Equilibrium Swelling Behavior of Collagen-Poly(HEMA) Copolymeric Hydrogels

INTRODUCTION

Hydrogels are a broad class of polymeric materials that are capable of imbibing large quantities of water without dissolution. These are among the best materials for a number of biomedical applications.[1-5] The ease of fabrication of hydrogels to various geometrical forms accounts for their wide range of applications. Hydrophilic gels display good tissue compatibility, nontoxicity and nonthrombogenicity. The particular suitability of hydrogel as biomaterial stems from the similarity of their physical properties to those of living tissues. This resemblance is based on their high water content, soft and rubbery consistency, and low interfacial tension. The high water content of the hydrogel allows the extraction of undesirable reaction byproducts prior to implantation and the easy penetration of small molecules such as water, electrolytes and metabolites into them *in vivo*. Hydrogels have been employed as drug delivery systems because of their biocompatibility, their ability to release entrapped drug in aqueous medium, and the ease of regulating such drug release by controlling water swelling and crosslink density. We have prepared and characterized collagen-poly(HEMA) hydrogels for the controlled release of anticancer drugs.[6] Equilibrium swelling measurements are of great importance in using hydrogels as drug delivery systems since there is a possible correlation between the rate of drug release and water swelling property of hydrogels. This paper discusses the results of the equilibrium swelling behavior of collagen-poly(HEMA) hydrogels with respect to hydrogel composition, pH, and solutes.

EXPERIMENTAL

Materials

Collagen. A ca. 200-day-old calf fetus was obtained from a local slaughter house, preserved in ice, and stored in a deep freezer until extraction of soluble collagen.

Chemicals. 2-Hydroxyethyl methacrylate (Fluka), sodium metabisulfite, and ammonium persulfate (Glindia) were used as obtained. The anticancer drugs used were bleomycin (Nippon Kayaku Co. Ltd., Japan), mitomycin C (Kyowa Hakko Kogyo Co. Ltd., Japan), and 5-fluorouracil (Sisco Research Laboratory, India).

All other chemicals used were of analytical reagent grade.

Methods

Preparation of Collagen-Poly(HEMA) Copolymeric Hydrogels. Soluble collagen was extracted from fetal calf skin by slight modification of standard method.[6] Hydrogels were prepared using 2% collagen solution in 1 m*M* HCl. In all preparations 2 mL of 2% collagen solution were used with varying concentration of hydroxyethyl methacrylate (HEMA) monomer (1 : 100, 1 : 50, 1 : 20, 1 : 15, and 1 : 10 collagen : HEMA (w/w). The detailed procedure for the preparation of hydrogel is reported elsewhere.[6] In brief, appropriate quantities of HEMA and ethylene glycol were added to the collagen solution. This was followed by the addition of ammonium persulfate and sodium metabisulfite. The contents were thoroughly mixed and incubated at 37°C for 3 h. The resulting smooth, cylindrical opaque hydrogels were then exhaustively washed with Tris-HCl buffer, pH 7.0 followed by acetone and water and stored at 4°C until further use. The anticancer drugs bleomycin (BLM), mitomycin C (MMC) and 5-fluorouracil (5-FU) were entrapped in the hydrogel matrix by adding the drug to the collagen solution before the addition of HEMA and mixing thoroughly. All other reagents were added exactly in the same fashion as in the preparation of placebo hydrogel. An outline of the steps involved in the preparation of collagen-poly(HEMA) hydrogels is given in Scheme 1:

Preparation of Poly(HEMA). Poly(HEMA) homopolymer was prepared exactly like that of collagen-poly(HEMA) hydrogel except that water was substituted for collagen. The monomer HEMA with all the other reagents were mixed well and incubated for 3 h at 37°C. The resulting smooth, cylindrical, opaque gels were then exhaustively washed with Tris-HCl buffer, pH 7.0 to remove residual monomer and ethylene glycol. The gels were then washed with water and stored at 4°C until use.

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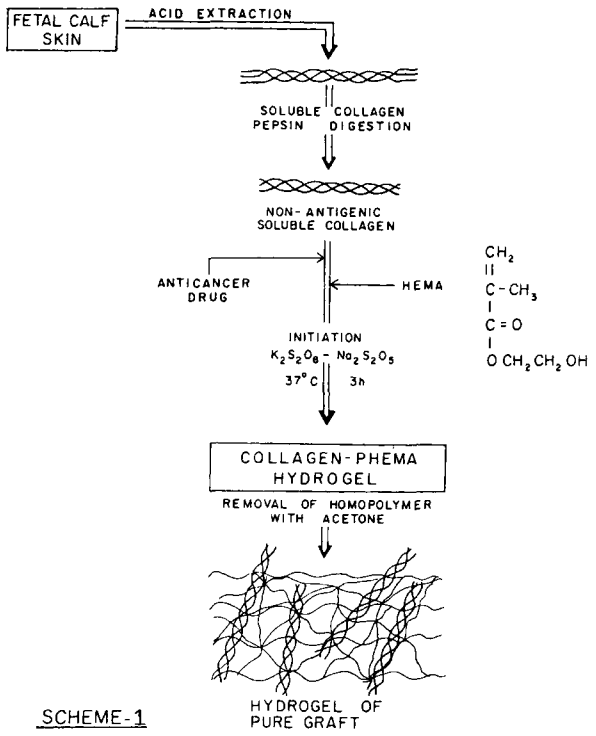

Scheme 1 Preparation of collagen–PHEMA hydrogels.

Equilibrium Swelling Studies. The water sorption capacity of the collagen–poly(HEMA) hydrogels was determined by swelling the hydrogel pellets in distilled water at room temperature (34 ± 1°C) until equilibrium was attained. The influence of pH on the swelling behavior of the hydrogels was determined by placing the hydrogel pellets in buffers of different pHs ranging from 2.0–9.0 at 37°C until equilibrium was attained.

Buffers used for the swelling studies were:

pH 2.0–0.012*N* hydrochloric acid

pH 4.0–0.05*M* potassium hydrogen phthalate

pH 7.4–0.01*M* phosphate buffer

pH 9.0–0.01*M* sodium tetraborate

Samples of bulk gel for determination of equilibrium water content or swollen volume were made by cutting pellets from a rod of hydrogel. The swollen weight of the pellet was determined by first blotting the pellet with filter paper to remove adsorbed water and weighed immediately on an electronic balance. The weights of the swollen pellets were recorded every 24 h until there was no further increase in weight (i.e.) when the gel had attained equilibrium.

Percent swelling of collagen–poly(HEMA) hydrogels at equilibrium was then calculated from the formula

$$E_{sw} = \frac{W_e - W_0}{W_0} \times 100$$

where E_{sw} is the percent swelling of hydrogel at equilibrium, W_e the weight of the hydrogel pellet at equilibrium swelling, and W_0 the initial weight of the hydrogel pellet.

RESULTS AND DISCUSSION

Water-sorption capacity of hydrogels is an important property for their biomedical applications particularly as drug delivery systems. Release of drugs by swelling-controlled mechanism takes place by diffusion of the solute/drug through an originally glassy polymer by countercurrent diffusion of water or biological fluid. The results of the equilibrium swelling behavior of collagen–poly(HEMA) hydrogels of different composition in water and at different pHs are discussed below. The effect of entrapped anticancer drugs on the swelling behavior of the hydrogels has also been carried out and the results are discussed at the end.

Effect of Hydrogel Composition on the Equilibrium Swelling Behavior of Collagen–Poly(HEMA) Hydrogels

The equilibrium swelling measurements in water for different hydrogel compositions are given in Figure 1. Homogeneous poly(HEMA) hydrogel swells in water to a maximum of 40% at equilibrium[7] independently of the way it was prepared. In the present study it was observed that poly(HEMA) was swollen in water to a maximum degree of 44% at equilibrium. However, poly(HEMA) hydrogel swells beyond its equilibrium in water when certain solutes interfere with the hydrophobic bonds. The same trend was also observed when composites of poly(HEMA) with a natural macromolecule like collagen was swollen in water. Collagen influences the swelling behavior of collagen–poly(HEMA) hydrogels as indicated clearly in Figure 1. The results showed that collagen values as low as 1% provided sufficient internal structural changes to allow great changes in volume to occur at the pH of distilled water. The equilibrium swelling values changed from 40% to as high as 275% with the change in the composition of collagen–poly(HEMA) hydrogels. The increasing trend with the swelling values was observed with decreasing concentrations of collagen in the hydrogels. At about 1% collagen concentration, the hydrogels showed maximum equilibrium swelling value of about 275%. On the other hand, pure poly(HEMA) without any collagen showed only 44% equilibrium swelling value. This appeared to be a very interesting observation since a high molecular weight natural macromolecule like collagen could influence the swelling behavior of poly(HEMA) to a significant extent even at a very low concentration.

Equilibrium swelling of collagen–poly(HEMA) hydrogels of different compositions in water, seemed to follow

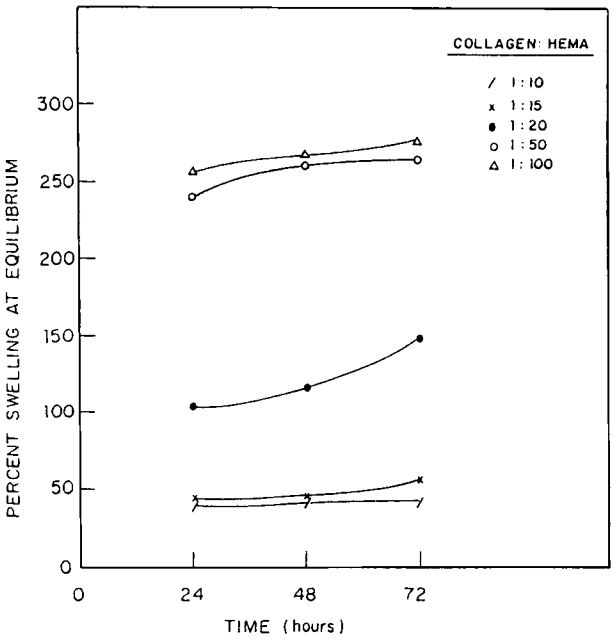

Figure 1 Effect of collagen concentration on the swelling behavior of collagen–poly(HEMA) hydrogel in water.

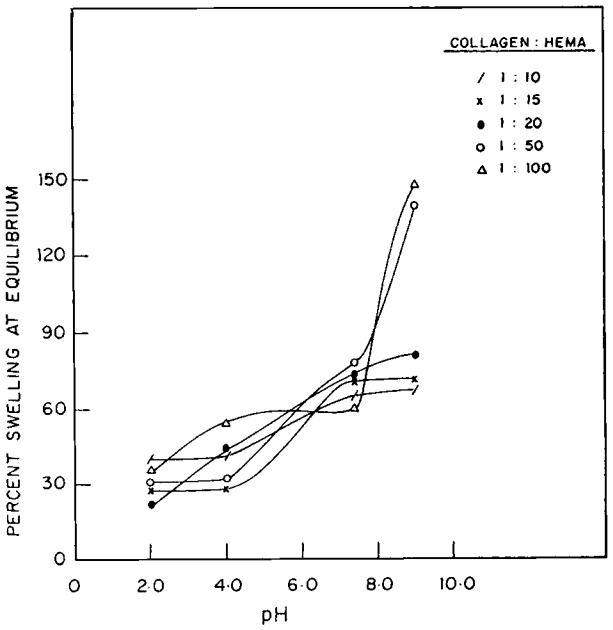

Figure 2 Effect of pH on the swelling behavior of collagen–poly(HEMA) hydrogel.

a definite pattern (Fig. 1). Hydrogels containing about 7 and 10% collagen (1 : 15 and 1 : 10 collagen : HEMA, w/w) showed a low level of percent hydration of about 50% whereas the hydrogels containing 5% collagen were swollen up to a middle level of ~ 150% at equilibrium. Hydrogels with much lower collagen contents (1 : 50 and 1 : 100 collagen : HEMA, w/w) had a higher level of swelling at about 250–275%.

Effect of pH on Swelling Behavior of Collagen–Poly(HEMA) Hydrogels

The swelling behavior of collagen–poly(HEMA) hydrogels in buffers of different pHs is shown in Figure 2. Gels that were equilibrated in the different pH solutions demonstrated critical point like behavior with the large change of volume occurring between pH 2.0 and 9.0. In acid pHs, the percent swelling at equilibrium showed lower values for all compositions of hydrogel as given in Table I. In most of the compositions, it was observed that the equilibrium swelling values increased with pH changes from the acid–alkaline range. It was interesting to note that at physiological pH (7.4) most of the hydrogels with different collagen compositions showed uniform swelling behavior of about 60–75%.

Table I Effect of pH on the Equilibrium Swelling of Collagen–Poly(HEMA) Hydrogels

Hydrogel (Collagen : HEMA)	Percent Swelling at Equilibrium			
	pH			
	2.0	4.0	7.4	9.0
1 : 10	40.0	41.2	64.7	67.0
1 : 15	28.0	27.5	70.0	71.0
1 : 20	22.0	42.2	72.6	81.0
1 : 50	30.0	32.0	77.0	138.5
1 : 100	36.0	54.0	60.0	148.0

Effect of Anticancer Drugs on the Equilibrium Swelling of Collagen–Poly(HEMA) Hydrogels

The effect of entrapped anticancer drugs such as BLM, MMC, and 5-FU on the swelling behavior of collagen–poly(HEMA) hydrogels in water is shown in Table II. After 72 h swelling in water, all the hydrogels reached equilibrium and the percent swelling was in the range of 35–54%. At equilibrium, the pHs of the swelling media were 3.9, 5.0, and 5.8 for 5-FU, MMC, and BLM, respectively. As observed in the case of swelling at different pHs, 5-FU containing hydrogels showed the lowest percent swelling (acid pH) at equilibrium while the swelling capacity of hydrogels increased with pH as seen for MMC and BLM. Further, the percent equilibrium swelling followed a pattern in that, with increasing molecular weight of the entrapped drugs, the equilibrium swelling also seemed to increase.

Table II Effect of Entrapped Anticancer Drugs on Equilibrium Swelling Behavior of Collagen–Poly(HEMA) Hydrogels in Water

Anticancer Drug	Percent Swelling at Equilibrium	pH of Swelling Medium at Equilibrium	Molecular Weight of Entrapped Drug
5-Fluorouracil	34.5	3.9	130
Mitomycin C	45.3	5.0	334
Bleomycin	53.5	5.8	1400

In conclusion, the swelling behavior of the collagen–poly(HEMA) hydrogels followed an interesting pattern, which in turn will have an effect on the drug release from these matrices. The presence of a natural macromolecule like collagen in the hydrogel was found to have a tremendous effect on the swelling behavior of the hydrogels, even at very low concentrations.

References

1. O. Wichterle and D. Lim, *Nature,* **165,** 117 (1960).
2. N. B. Graham and M. E. McNeill, *Biomaterials,* **5,** 27 (1984).
3. J. Heller, R. F. Helwing, R. W. Baker, and M. E. Tuttle, *Biomaterials,* **4,** 262 (1983).
4. J. Host and R. Langer, in *Hydrogels in Medicine and Pharmacy,* N. A. Peppas, Ed., CRC Press, Boca Raton, FL, 1987, Chap. III, p. 96.
5. J. D. Andrade, in *Hydrogels for Medical and Related Applications,* ACS Symposium Series 31, Am. Chem. Soc., Washington, DC, 1976.
6. R. Jeyanthi and K. Panduranga Rao, *Biomaterials,* **11,** 238 (1990).
7. M. F. Refojo, *J. Biomed. Mater. Res.,* **3,** 333 (1969).

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