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RESEARCH PAPER

Swelling and Ketoprofen Release Characteristics of Thermo- and pH-Responsive Copolymer Gels

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ABSTRACT

Swelling-controlled drug delivery copolymer gels were newly synthesized by introducing thermo- and pH-responsive methacryloyl-glycine (MA-Gly) or pH-responsive methacrylic acid (MA-Ac) for comparison with thermoresponsive acryloyl-L-proline ethyl ester (A-ProOEt). A homopolymer gel of A-ProOEt was kept at degrees of swelling that were less than 0.5 at a pH from 2.5 to 7.5 at 37°C. The thresholds of swelling for copolymer gels consisting of A-ProOEt/MA-Gly and A-ProOEt/MA-Ac with a composition of 40/60 mol% were found to be pH 3.0 and pH 5.5, respectively, in buffer solutions at 37°C. The diffusion characteristics of 2-(3-benzoylphenyl)propionic acid (ketoprofen) from such copolymer gels was evaluated in buffer solutions at pH's more than 5.5, and it was found that A-ProOEt/MA-Gly gel possesses a case II transport mechanism that is completely linear time dependent in both the amount diffused and the penetrating swelling front position. On the other hand, A-ProOEt/MA-Ac gel exhibited a non-Fickian (or anomalous) diffusion behavior under the same conditions.

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INTRODUCTION

Most gels with weak polybases and polyacids show a characteristic pH-responsive function (1–5) in which a conformational transition is caused from the collapsed state to the swollen state on ionization in polar media. Such gels include hydrophobically modified weak polyacids consisting of acrylic acid and *n*-alkyl acrylate (6); polyampholyte gels containing methacrylic acid, dimethylaminoethyl methacrylate, and 2-hydroxyethyl methacrylate (7); positively charged gels containing *N*-isopropylacrylamide and *N,N′*-dimethylaminopropyl methacrylamide (8); segmented poly(amine ureas) with a repetitive array of polar (*N,N′*-diethylethylenediamine) and polar (diethylenephenylene) units (9); and amphiphilic poly(2-ethylacrylic acid) (10). Therefore, pH-responsive polymer gels are particularly useful as potential drug carriers for oral delivery.

When orally administered (11), drug-loaded gels reach the stomach (pH 1.0–2.5) through gullet, then pass into the terminal ileum (pH 7.5 ± 0.4) from the proximal small intestine (pH 6.0 ± 0.5) and colon (pH 6.5), where the release of water-soluble drugs usually involves the simultaneous absorption of gastrointestinal juice resulting largely from pH and desorption of the drug via a swelling-controlled diffusion mechanism.

The final goal of our research in this field is to design a novel bifunctional gel for application in colon delivery systems. To achieve this goal, we assigned functions to gels as described below. In the first function, drug-loaded gels do not respond to stimulus at all in the stomach, no gastric juice is absorbed, and the drug is not desorbed. In the second function, swelling of the gel alone specifically in the intestine due to uptake of intestinal juice at pH 7.5 ± 0.4 at the terminal ileum and at pH 6.0 ± 0.4 at the inlet of the intestine occurs without drug desorption. In the third function, pH-triggered reshrinking occurs in the colon, which absorbs only the intestinal juice because of excretion. The slightly lower pH at the inlet of the colon (pH 6.5) compared to that at the terminal ileum (pH 7.5 ± 0.4) is the most important factor in which gels swollen sufficiently without desorption of the drug while passing through the intestine should reshrink at a stretch accompanying a desorption of drug.

We chose a novel gel based on pendant amino acid residues consisting of a combination of thermoresponsive acryloyl-L-proline ethyl ester (A-ProOEt) and thermo- and pH-responsive methacryloyl-glycine (MA-Gly) or pH-responsive methacrylic acid (MA-Ac) for comparison. A homopolymer gel of A-ProOEt, which shows a lower critical solution temperature of 2°C in water, was kept in a shrunken state in the pH 2.5 to pH 7.5 region at 37°C. We then attempted to cause A-ProOEt gel deswollen in the pH 2.5 to pH 7.5 range at 37°C to return to a deswollen state in the colon from a swollen state in the intestine by introducing MA-Gly or MA-Ac gel.

In this report, we discuss the first results with regard to release of 2-(3-benzoylphenyl)propionic acid (ketoprofen) by thermo- and pH-responsive copolymer gels based on L-proline ethyl ester and glycine residues for application in colon delivery systems.

MATERIALS AND METHODS

Materials

The A-ProOEt, $R_f = 0.60$ (ethyl acetate, Merck kieselgel $60F_{254}$ plate) and $[\alpha]_D = -196.5°$ ($c = 1$ in methanol) was synthesized by a coupling reaction of L-proline ethyl ester hydrochloride with acryloylchloride (12,13). A ^{1}H-NMR (nuclear magnetic resonance) spectrum of A-ProOEt used in this study is shown in Fig. 1. The MA-Gly ($R_f = 0.48$) (methanol/chloroform, 1/1) was synthesized by saponifying methacryloyl glycine methyl ester (MA-GlyOMe) ($R_f = 0.55$) (ethyl acetate, Merck kieselgel $60F_{254}$ plate), which was obtained by a coupling reaction of glycine methyl ester hydrochloride with methacryloylchloride. The commercial MA-Ac and ketoprofen were purchased from Wako Pure Chemical

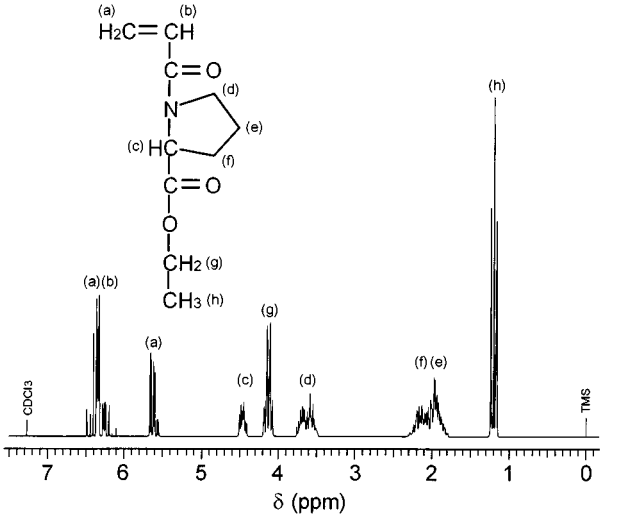

Figure 1. A ^{1}H-NMR spectrum of the A-ProOEt monomer used in this study.

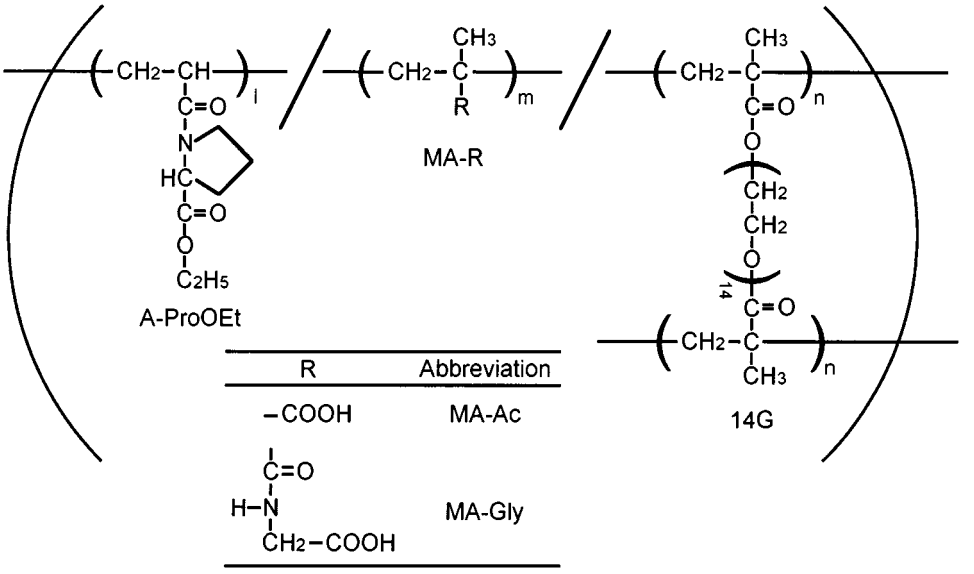

Figure 2. Structural formulas of gels based on polymer networks consisting of A-ProOEt, MA-R, and 14G.

Industries Limited (Osaka, Japan). Other chemicals were reagent grade.

Synthesis of Gels

The synthesis of copolymer gels consisting of A-ProOEt/MA-Gly and A-ProOEt/MA-Ac (structural formula shown in Fig. 2) by radiation-induced polymerization in the presence of a slight amount of cross-linking agent tetradecaethylene glycol dimethacrylate (14G) is as follows. A-ProOEt/MA-Gly (15 mmol, 40/60 mol%) or A-ProOEt/MA-Ac (15 mmol, 40/60 mol%) was mixed with 4 ml of acetone/water (50/50 wt%) containing 0.03 mmol of 14G. Dried nitrogen gas was bubbled through these monomer solutions, and they were charged into a 5-mm inner diameter glass ampoule. The ampoule was irradiated at 30 kGy at a dose rate of 10 kGy/hr at 0°C (ice-water system) under a nitrogen atmosphere using γ-rays from a ^{60}Co source to obtain loosely cross-linked copolymer gels. The gels obtained were washed with acetone to remove unreacted monomer, cut in a round slice, and lyophilized after being placed in water.

Preparation of Ketoprofen-Loaded Gels

The gels lyophilized without drug loading were immersed in aqueous solution and saturated with ketoprofen at 5°C until equilibrium was reached, and the drug-loaded gels were lyophilized again.

Swelling of Gels

The gels lyophilized without drug loading were immersed in vials filled with buffer solutions ranging in pH from 2.5 to 7.5 at 37°C until equilibrium was reached. The degree of equilibrium swelling S_w was estimated as

$$S_w = \frac{W - W_0}{W_0}$$

where W and W_0 are the weights of swollen gel and dried gel, respectively. The 0.01 M citric acid buffer solutions at pH 2.5, 3.0, 4.0, and 5.0 and 0.01 M potassium dihydrogenphosphate buffer solutions at pH 5.5, 6.0, 6.5, 7.0, and 7.5 were obtained by adjusting pH with sodium hydroxide. In this case, the ionic strength of the buffer solution was kept at 0.1 M by adding the appropriate amount of sodium chloride.

Release of Ketoprofen

Ketoprofen-loaded gels in a dried state were first put into a vial filled with a pH 3.0 buffer solution at 37°C for 1 hr to release the drug absorbed by the surface of the gel. These samples were then transferred into vials filled with 50 ml of buffer solutions at pH 3.0, 5.5, and 7.5 at 37°C. At a designated time interval, 1 ml of the medium was withdrawn, and the amount of released drug

was assayed at 260 nm with a Shimazu UV-240 spectrophotometer (Kyoto, Japan).

Instruments

A ^{1}H-NMR spectrum of the A-ProOEt monomer synthesized in this study was obtained at 200 MHz on a Varian Gemini-200 spectrometer using $CDCl_3$ with trimethylsilane (TMS) as a reference. The surface microstructures of copolymer gels treated in buffer solutions at pH 3.0 and 7.5 at 37°C were observed with a Jeol JXA-733 scanning electron microscope (SEM) (Tokyo, Japan).

RESULTS AND DISCUSSION

Thermoresponsive characteristics of gels used in this study were first evaluated from the changes in S_w in deionized-distilled water (Fig. 3). It was confirmed by transmittance experiments at 500 nm (14) that linear polymers of A-ProOEt and MA-Gly showed cloud points around 2°C and 13°C in water, respectively, as these points correspond to a lower critical solution temperature (LCST). These copolymer gels, consisting of cross-linked networks, underwent the volume phase transition around LCST in water, swelling below LCST, but shrinking above it. For example, the S_w values of A-ProOEt gel were 9 in a swollen state at 0°C and 0.5 in a collapsed state at 5°C. In contrast, MA-Ac gel showed no LCST and remained swollen around S_w 300 in water at 0°C to 40°C. On the other hand, copolymer gels obtained by introducing 60 mol% MA-Gly with both thermo- and pH-responsive functions or 60 mol% MA-Ac with a pH-responsive function alone into 40 mol% A-ProOEt with a thermoresponsive function alone exhibited the shrunken state at S_w 1 at 5°C to 40°C. This suggests that no copolymer gels obtained with 60 mol% MA-Gly or MA-Ac swell in water because of the strong hydrophobic effect of pendant ProOEt residues. We attempted to cause such copolymer gels in a collapsed state at 37°C to swell using pH of the solution to cause ionic repulsion between pendant COOH residues.

Figure 4 clearly shows the pH dependence of the swelling of copolymer gels A-ProOEt/MA-Gly and A-ProOEt/MA-Ac with a composition of 40/60 mol% treated for 12 hr at 37°C. These copolymer gels exhibited different pH-responsive swelling behaviors. The A-ProOEt/MA-Gly-based copolymer gel was found to swell in buffer solutions with pH more than 3.0. This is because the volume change ratio of $V_{swollen\ at\ pH\ 7.5}/V_{collapsed\ at\ pH\ 3.0}$ ($V_{7.5}/V_{3.0}$) attains 116 at this pH. However, this pH threshold shifted to the right in the A-ProOEt/MA-Ac copolymer gel to pH 5.5 ($V_{7.5}/V_{3.0} = 35$). As a general rule, pH-triggered gel swelling requires mutual

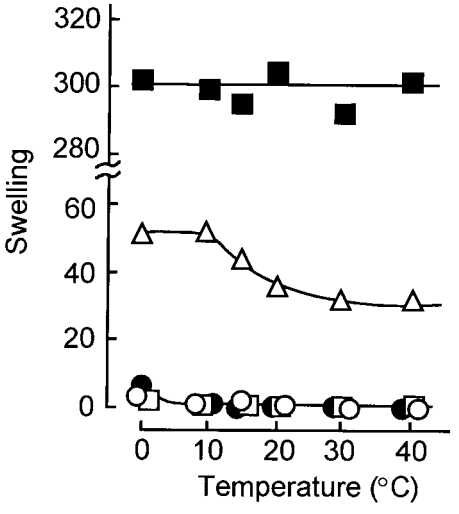

Figure 3. Temperature dependence of the equilibrium swelling of gels including ○, copoly(A-ProOEt/MA-Gly), 40/60 mol%; □, copoly(A-ProOEt/MA-Ac), 40/60 mol%; △, poly(MA-Gly); ●, poly(A-ProOEt); and ■, poly(MA-Ac) in water.

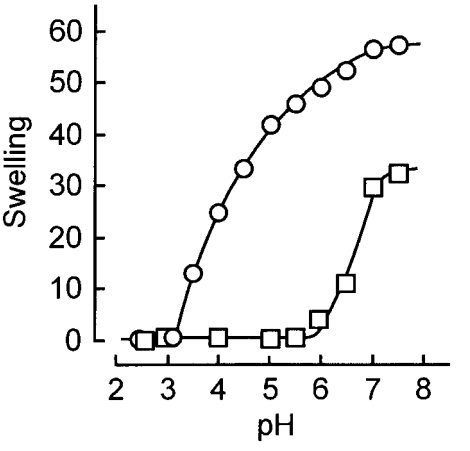

Figure 4. pH dependence of the swelling of copolymer gels, including ○, A-ProOEt/MA-Gly, and □, A-ProOEt/MA-Ac, with a composition of 40/60 mol% treated for 12 hr at 37°C.

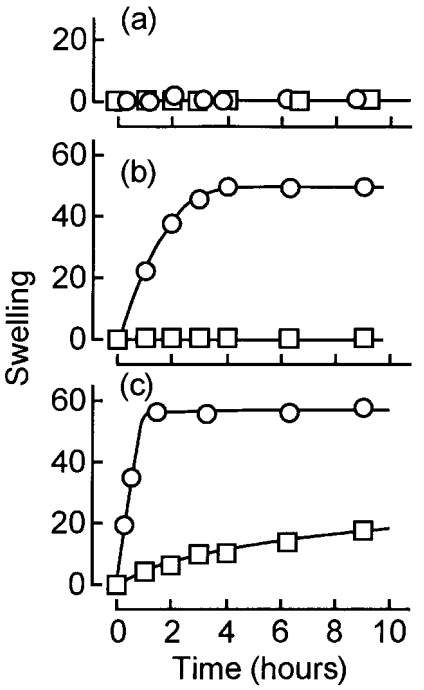

Figure 5. Time dependence of the swelling of copolymer gels, including ○, A-ProOEt/MA-Gly, and □, A-ProOEt/MA-Ac, with a composition of 40/60 mol% treated in buffer solutions with (a) pH 3.0, (b) pH 5.5, and (c) pH 7.5 at 37°C.

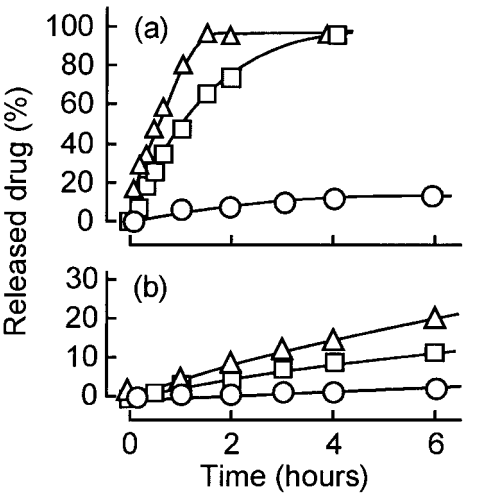

Figure 6. Cumulative amount of ketoprofen released from copolymer gels including (a) A-ProOEt/MA-Gly and (b) A-ProOEt/MA-Ac with a composition of 40/60 mol% treated in buffer solutions with pHs of (○) pH 3.0, (□) pH 5.5, and (△) pH 7.5 at 37°C.

ionic repulsion between the pendant COOH residues in the gel. Glycine has two dissociation constants, pK_1 = 2.34 and pK_2 = 9.60. It was confirmed that the threshold conditions for swelling of homopolymer MA-Gly gel was pH 2.5 and 37°C. The pH of this threshold was brought up by introducing A-ProOEt into MA-Gly. The thresholds of swelling for the copoly(A-ProOEt/MA-Gly) gel obtained at compositions of 40/60, 60/40, 80/20, and 90/10 mol% were pH 3.0, pH 4.0, pH 5.5, and pH 6.5, respectively (M. Negishi and M. Yoshida, private communication, March 1998). On the other hand, the pK_a of MA-Ac monomer is 4.65 (15). The swelling threshold of this homopolymer gel was pH 4.7 at 37°C, while copolymer gel consisting of 40 mol% A-ProOEt and 60 mol% MA-Ac showed a threshold of pH 5.5.

The time dependence of the swelling of 40 mol% A-ProOEt containing the copolymer gels A-ProOEt/MA-Gly and A-ProOEt/MA-Ac is shown in Fig. 5 as a function of pH. At pH 3.0, these gels remained in a shrunken state, with S_w values below 0.5. At pH 5.5, no A-ProOEt/MA-Ac gel was swollen; however, A-ProOEt/MA-Gly gel showed a marked increase of swelling parabolically with the time until equilibrium was reached 4 hr after the start of the experiment (S_w = 46). Equilibrium swelling was reached by the second hour of the experiment when the sample was treated in a pH 7.5 buffer solution (S_w = 58). In contrast, A-ProOEt/MA-Ac gel swelled gradually for the entire 9-hr experimental period in pH 7.5 buffer solution (S_w = 18). From these results, it is reasonable to conclude that the introduction of MA-Gly in copolymers induces a strong ionic repulsion and a quick increase in swelling when compared to MA-Ac.

Last, the swelling characteristics of copolymer gels in relation to ketoprofen release were investigated using buffer solutions with pH 3.0, 5.5, and 7.5 at 37°C (Fig. 6). The cumulative amount of ketoprofen released from copoly(A-ProOEt/MA-Gly) gel reached 100% at 1.5 hr after the start of the experiment in pH 7.5 buffer solution and 4 hr after in pH 5.5 buffer solution. At pH 3.0, the cumulative amount of released drug was only 14% even after 6 hr had passed due to gel shrinkage. These findings agree with the swelling pattern shown in Fig. 5a. The correlation between gel swelling and drug release due to changes in pH was also observable in copolymer A-ProOEt/MA-Ac gel.

Table 1

Characteristic Parameters of Ketoprofen Released from Thermo- and pH-Responsive Copolymer Gels

	A-ProOEt/MA-Gly			A-ProOEt/MA-Ac		
	pH 3.0	pH 5.5	pH 7.5	pH 3.0	pH 5.5	pH 7.5
n	0.59	1.08	1.03	0.83	0.80	0.90
k	5.02	53.04	69.14	1.19	2.89	3.83
r	0.997	0.992	0.999	0.996	0.992	0.99

r = correlation coefficient.

The release of drugs from gels is generally known to follow the Higuchi relationship (16), in which release kinetics can be expressed as

$$\frac{M_t}{M_\infty} = kt^n$$

where M_t and M_∞ express the amount of the drug released at time t and at infinite time ∞, k is the proportionality constant, and n is the exponent characteristic of the mode of transport of the drug (17–23).

Release parameters n and k, calculated from the slope of the curve of cumulative amount of released drug and time as a function of pH shown in Fig. 6, are summarized in Table 1. Water adsorption characteristics of gel exhibited anomalous behavior, ranging between Fickian and case II extremes, depending on experimental temperature and thermodynamic compatibility of the solvent and gel. Typically, both the amount diffused and the penetrating swelling front position in case II transport are completely time dependent in a linear fashion, whereas Fickian diffusion is square root time dependent. An intermediate situation, known as non-Fickian or anomalous diffusion, occurs whenever the rates of Fickian diffusion and polymer relaxation are comparable. Based on values of n given in Table 1, the characteristics of ketoprofen diffusion from copolymer gels consisting of A-ProOEt/MA-Gly and A-ProOEt/MA-Ac swollen at pH more than 5.5 were shown to have a case II transport mechanism ($n > 1.0$) and non-Fickian (or anomalous) diffusion mechanism ($0.5 < n < 1.0$), respectively.

To investigate differences in ketoprofen release between A-ProOEt/MA-Gly and A-ProOEt/MA-Ac, their surface structures were observed microscopically in buffer solution with different pH (Figs. 7 and 8). The surface structure of A-ProOEt/MA-Gly gel treated in a pH 3.0 buffer solution was covered by a rigid membranelike barrier, which blocked drug release from the interior matrix (Fig. 7). The type of formation was also observed in copolymer A-ProOEt/MA-Ac gel at pH 3.0. However, drug release markedly increased at pH 7.5, although differently in each gel. The cause for this difference was found to be the formation of different size laby-

Figure 7. A surface SEM view of copoly(A-ProOEt/MA-Gly) (40/60 mol%) gel treated for 4 hr in a pH 3.0 buffer solution at 37°C.

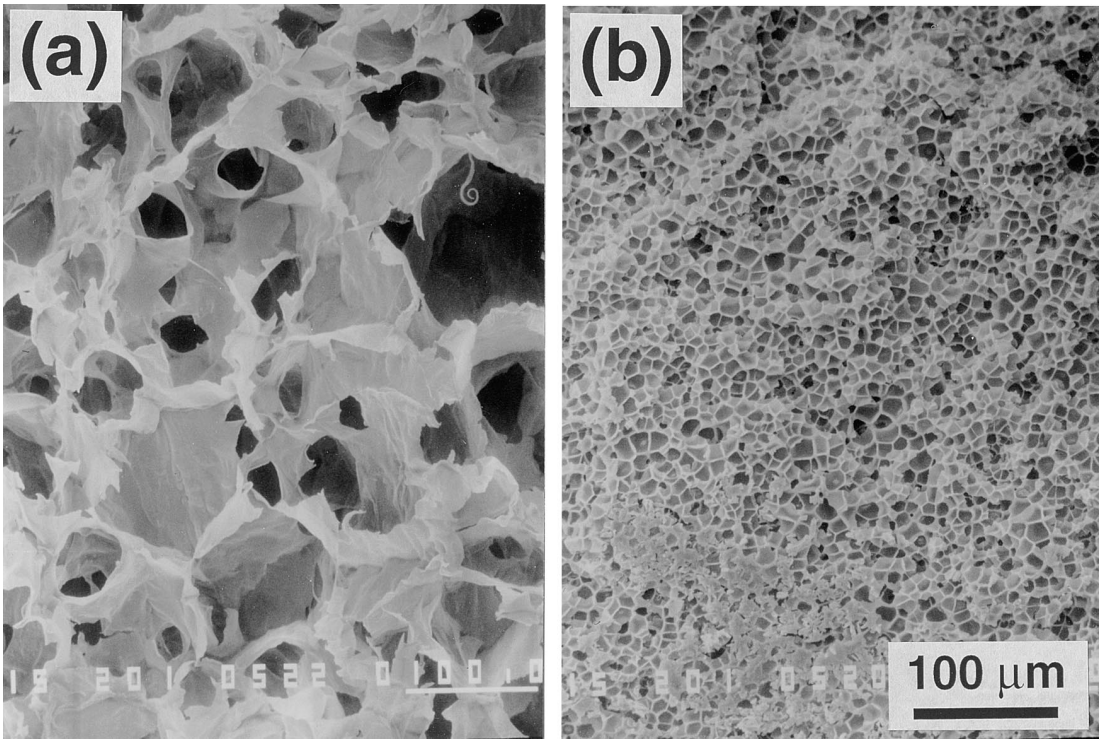

Figure 8. Surface SEM views of copolymer gels, including (a) A-ProOEt/MA-Gly and (b) A-ProOEt/MA-Ac, with a composition of 40/60 mol% treated for 4 hr in a pH 7.5 buffer solution at 37°C.

rinthlike pores, which increase drug release as pore size increases.

In conclusion, we synthesized a novel bifunctional gel by a combination of thermoresponsive A-ProOEt and thermo- and pH-responsive MA-Gly or pH-responsive MA-Ac. A homopolymer A-ProOEt gel maintained a shrunken state below S_w 0.5 from pH 2.5 to 7.5 at 37°C due to the effects of a thermoresponsive function (LCST = 2°C). In the case of copolymer gels obtained by introducing 60 mol% MA-Gly or MA-Ac, the threshold of swelling was found to be pH 3.0 for A-ProOEt/MA-Gly gel and pH 5.5 for A-ProOEt/MA-Ac gel. The release of ketoprofen from these copolymer gels was closely related to swelling of the gels in response to pH changes. We anticipate the further development of such bifunctional gels based on pendant amino acid residues for application in colon delivery systems.

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