 0.6. The straight-line relationship in our polymeric system seems to be occurring only due to the phase separation of these hydrogels. The total release vs. time and the incremental rate of release vs. time show that the release rates are almost uniform after 2–3 h of the initial release. The above figures and the release data indicate that the variation in the degree of cross-linking is a practical method to control the diffusivity of a drug.

The release profile of $^3$H-estradiol for 0.75*M*, 1*M*, 2*M*, 3*M*, and 4*M* PEG 6000 show that $t^{0.5}$ is 10 h for 0.75*M* PEG 6000, 11 h for 1*M* PEG 6000, 13 h for 2*M* PEG 6000, 15.5 h for 3*M* PEG 6000, and 16 h for 4*M* PEG 6000. Figure 4 indicates the effect of the cross-linking agent on the equilibrium swelling uptake of water. The concentration of the polymer reduces the water content due to the chain entanglements. In Figure 5, the log $Qt/Q\alpha$ vs. log time shows a straight-line relationship that indicates the

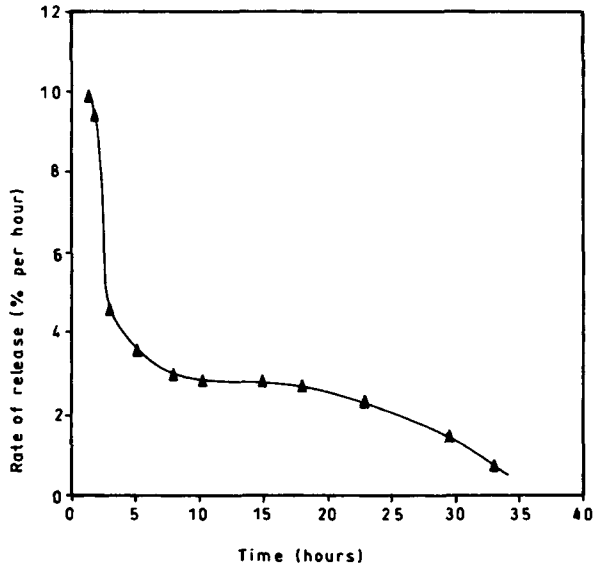


**Figure 2** Rate of release vs. time: (▲) 1*M* PEG 6000.

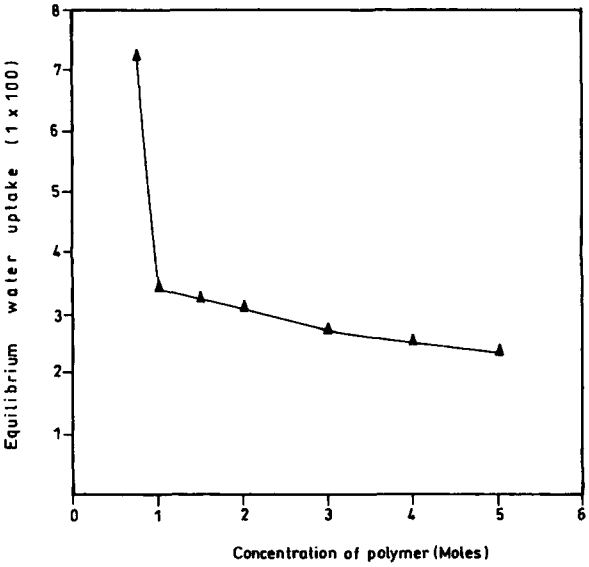


**Figure 4** Equilibrium water uptake vs. concentration of polymer: (▲) hydrogels from PEG 6000.

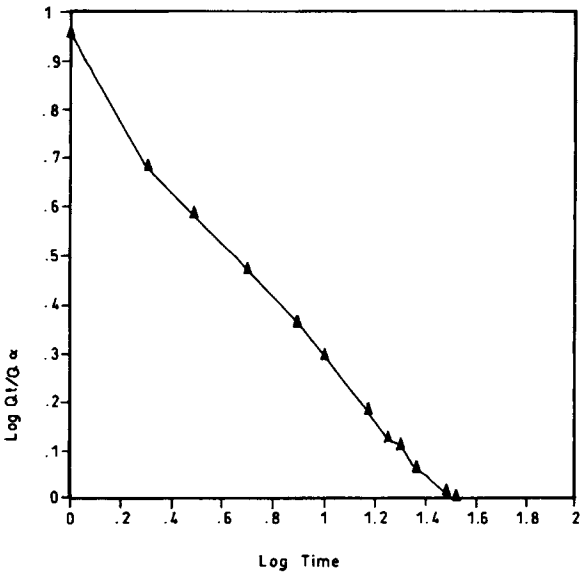


**Figure 5** Log $Qt/Q\alpha$ vs. log time: (▲) hydrogels from PEG 6000.

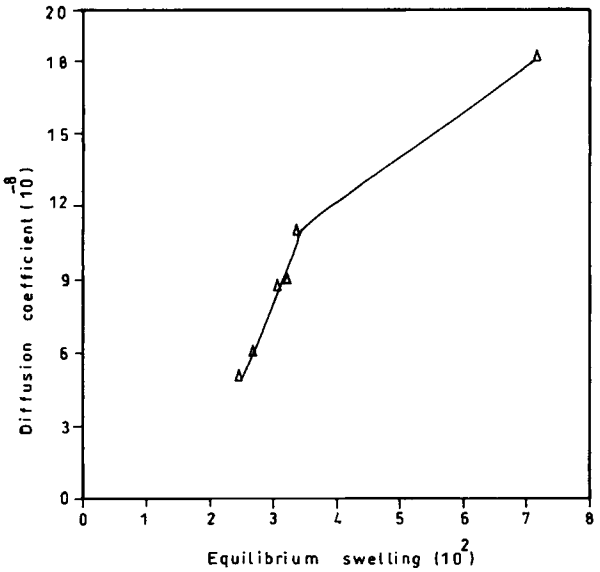


**Figure 6** Diffusion coefficient vs. equilibrium swelling: (△) hydrogels from PEG 6000.

effectness of the cross-linking agent in this polymer system. These data clearly demonstrate the effect of the proportion of the cross-linking agent and the related equilibrium swelling of the hydrogels on the diffusion coefficient ($D$) (Table I), which normally follows the well-described release equation[25]:

$$Qt/Q\alpha = 4(Dt/r^2)^{0.5}$$

where $Qt$ is the fraction of drug released at time ($t$); $Q\alpha$, the amount of drug released at $t\alpha$; $D$, the apparent diffusion coefficient of the drug through the swollen polymer; and $r$, the half-thickness or radius of a cylinder. The equilibrium swelling of the hydrogels indicates that the diffusion coefficient is increased as the entanglement of the polymer system is decreased (Fig. 6). As we expected, the $D$ values decreased with increase in the molar ratio of the polymer (Fig. 7). Cross-linking reduces the mobility of polymer chains and, consequently, the diffusion of any active species through the networks would be difficult.

Unlike the first hour during which the release of $^3$H-estradiol is delayed due to the slow dispersion of the steroid from the ethanol/water system in the gel to the surrounding water, in the second hour, the rapid release of the order of 6% is apparently attributed to an increase as the water content of the gel increases. These observations also suggests that the amount of water present in the hydrogel may have a significant effect on the release profile. As pointed out earlier in the swelling experiment and by other research workers,[12,21] there are two types of water present in these networks, namely, bound and free water. Graham et al.[10] showed that the bound water in PEO gel is strongly associated with the polymer in the form of a stable trihydrate complex. The free water has been shown to be similar to bulk water in an aqueous solution.

**Table I Diffusion Coefficient of Fully Swollen Hydrogels**

| Composition of Polymer | $t$ (s) ($\times 10^{-4}$) | $Qt/Q\alpha$ | Radius of Cylinder (cm) | Diffusion Coefficient $Mt/M\alpha = 4\ (Dt/r^2)^{0.5}$ ($\times 10^{-8}$) |
|---|---|---|---|---|
| 0.75$M$ PEG 6000 | 3.60 | 0.52 | 0.350 | 18.05 |
| 1$M$ PEG 6000 | 3.90 | 0.54 | 0.275 | 10.92 |
| 1.5$M$ PEG 6000 | 4.50 | 0.55 | 0.250 | 8.24 |
| 2$M$ PEG 6000 | 4.70 | 0.57 | 0.250 | 8.51 |
| 3$M$ PEG 6000 | 5.60 | 0.58 | 0.225 | 5.98 |
| 4$M$ PEG 6000 | 5.80 | 0.60 | 0.200 | 4.90 |

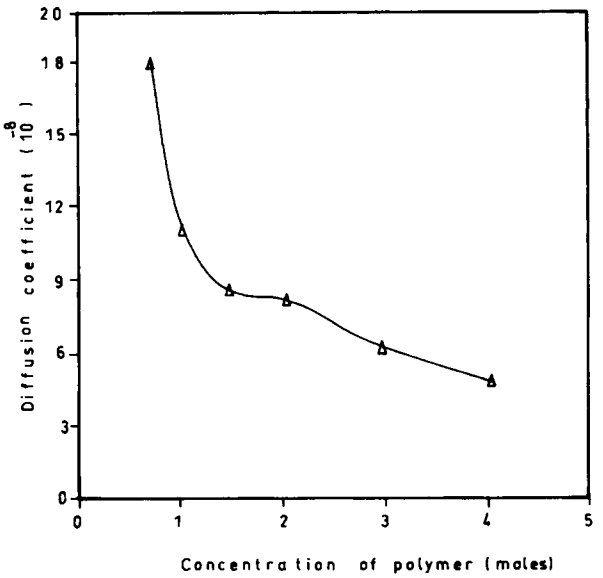


**Figure 7** Diffusion coefficient vs. concentration of polymer: (△) hydrogels from PEG 6000.

## CONCLUSION

Hydrogels find extensive biomedical applications combining the properties of hydrophilic and cross-linked materials. Polymer networks were prepared from PEG 6000 cross-linked by 1,1,1-tris(hydroxy methyl)ethane and the stoichiometric equivalence of hexamethylene diisocyanate as a coreactant to form infinite urethane-linked networks. By varying the amount of cross-linking agent, the degree of swelling and the release studies were examined. A rapid procedure was developed for the study of *in vitro* release characteristics of radio-labeled drugs in a sustained delivery system. The scintillation spectroscopy was used for simultaneous determination of percent release of each labeled drug from the single sustained action hydrogel. Polymer in the form of a fully swollen drug containing a cylinder provides a monolithic delayed-release device that normally follows the constant rate of diffusion. The relationship among the diffusion coefficient, swelling, and the drug-release studies shows the uniform behavior of the polymer networks in this study.

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