Polyurethane Networks Based on Poly(ethylene oxide)

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SYNOPSIS

A wide range of infinite urethane polymer networks were prepared from poly(ethylene glycol) (PEG) and hexamethylene diisocyanate (HMDI) using 1,1,1-tris-(hydroxymethyl)ethane (THME) as the cross-linking agent. The influence of temperature, cross-linking, and crystallinity on the swelling character of the hydrogel has been discussed. The toxicity of the network polymer by intravaginal implants in rats was studied. Implantation of the polymer did not result in alteration in behavior and feed intake or any pathological changes in the tissue. Vaginal fluids from the polymer-implanted rats or the polymer extract when inoculated on a listeria monocytogene culture plate were unable to inhibit the bacterial growth. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

There is a substantial commercial interest in synthetic hydrogels of poly(ethylene oxide) (PEO).[1] These have been shown to be quite promising materials, particularly for artificial organs and other biomedical application.[2-6] Recently, the hydrogels based on PEO have been used as vaginal pessaries for the target of prostaglandin E_2 (PGE2) delivery as well as rectal pessaries for the delivery of morphine hydrochloride.[7] These hydrogels have been shown to absorb large amounts of water, depending on the extent of cross-linking and temperature. This behavior of the polymer has been reported to be very useful in the control of drug released from the network.[8,9] In the above preparation, the networks were obtained either by radiation cross-linking or through urethane bond formation using methylene diphenyl diisocyanate or methylene dihexyl diisocyanate as coreactant. The aromatic diisocyanates are very reactive and do not allow enough time to fill the molds. To retard the rate of the reaction, materials like benzyl chloride were added.[9] On the other hand, for methylene dihexyl diisocyanate, ferric chloride was used as a catalyst to improve the rate of reaction. These problems impose difficulty in obtaining reproducible materials.

The present study explored the possibility of using hexamethylene diisocyanate (HMDI) as coreactant for poly(ethylene glycol) (PEG) to obtain reproducible hydrogels. The toxicology of the materials is also examined.

EXPERIMENTAL

The hydrogel system consists of poly(ether urethane) based on HMDI, PEG, and 1,1,1-tris(hydroxylmethyl)ethane (THME) as a cross-linking agent. The expected structure of the network is shown in Figure 1 (with the elimination of minor processes of allophanate and biuret groups). The notation x and y in Figure 1 represents the number of repeat unit in PEG and the network, respectively.

The chemistry of the system allowed the ratio of triol to PEG to be varied within fairly wide limits for obtaining a range of compositions. The following nomenclature was used for identifying the various structural elements in a given polymer. The first number followed by letter "M" represents the ratio of the number of moles of THME to the number of moles of diol (PEG 6000, a commercial code for the poly(ethylene glycol) and is identified with x in the formula. Thus, 1M-PEG 6000 is the hydrogel based on poly(ethylene glycol) 6000 and HMDI cross-linked with 1 mol (equivalent to the actually measured $\overline{M_n}$ of PEG 6000) of 1,1,1-tris (hydroxylmethyl)ethane. The term PEG is used for

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the starting material containing terminal hydrogels, whereas PEO is used when the hydroxyl group has been reacted and the unit is an integral part of polymeric network. In the proceeding sections, the dry network is referred to as xerogel, whereas the swollen polymer, as hydrogel.

Purification of Material

1,6-Hexamethylene Diisocyanate (E. Merck)

It was used as supplied by the manufacturer.

Poly(ethylene glycol) (BDH)

The molten PEG was filtered through an activated charcoal column at 80°C to remove the residual catalyst. It was dried at 100°C under reduced pressure, while bleeding a slow stream of dry nitrogen at regular intervals for 4 h. The hydroxyl and acid numbers were determined by standard methods[10,11] and the $\overline{M_n}$ of PEG 6000 was 8850. The above value of $\overline{M_n}$ was used to calculate the molar equivalent of diisocyanate and the cross-linker (THME) supplied by BDH.

1,1,1-Tris(hydroxymethyl)ethane (BDH)

The cross-linking agent was dried under vacuum at 80°C for 8 h and stored in a desiccator.

Formulation

Various molar compositions of the triol were used to obtain xerogels with different extents of cross-linking. A typical formulation is described below:

THME (0.6785 g) was added to 1 molar equivalent of PEG 6000 (50 g) in a beaker and maintained at 85°C. Molten HMDI (4.0721 g) was poured into the mixture of glycols and vigorously stirred for 1 min, and the homogeneous mixture was transferred into a preheated polypropylene tubing and cured in an oven at 85°C for 4 h. The cooled tubing mold was stored over anhydrous calcium chloride in a desiccator.

Purification of Hydrogels

The polypropylene tubes containing hydrogels were cut into pieces of 1.5 cm. The cylindrical xerogel samples were placed in distilled water for 24 h followed by boiling in fresh distilled water to remove any unreacted and extractable materials. The extraction was monitored by a UV spectrophotometer. The swollen hydrogel was dried in air for 4 h and then under vacuum at 60°C to a constant weight. It was stored in a desicator for subsequent use.

Procedure

Swelling experiments were carried out to estimate the water uptake of xerogels. An accurately weighed

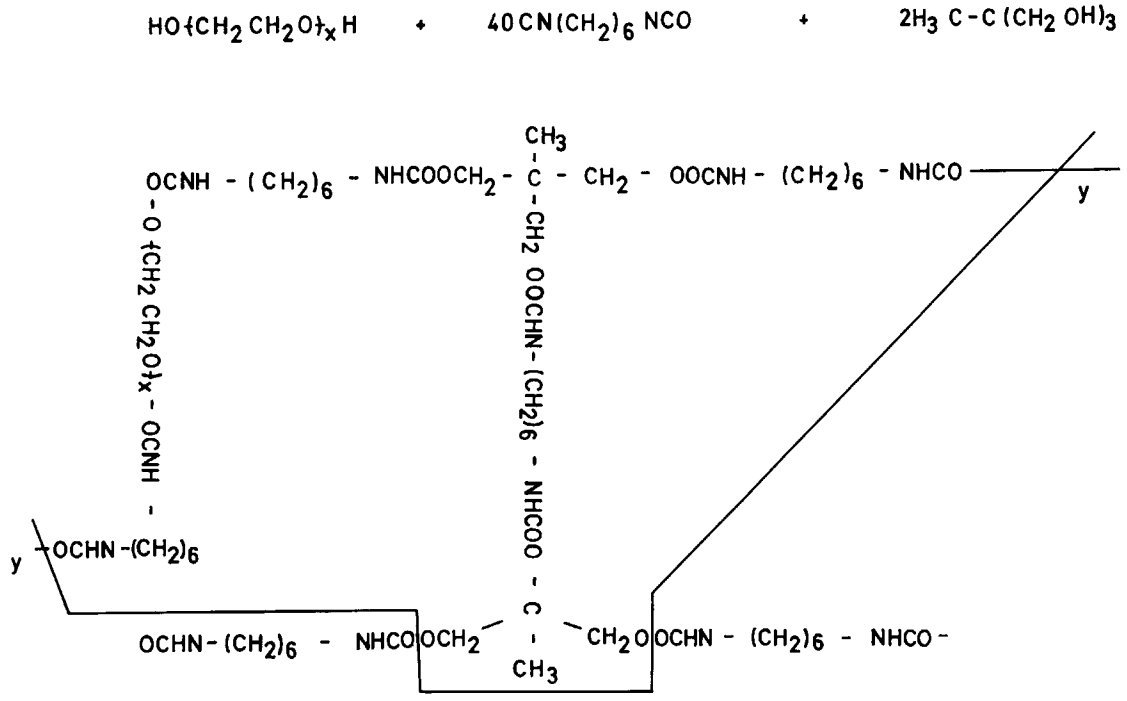


Figure 1 Representation of hydrogel network formation when PEG and THME react with a stoichiometric amount of HMDI.

xerogel was placed in a bottle containing a large excess of double-distilled water at a desired temperature in a thermomix and allowed to swell. At fixed intervals, the sample was removed from the bottle, water-wiped off with tissue, and immediately reweighed in a stoppered weighing bottle. The water uptake in terms of parts per hundred (pph) of the initial dry polymer was evaluated by the following relationship:

$$\text{Swelling or water uptake (pph)} = (W_s - W_d/W_d) \times 100$$

where W_s is weight in grams of the hydrogel sample and W_d is weight in grams of the xerogel sample.

The heat of fusion (ΔH_f) was obtained using a DuPont 990 thermal analyzer with a DuPont 910 differential scanning calorimeter (DSC) cell. The heat of fusion was calculated using the procedure described elsewhere for similar materials.[8] The DSC instrument was calibrated with pure indium metal (ΔH = 24.8 J g^{-1}) taken as a standard.[12] The heat of reaction ΔH value[13] of 100% crystalline PEG was taken as 9.24 J/g to calculate fractional crystallinity. The percentage crystallinity was calculated using the weight of the crystalline fraction incorporated into the polymer test sample. One endotherm for each composition was obtained and the area of the melting endotherm was measured in triplicate using a planimeter and the results averaged. Heats of fusion were calculated using the following relation:

$$\Delta H_f = [A \cdot B \cdot E \cdot S \times 60/\text{m}]\,\text{J g}^{-1}$$

where A is the endotherm area (cm^2); B, the recorder time base (min cm^{-1}), and S, its sensitivity of trace (mV cm^{-1}); E, the calibration coefficient of DSC cell (mW mV^{-1}); and m, the weight of PEG in the hydrogel sample (mg). The degree of crystallinity (%) is given by the following equation:

$$\text{Crystallinity \%} = [\Delta H_f/\Delta H_{\text{std}}] \times 100$$

where ΔH_{std} is the heat of fusion of 100% crystalline PEG.

The sterilized xerogels were subjected to toxicological tests. However, for comparison purposes, the extracted material from the hydrogel was also implanted in the rats to examine its toxicity. Six adult (9–12-month-old, weight range 190–250 g) female Sprague Dawley rats were implanted intravaginally with the test polymer, whereas a similar number of rats of same age and weights were implanted with pieces of Teflon tubing.

The rats selected for treated and control animals were found to be of almost similar body weight. The polymeric implants and the Teflon pessaries were of 3–4 × 1 mm in size and 0.11–0.17 g in weight. Each test animal was kept in a separate cage in light for a period of 18 h and 6 h in the dark. Rat chow and water was provided to both the treated and control animals. Changes in behavior and basal body temperature were observed at 6 h intervals for a period of 24 h. Fifty microliters of 0.9% sterilized sodium chloride solution was introduced into the vagina by a pipette and sucked back as vaginal fluid after an interval of 6 h. Behavioral changes like posture, gait, feeding, watering, urination, and defecation were observed and recorded. The vaginal fluid samples were deposited on selected points on tryptose agar plates, cultured with listeria monocytogene pure culture, incubated at 37°C for 24 h, and examined for growth inhibition and adverse effect on colony formation and cell morphology. After 2 days *in situ* retention of the polymer and Teflon pessaries, all the animals were killed by cervical dislocation and were observed for necropsical changes. Vaginal tissues from treated and control rats were dissected and fixed in Bouin's fixative, dehydrated in propanol, and embedded in paraffin. Sections were cut at 6 μm thickness and slides of vaginal tissue were stained with Harris's hematoxyline. The extract (2 mg) was dissolved in (4 mL) normal saline to examine specifically the toxicity of extract from the sterilized polymer. A 100 μL aliquot of this solution was injected, subcutaneously, into three female rats. Another three animals were inoculated with normal saline to serve as a control. Behavioral changes like posture, gait, feeding, watering urination and defecation, body temperature, and feed consumption were noted for a period of 16 h. The dissolved polymer extract was also inoculated into the broth culture tubes of listeria monocytogenes. Physiological saline was inoculated in separate culture tubes containing nutrient broth to serve as a control. All the inoculated plates were incubated at 37°C for 24 h and results were recorded.

RESULTS AND DISCUSSION

The hydrogels based on PEO are a prospective material for drug-delivery systems. Therefore, we have examined their crystallinity, swelling, and toxicology.

Crystallinity

Figure 2 shows the dependence of crystallinity on the molar ratio of THME to PEG for the polyure-

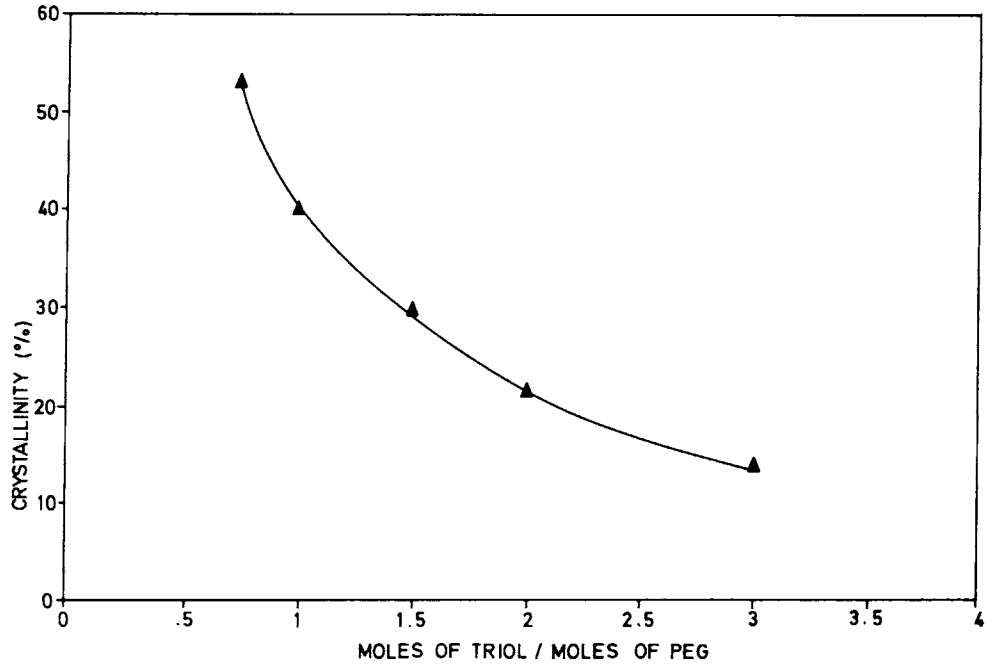


Figure 2 Crystallinity vs. triol concentration: (▲) hydrogels from PEG 6000.

thane xerogels. It is obvious from the figure that a reduction in fractional crystallinity of xerogels occurs as concentration of the triol is increased. This decrease may be due to chain entanglement with increasing concentrations of cross-linker in the network. The entanglement lowered the degree of freedom, thus making the formation of ordered molecules relatively difficult. The decrease in crystallinity can also be attributed to the structural disruption occurring, possibly, because of the formation of microdomains. However, the system appears to be complex and it is difficult to quantify the effect of individual parameters like cross-linking and microdomain effects. The hydrogel based on poly(ethylene

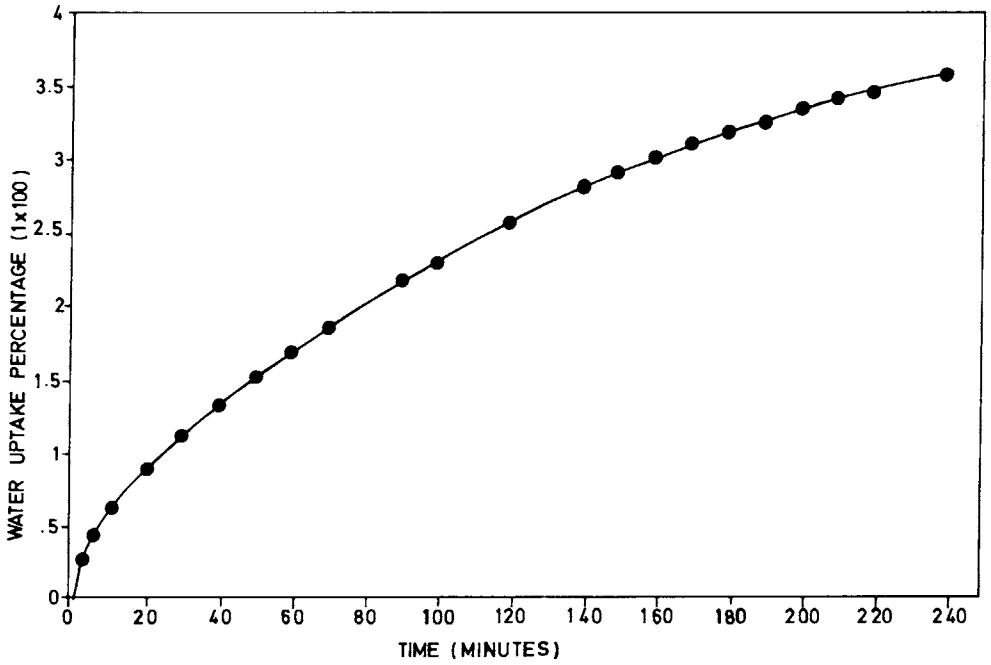


Figure 3 Swelling of hydrogel in water at 30°C: (●) 1M-PEG 6000.

oxide) and methylene diphenyl diisocyanate have been reported[9] to yield crystalline material, e.g., an analogous 1M-PEG 6000 is reported to be 28% crystalline, whereas the corresponding 1M-PEG 6000 in the present study is 49% crystalline. This shows that the networks prepared from PEG and HMDI are more crystalline compared to those obtained from PEG and methylene diphenyl diisocyanate (MDI). HMDI is an alliphatic diisocyanate and contains methylene groups similar to PEG. The presence of methylene groups in the entire network enhances the possibility of regular arrangement of molecules as compared to the noncompatibility of phenyl and methylene groups in the case of hydrogel based on PEG and MDI.[14]

Swelling Isotherm

A typical swelling isotherm of 1.0M-PEG 6000 at 30°C is presented in Figure 3. It indicates that the swelling of the polymer starts as soon as it comes into contact with water and the absorption reaches to a maximum value of 341% after 220 min. However, the swelling rate plot (Fig. 4) indicates that initial water uptake decreases after 40 min and then a steady rate of water diffusion is achieved up to 140 min. After this, the swelling rate again starts decreasing. This behavior can only occur if the initial water uptake is on the free surface of the gel. This follows a gradual diffusion of water into the polymer matrices.[15] After the maximum penetration of water into the matrices, its uptake slows down. Similar suggestions have been made for water-swellable polymers.[8] In the present studies, it was further determined that the equilibrium water uptake (pph) of 0.75M-PEG 6000 was 720%; of 1M-PEG 6000, 341%; of 1.5M-PEG 6000, 325%; of 2M-PEG 6000, 311%; of 3M-PEG 6000, 272%; of 4M-PEG 6000, 252%; and of 5M-PEG 6000, 235%. This indicate that equilibrium water uptake decreases as the chain length of PEO is decreased between the cross-links. The xerogel made from PEO has been shown[9,16] to behave in a fashion similar to our study, whereas other hydrophillic polymers made from acrylamide,[17,18] cellulose,[19-21] HEMA,[22] poly(vinyl alcohol),[23] poly(ethylene terephthalate),[24] and nylon[25] exhibit a reverse behavior.

This typical hydration process in PEO hydrogel has been explained on the basis[8] that, in the dry state, regions of chain-extended crystalline and amorphous PEO exist, held together by the cross-linker. When water penetrates into the network, the hydrophobic domain (THME + HMDI) is pushed apart but still provides the matrix with a structural integrity. The crystalline and amorphous PEO chains become hydrated and stretched, but, restrained by the cross-links, retain some flexible yet ordered arrangement, creating a "pore" structure through which water can penetrate freely. The PEO chains are believed to contain water ordered into a

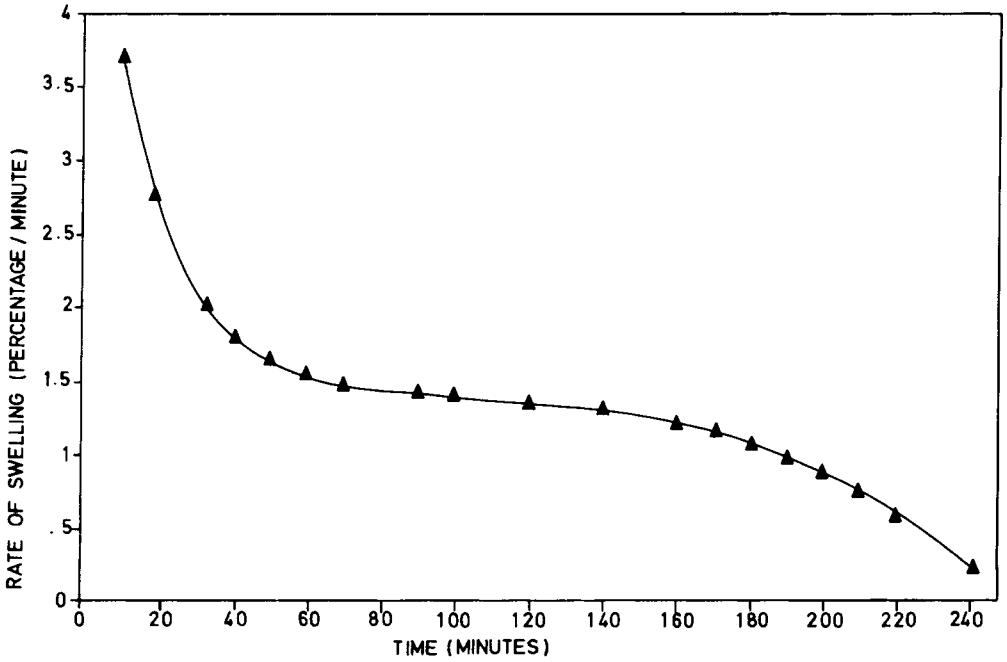


Figure 4 Rate of swelling in water at 30°C: (▲) 1M-PEG 6000.

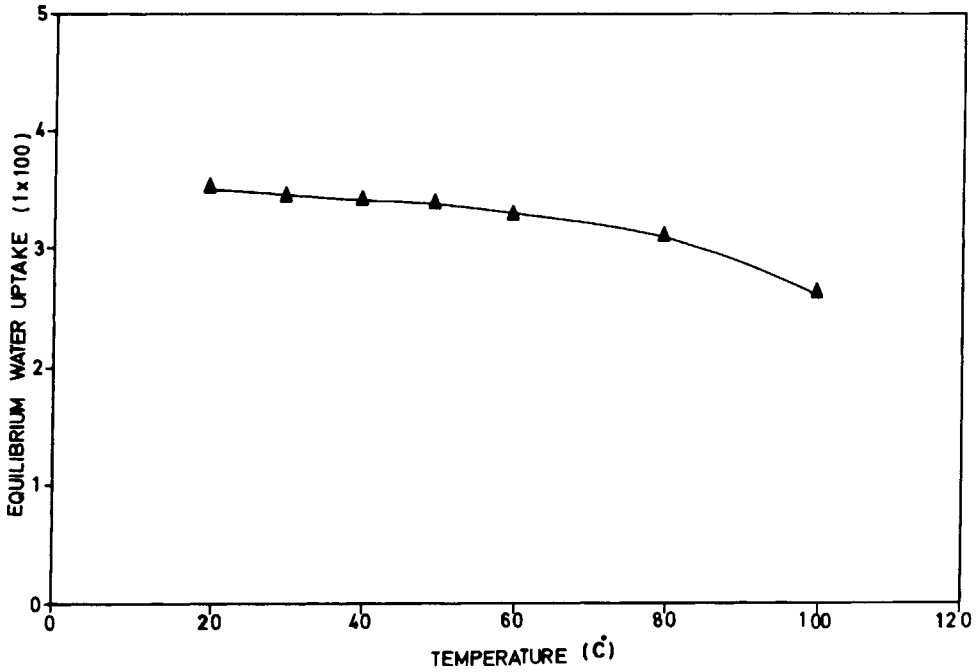


Figure 5 Effect of temperature on equilibrium swelling: (▲) 1M-PEG 6000.

specific trihydrate as well as free water in between.[8] This swelling phenomenon was further studied and experiments at various temperatures showed that the maximum uptake of water by the hydrogels 1M-PEG 6000 decreased with an increase of temperature, as depicted in Figure 5. It is also obvious from this figure that the xerogel of 1M-PEG 6000 equilibrates with water in 326 min at 30°C, whereas the same material required only 214 min at 100°C. This indicates a faster rate of swelling as a function of temperature. In other words, at higher temperatures, the crystalline polymer becomes amorphous, allowing a faster rate of penetration of water.[26]

Figure 6 indicates that as the temperature is increased the maximum water uptake is reduced. This implies that the water imbibed by the polymer may have various forms. Thus, at higher temperature, free water is occluded from the matrix, whereas the bound water is retained by the PEO chain. The presence of free and bonded water in PEO gels has been reported earlier.[8,27]

Toxicology

Twenty-four hour intravaginal implantation of the test polymer or the Teflon (control) pessaries did not affect the normal behavior of the rats. Posture and alertness was normal both in the treated and the control rats. No lordosis or difficulty in walking was observed and the gait was normal in both groups. During 24 h observation, per animal feed consumption in the treated and control rats was 16.5 ± 2.0 g and 14.2 ± 2.0 g, respectively. Rectal temperature of the treated and control animals remained in the normal physiological range (37.8–39.2°C). Mean body temperature in the treated animals varied from 37.8 ± 0.1 to 38.0 ± 0.1°C during the observation period. In the control animals, mean body temperature varied from 37.9 ± 0.1 to 38 ± 0.3°C. No febrile reaction was observed in any of the individual rats (Table I).

Necropsical examination after the 24 h observation did not reveal any pathological lesions on the viscera of the treated or the control rats. No inflammation of the kidney or the liver was observed in any rat. A variable degree of local hyperanemia at the vaginal orifice was present in some of the control and treated rats and no discharge was observed. The morphological appearance of vaginal mucosal from the polymer implanted and the control rats is shown in Figure 7. Figure 7(a) shows the experimental section through the mucosa of the vagina ×100. Stratified squamous epithelium is vague. At the basal layer of the epithelium, some papillae are also visible. Lamina propria is loosely packed with connective tissue and no leukocytes or polymorphonuclear cells are present in the propria. Figure 7(b) indicates the experimental section through the mucosa of the vagina ×100. Stratified squamous epithelium is well developed. Cells are partly keratinized. Transverse

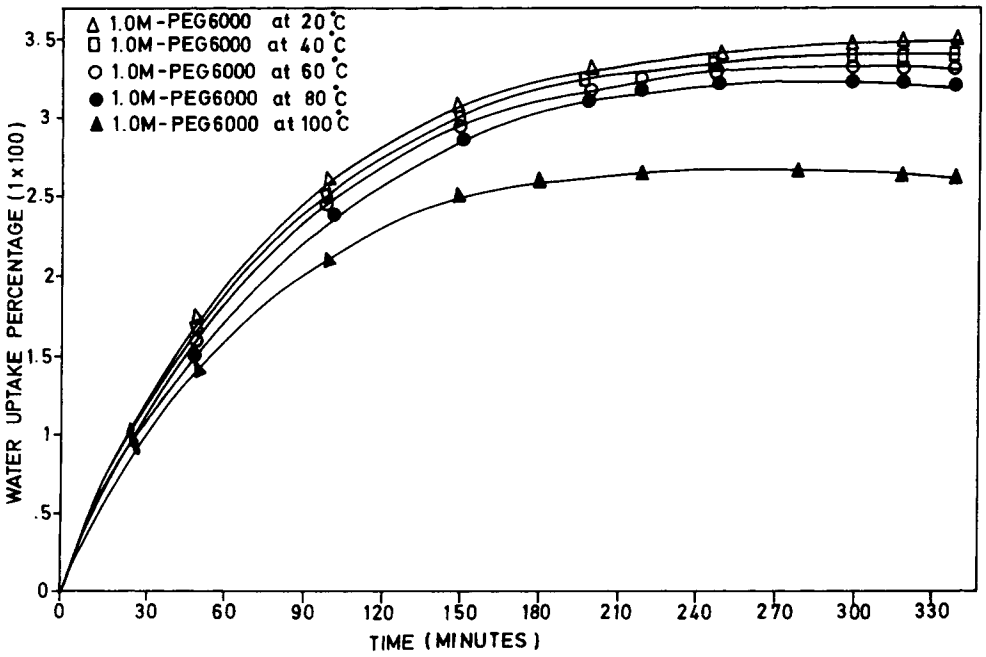


Figure 6 Water uptake vs. time of 1M-PEG 6000 at various temperatures: (△) 20°C; (□) 40°C; (○) 60°C; (●) 80°C; (▲) 100°C.

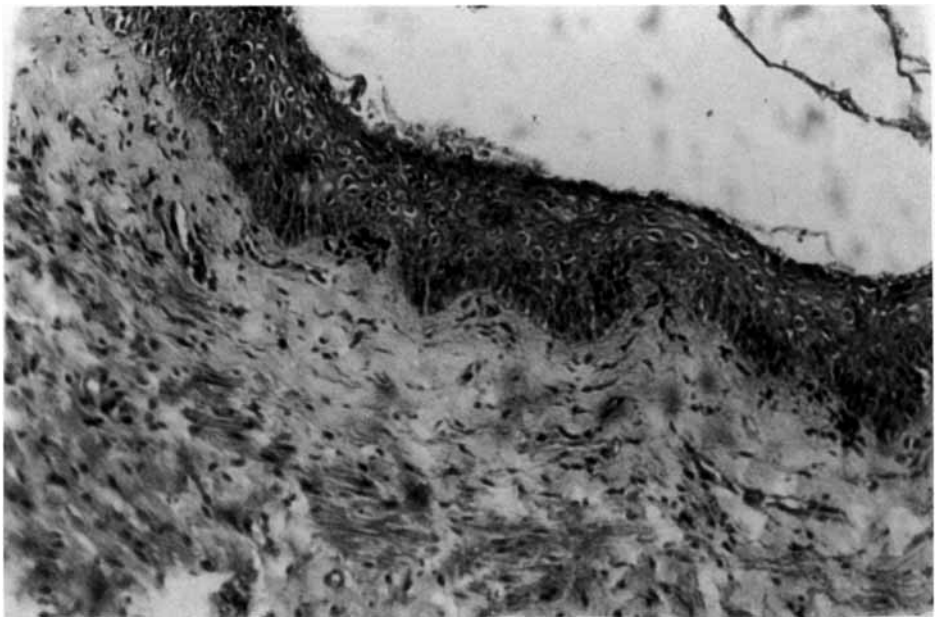
folds are vague and less marked. Lamina propria is loose. Circular and transverse smooth muscle fibers are present beneath the propria. In both the cases, no degenerative or inflammatory changes are discernible.

Treatment with polymer extract did not result in significant alteration in the behavior of the rats. The animals remained active and alert and no lethargy was observed during the observation period. Feed consumption was 9.3 and 8.3 g in the treated and

Table I Data of Feed Consumption and Body Temperature of Rats

Animal	Body Weight (g)	Feed Consumed (g)	Body Temperature (°C) Time (h) 1200	1800	2400	600	1200
Experimental							
E1	250	20	37.9	37.8	37.7	37.8	37.8
E2	250	22	36.7	34.7	37.8	36.8	37.8
E3	230	20	38.2	34.7	37.8	37.8	38.0
E4	190	12	37.8	37.2	37.2	38.9	38.0
E5	210	15	37.8	37.5	36.9	38.4	38.0
E6	200	10	37.8	37.5	36.7	38.0	38.0
Control							
C1	190	15	38.0	37.8	37.9	37.3	37.8
C2	190	10	37.2	37.8	37.9	36.7	38.0
C3	270	10	34.7	38.4	37.9	37.2	38.0
C4	210	20	37.8	34.7	37.3	37.0	38.0
C5	240	10	37.8	37.5	37.2	36.8	38.3
C6	180	20	37.2	37.2	37.2	36.8	37.8

(a)



(b)

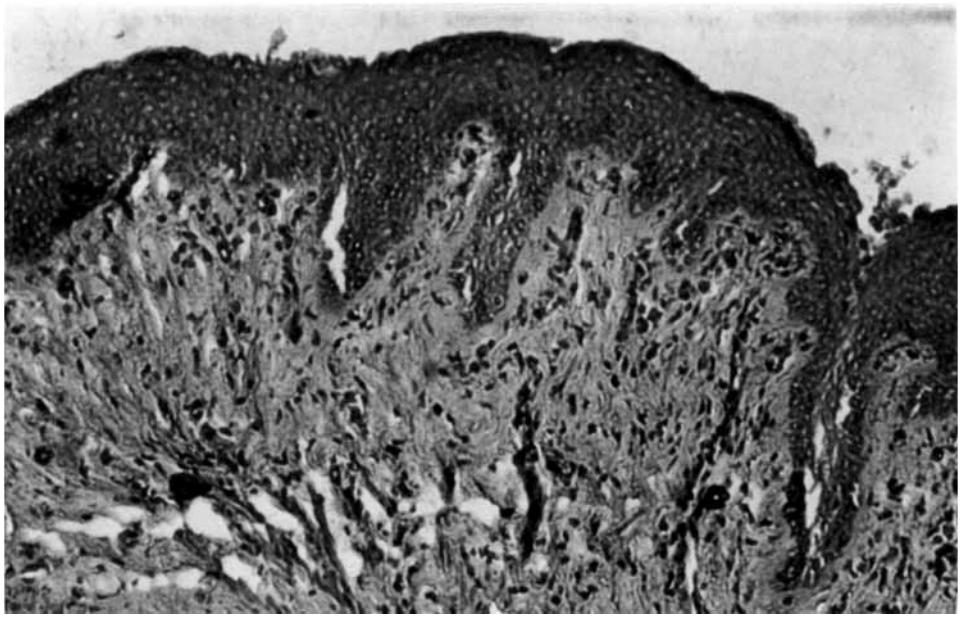


Figure 7 (a) Morphological appearance (×100) of vaginal mucosal from polymer implanted rat. (b) Morphological appearance (×100) of vaginal mucosal from polymer control rat.

control rats, respectively. No local tissue reaction at the site of injection was observed in any of the animals. No febrile reaction resulted and the change in the basal body temperature of treated and control animals was in normal limits. Inoculation of the polymer extract or the normal saline in the listeria monocytogene culture plates did not result in bacterial inhibition. Details of feed consumption and body temperature of treated and control rats are given in Table I.

CONCLUSION

A wide range of hydrogels with a greater degree of formulation control can be prepared from PEG and HMDI. The network prepared from aliphatic, consequently, is more crystalline and, hence, stronger than the polymer prepared from aromatic diisocyanate and PEG. The hydrogels with varying swelling characteristics can be prepared by altering the composition of the network. The swelling phe-

nomenon is influenced both by the extent of crosslinking and the temperature. The water-sterilized hydrogels were found to be nontoxic to rats. Even the low molecular weight extract of sterilized hydrogel did not show any abnormality when implanted in rats.

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