



Poly(ethylene glycol)-containing Hydrogels for Oral Protein Delivery Applications

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Abstract. Novel pH-sensitive hydrogels were developed as suitable candidates for carriers in bioMEMS devices as well as for oral delivery of therapeutic peptides and proteins due to their ability to respond to environmental pH change. Macromonomers containing various PEG molecular weights were synthesized and used to prepare P(MAA-g-EG) hydrogels were by photopolymerization. P(MAA-g-EG) hydrogels showed a drastic change of the equilibrium swelling ratio between pH 2.2 and 7.0. At pH 7.0, hydrogels with PEGMA2000 exhibited higher swelling ratio than hydrogels with PEGMA1000. For both hydrogels with PEGMA1000 and PEGMA2000, the swelling mechanism became more relaxation-controlled as the environmental pH changed from 2.2 to 7.0 due to the ionization of the functional groups in polymer networks at high pH. *In vitro* release studies of insulin were conducted. P(MAA-g-EG) hydrogels exhibited drastic increase of insulin release as the pH of the medium was changed from acidic to basic. Insulin release from P(MAA-g-EG) hydrogels with PEGMA2000 was slower than from hydrogels with PEGMA1000 at both low and high pH. These results were used to design and improve protein release behavior from these carriers.

Key Words. poly(ethylene glycol), macromonomers, pH-sensitive, hydrogel, insulin release

1. Introduction

Microelectromechanical systems (MEMS) are created by utilizing microfabrication technologies to integrate mechanical and electronic elements on a common substrate. Micromachining of MEMS can be surface or bulk micromachining, both based on IC manufacturing technologies. These fabrication techniques have been utilized to manufacture a wide range of sensors, actuators, and microdevices, including pressure sensors, accelerometers, flow sensors, ink jet printer heads, and micromirrors for projection. In particular, the fabrication of microsensors is an area of immense interest in MEMS research.

Sensors measure a specific entity in their environment using a sensing element and then via a transducer(s) provide an output relating to what they measure. A

particularly promising technique for sensor development is based on the deposition of thin polymer gels on microdevices. Especially important are gels exhibiting molecular biorecognition. This is done by a process that imprints the chemical identity of a biomolecular substance on the surface of a gel. The treated gel then attracts and captures that same substance. We are exploring applications of the process, called biorecognition, to treat disease by subtraction.

In this work, our goal was to devise a system that would recognize these undesirable compounds and remove them from the blood by molecular recognition. In all these gels, the use of poly(ethylene glycol) (PEG) in the process creates “stealth conditions,” allowing these particles circulating in the blood to go unrecognized by the body’s defenses. Eventually then, these systems could lead to new delivery systems.

Delivery of therapeutic peptides and proteins by oral or anolyte-inducing means is one of the great scientific challenges in the pharmaceutical field. In development of oral delivery systems for peptides and proteins, major barriers are the degradation of proteins by proteolytic enzymes and the acidic environment in the gastrointestinal (GI) tract and the low penetration of proteins across the lining of the intestine into the blood stream (Lee et al., 1990; Woodley, 1994).

To solve these problems, considerable research efforts have been made in recent years. For instance, entrapment of proteins within carriers, such as liposomes, nanoparticles, multiple emulsions, and polymers, has been used to protect proteins from proteolysis in the GI tract. Alternatively, the design of delivery systems targeting the colon, where proteolytic activity is relatively low, has been pursued. In addition, permeation enhancers are being explored to improve uptake and transport through the intestinal wall, and peptidase inhibitors and enzyme-resistant peptide analogs are used to protect peptides

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molecular weight 2,000 (PEGMA2000) was synthesized as described previously. Tetra(ethylene glycol) dimethacrylate (TEGDMA, Polysciences, Warrington, PA) was used as a crosslinking agent without further purification.

To initiate the reaction, 1-hydroxycyclohexyl phenyl ketone (otherwise known as Irgacure[®] 184, Ciba-Geigy, Hawthorne, NY) was used as a UV-light sensitive initiator. Monomer mixtures with feed molar ratio of 1:1 EG:MAA using PEGMA1000 and PEGMA2000 were mixed and the TEGDMA was added in the amount of 1.2 mol% of total monomers. The initiator was added in the amount of 0.1 wt% of the total monomers and the resulting mixtures were diluted to 60 wt% with a 1:1 mixture by weight of ethanol and water. Nitrogen was bubbled through the mixture for 15 minutes to remove dissolved oxygen that would act as an inhibitor for the reaction. The mixture was cast between glass slides (size 75 × 50 × 1 mm) and was exposed to UV light (intensity 15.0 ± 0.5 mW/cm²) for 30 minutes in a nitrogen environment. The ensuing hydrogel films were cut into disks of 1 cm diameter placed in deionized water for 7 days, and the water was changed every 12 hours in order to remove any unreacted monomers, crosslinking agents, and initiators. Then, the disks were dried in air for 1 day and placed in a vacuum oven at 25 °C until their weight remained constant within 0.1 wt% over 24 hours. For insulin incorporation and *in vitro* release studies, dried hydrogels were ground in particles which were passed through sieves in the range between 150 and 212 μm.

2.3. Swelling studies

To determine relevant swelling properties, the dried hydrogel disks were weighed and placed in phosphate–citrate buffer solutions of pH values of 2.2 and 7.0 at 37 °C. The ionic strength of each buffer solution was adjusted to 0.5 M by the addition of KCl. After swelling, the samples were taken out of the buffer solutions, blotted to remove surface water and weighed. The swelling of the hydrogels was expressed as the weight swelling ratio, q , determined as the ratio of the weight of the swollen hydrogel, W_s , to the weight of the initially dry hydrogel, W_d . The equilibrium weight swelling ratio was obtained when the weight of the swollen hydrogel reached constant value ($\pm 1\%$).

To determine the dynamic swelling behavior, at specified time intervals the weight swelling ratio, q , was calculated as described previously.

2.4. Insulin loading

A sample of 50 mg of bovine insulin (28.1 U/mg, Sigma, St. Louis, MO) was first dissolved in 10 ml of 0.1 N HCl, the solution was neutralized with 10 ml of 0.1 N NaOH and it was diluted with 80 ml of pH 7.4 phosphate

buffered solution to make a 50 mg/ml of insulin stock solution. Incorporation was accomplished by soaking 140 mg of each set of the dried microparticles in 20 ml of insulin stock solution. At specific time points, 0.3 ml samples were withdrawn from the solution with a syringe to determine the insulin incorporation ratio.

After 6 hours, 20 ml of 0.1 N HCl was added to the loading solution to collapse the microparticles, the entire solution was passed through filter paper (Whatman 4, 42.4 mm ϕ , particle retention > 25 μm, Clifton, NJ) and the particles were washed with 100 ml of 0.1 N HCl and 100 ml of deionized water to eliminate the remaining buffer and HCl solution. The insulin-incorporated microparticles were dried under vacuum and stored at 4 °C prior to use.

2.5. In vitro release studies

Release experiments were performed using a USP dissolution apparatus (model 2100B, Distek, North Brunswick, NJ). The pH of the external medium was varied from 2.2 to 6.5 to mimic the physiological conditions of the GI tract. Microparticles containing insulin were placed in the dissolution test unit with 50 ml of pH 2.2 phosphate–citrate buffer solution. After 1 hour, the pH of the solution was changed to 6.5 by adding 1 N NaOH. During the experiment, the temperature and stirring speed were maintained at 37 °C and 100 rpm, respectively. At specific time intervals, 0.3 ml of samples was taken out with a syringe and insulin concentration was analyzed.

The concentration and stability of insulin were measured by reverse-phase high performance liquid chromatography (RP-HPLC) and bovine insulin ELISA kit. The RP-HPLC used in this analysis consisted of a Waters 2690 separations module, a Waters 2487 dual λ absorbance detector (Waters, Milford, MA), and a column (model 218TP54, C₁₈, 25 × 0.46 cm, 300 Å, 5 μm particle size, Vydac, Hesperia, CA). The mobile phase consisted of solution A which was water with 0.1 v/v% trifluoroacetic acid (TFA, Sigma, St. Louis, MO) and solution B which was acetonitrile (Mallinckrodt, Paris, KY) with 0.085 v/v% TFA. The flow gradient was from 72.5 to 64.2% of solution A over 10 minutes and the flow rate was 1.5 ml/min. UV detection was performed at dual wavelength of 215 and 276 nm.

3. Results and Discussion

3.1. Characterization of the PEG-containing macromonomers

The DCCI method, which was used to synthesize PEG-containing macromonomers in this work, is one of the standard methods of peptide synthesis and falls in the

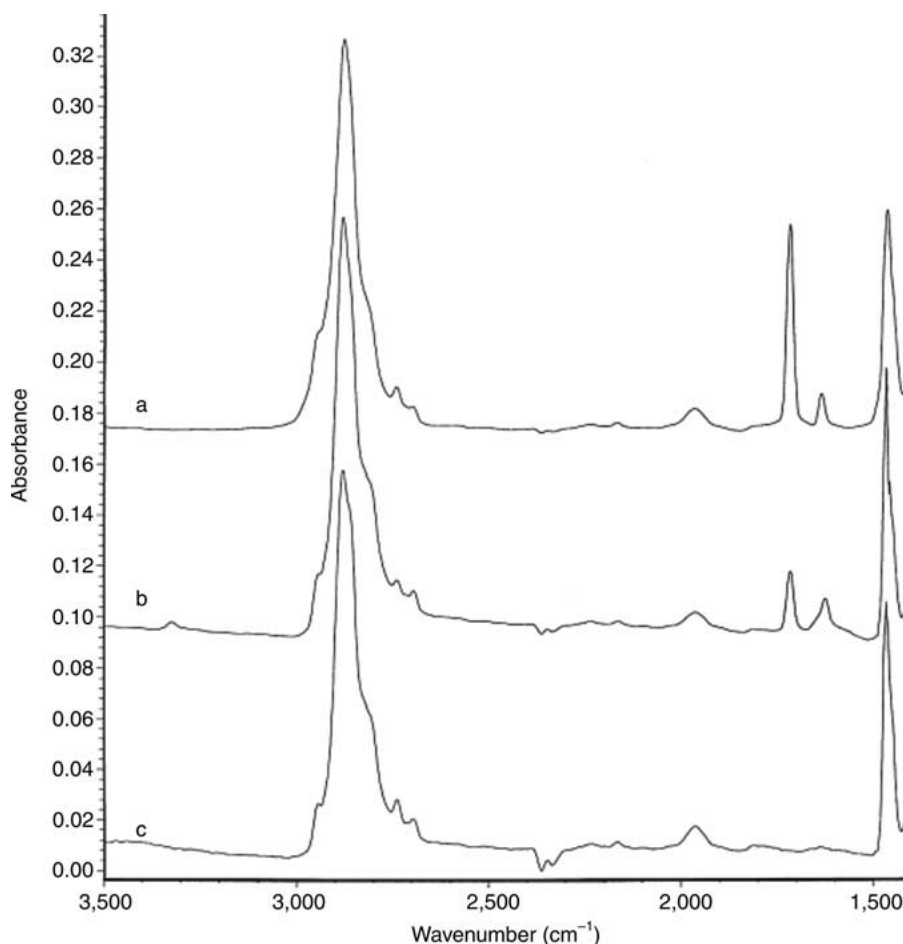


Fig. 2. ATR-FTIR spectra of PEGME and PEGMA macromonomers; (a) spectrum of commercially available PEGMA1000 (Polysciences Inc.) as a reference; (b) spectrum of laboratory-synthesized PEGMA2000; (c) spectrum of PEGME2000.

category of esterification reactions of methacrylic acid. The advantages of this reaction are: (i) insensitivity to the presence of moisture; (ii) nearly quantitative yields; and (iii) no side reactions (Gnanou et al., 1987).

Figure 2 shows the FTIR spectra of PEGME2000, laboratory-synthesized PEGMA2000, and commercial PEGMA1000 as a reference. The most distinct peaks in these spectra were the peak of the carbonyl group (C=O) observed in the region of 1850–1400 cm^{-1} and the peak of the C=C observed in the region of 1690–1600 cm^{-1} . These peaks contain information about the presence of methacrylate end groups in the macromonomers. The spectrum of PEGME2000 (Figure 2c) contains no peaks of C=O and C=C. However, the spectra of PEGMA1000 (Figure 2a) and PEGMA2000 (Figure 2b) exhibit the C=O and C=C peaks at 1717 and 1630 cm^{-1} , respectively. In addition, spectra of PEGMA1000 and PEGMA2000 were identical.

The macromonomers prepared in this work were also characterized using $^1\text{H-NMR}$ spectroscopy. The spectrum of the laboratory-synthesized PEGMA2000 was

compared with the spectrum of commercial PEGMA1000 as a reference. Figure 3 exhibits the $^1\text{H-NMR}$ spectra of PEGMA2000 and PEGMA1000 synthesized in our labs. It is clear that the methacrylate end group was indeed present and two spectra were identical.

3.2. Swelling behavior of the *P(MAA-g-EG)* hydrogels

We have shown that *P(MAA-g-EG)* hydrogels exhibit pH-responsive swelling behavior. This results from the ionization or deionization of the functional groups in the polymer networks responding to environment pH changes. At pH values lower than the pK_a of the gel, the carboxylic acid groups of the hydrogel are protonized forming hydrogen bonding with adjacent oxygens whereas at pH values higher than the pK_a of the gel, carboxylic acid groups become ionized leading to swollen networks due to the electrostatic repulsion between charged groups. Several studies have been presented concerning the effect of PEG molecular weight

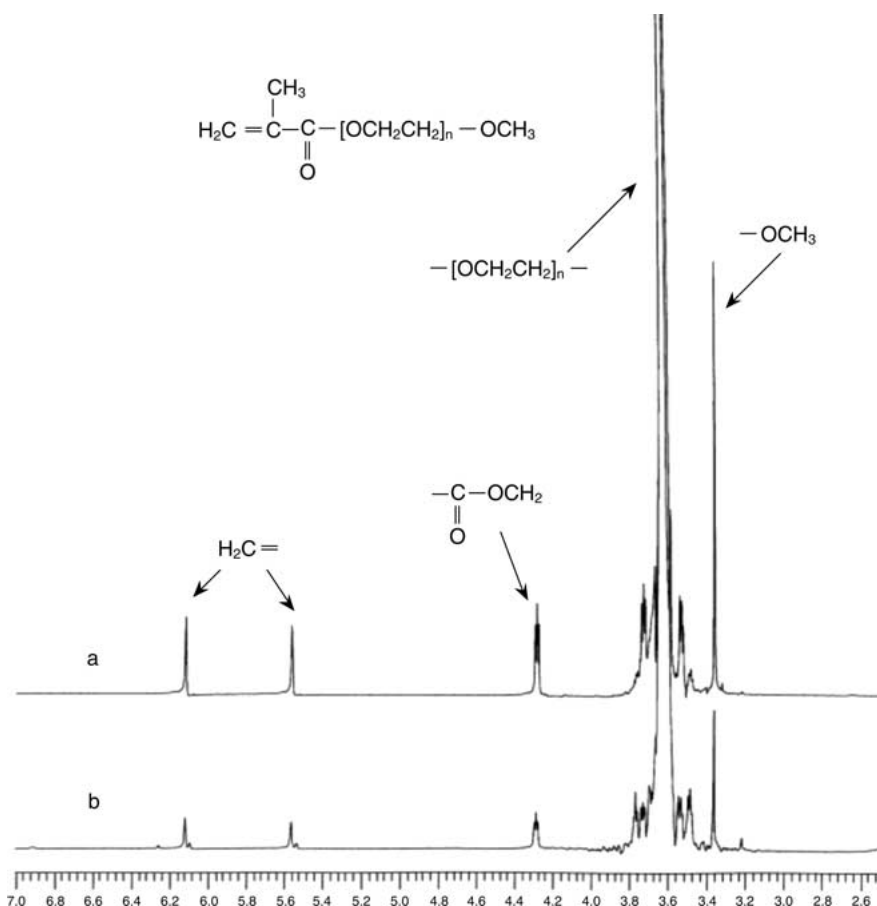


Fig. 3. $^1\text{H-NMR}$ spectra of PEGMA macromonomers showing functional groups, (a) spectrum of commercially available PEGMA1000 (Polysciences Inc.) as a reference; (b) spectrum of laboratory-synthesized PEGMA2000. The macromonomers were dissolved in deuterochloroform.

lower than 1,000 on the swelling behavior of the P(MAA-g-EG) hydrogels (Bell et al., 1996; Lowman et al., 2000; Kim et al., 2003). In this work, emphasis was given to hydrogels with grafted PEG molecular weight higher than 1,000.

The effects of the pH of the surrounding medium and of the grafted PEG molecular weight on the equilibrium swelling behavior of the gels is shown in Figure 4. In all cases, the swelling weight reached its equilibrium after 6 hours. The equilibrium swelling ratio of the hydrogels was a function of the network structure, crosslinking ratio, hydrophilicity, and degree of ionization of the functional groups. As expected, there was a drastic change in the equilibrium swelling ratio of P(MAA-co-MEG) hydrogels between pH 2.2 and 7.0. At pH 2.2, the hydrogels were in a relatively collapsed state, while at pH 7.0, they swelled from 27 to 32 times of their initial dry weight.

This sharp transition between the collapsed and the swollen states at pH 2.2 and 7.0 indicates that in

pharmaceutical and bioMEMS applications where these systems will be used as controlled release carriers, they can swell and collapse rapidly responding to the physiological pH change of the human GI tract. Thus, when the drug-incorporated gels reach the upper small intestine, increase of the pH can induce gel swelling immediately leading to release of drug.

At pH 2.2, there was no significant difference of the swelling ratio regardless of the grafted PEG molecular weight. However, at pH 7.0, hydrogels prepared from PEGMA2000 exhibited a higher swelling ratio than hydrogels with PEGMA1000. This was because hydrogels with PEGMA2000 have several proximate carboxylic groups; thus, there was more electrostatic repulsion between ionized carboxylic acid groups at high pH, thus rendering these networks more swellable.

To investigate the time-dependent swelling behavior of P(MAA-g-EG) hydrogels, dynamic swelling studies were performed. P(MAA-g-EG) disks with different grafted PEG molecular weight were tested in pH 2.2 and

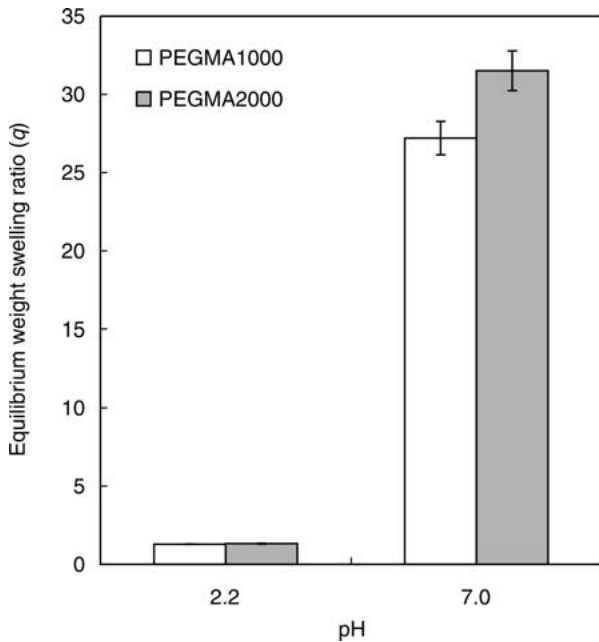


Fig. 4. Equilibrium weight swelling ratio of P(MAA-g-EG) hydrogels at pH 2.2 and 7.0 with different grafted PEG molecular weight.

7.0 buffer solutions. Figure 5 presents the weight swelling ratio, q , of P(MAA-g-EG) with PEGMA1000 and PEGMA2000 as a function of the time in pH 2.2 and 7.0 buffer solutions at 37 °C.

It was observed that at pH 7.0, the amount of absorbed water in the hydrogels was larger than at pH 2.2. The portion of the water absorption curve with $M_t/M_x < 0.60$ was analyzed (Ritger et al., 1987) by the following equation.

$$\frac{M_t}{M_x} = kt^n \tag{1}$$

Here, M_t is the mass of water absorbed at time t , M_x is the mass of water absorbed at equilibrium, k is a characteristic swelling constant, and n is a characteristic exponent describing the mode of water transport. For a hydrogel film, a value of $n = 0.5$ indicates Fickian diffusion, a value of $n > 0.5$ indicates non-Fickian or anomalous transport and $n = 1$ implies Case II (relaxation-controlled) transport.

The constants n and k were calculated from the slopes and intercepts of the plots of $\ln(M_t/M)$ vs. $\ln t$ from the experimental data shown in Figures 6 through 9; they are summarized in Table 1. The same figures indicate the best fits of the data by equation (1).

We concluded that the swelling mechanism of P(MAA-g-EG) became more relaxation-controlled as the environmental pH changed from 2.2 to 7.0. This was important because it established the mechanism of

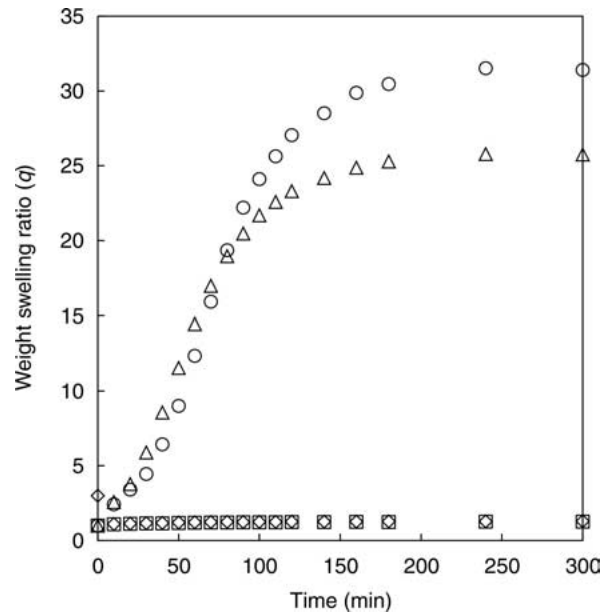


Fig. 5. Dynamic swelling behavior of P(MAA-g-EG) hydrogels (1 : 1 EG : MAA) swollen in buffer solutions at 37 °C; PEGMA1000/pH2.2 (\diamond), PEGMA2000/pH2.2 (\square), PEGMA1000/pH7.0 (\triangle), and PEGMA2000/pH7.0 (\circ).

swelling (and therefore response) of bioMEMS systems in which these gels would be used. At pH 2.2, the values of the exponent n were approximately 0.5 regardless of molecular weight of the grafted PEG, thus indicating a Fickian diffusion process. At pH 7.0, the values of n were

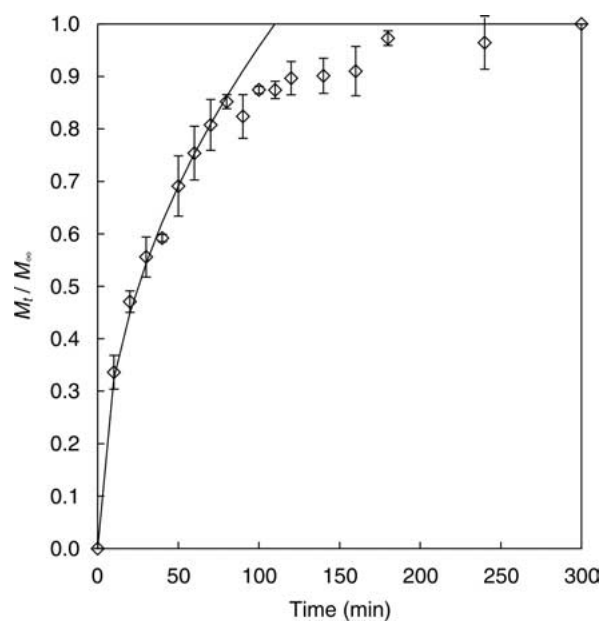


Fig. 6. Fractional water uptake, M_t/M_∞ , of P(MAA-g-EG) hydrogels with PEGMA1000 (1 : 1 EG : MAA) swollen in pH 2.2 buffer solution at 37 °C; experimental data (\diamond) and equation (1) (—) (average \pm S.D., $n = 3$).

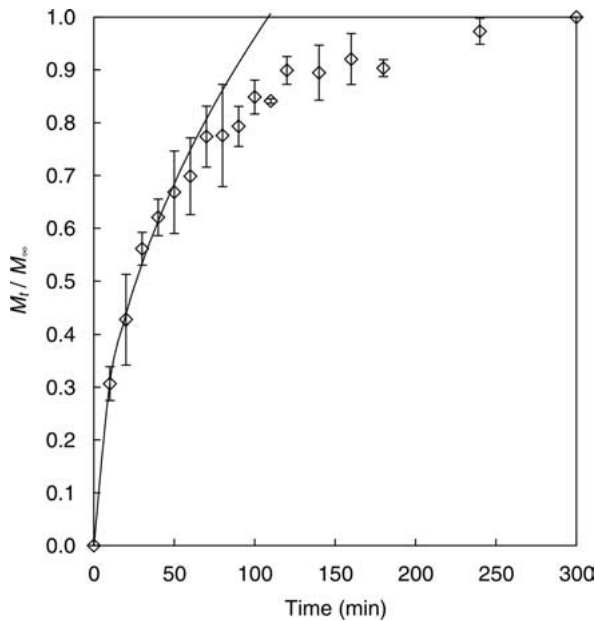


Fig. 7. Fractional water uptake, M_t/M_∞ , of P(MAA-g-EG) hydrogels with PEGMA2000 (1:1 EG:MAA) swollen in pH 2.2 buffer solution at 37°C; experimental data (\diamond) and equation (1) (—) (average \pm S.D., $n=3$).

about 1.5. Additionally, the water transport mechanism of P(MAA-g-EG) showed little dependence on the molecular weight of grafted PEG at the same pH.

The swelling mechanism of crosslinked polymers is dependent on the relative contribution of penetrant diffusion and polymer relaxation. In ionic polymer networks, the polymer relaxation is affected by the ionization of the functional groups of the polymer. Increase of ionization contributes to the electrostatic repulsion between adjacent ionized groups leading to chain expansion, which in turn affects macromolecular chain relaxation. Therefore, the swelling mechanism becomes more relaxation controlled as gel ionization becomes prominent. This explains the reason why at pH 7.0 the swelling mechanism of P(MAA-g-EG) networks became more relaxation controlled. On the other side, at pH 2.2, the ionization was not significant and there were no interactions between ionized functional groups. Thus, the overall transport mechanism was not affected as much by relaxation and the result was Fickian (diffusion-controlled) transport with n values approaching 0.5.

Table 1. Parameters n and k of equation (1) for P(MAA-g-EG) hydrogels with PEGMA1000 and PEGMA2000 (1:1 EG:MAA) swollen in pH 2.2 and 7.0 buffer solutions at 37°C (average \pm S.D., $n=3$)

MW of grafted PEG	n		$k \times 10^2$ (min ⁻ⁿ)	
	pH 2.2	pH 7.0	pH 2.2	pH 7.0
1,000	0.47 (\pm 0.02)	1.48 (\pm 0.04)	10.99 (\pm 0.57)	0.14 (\pm 0.06)
2,000	0.49 (\pm 0.05)	1.49 (\pm 0.10)	10.06 (\pm 0.27)	0.08 (\pm 0.03)

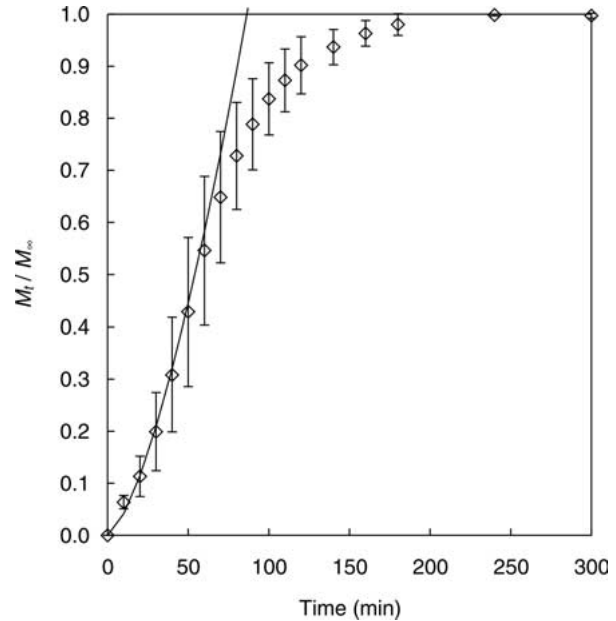


Fig. 8. Fractional water uptake, M_t/M_∞ , of P(MAA-g-EG) hydrogels with PEGMA1000 (1:1 EG:MAA) swollen in pH 7.0 buffer solution at 37°C; experimental data (\diamond) and equation (1) (—) (average \pm S.D., $n=3$).

Plotting the fractional sorption data as a function of the square root of time provides valuable information for distinguishing between Fickian and Case II transport mechanisms since the Fickian diffusion curve exhibits a monotonic inflection-free approach to equilibrium while the Case II curves clearly sigmoidal (Ensore et al., 1977). Figure 10 presents the fractional water uptake of P(MAA-co-MEG) hydrogels as a function of the square root of time at pH 2.2 and 7.0. The difference of curve shapes between pH 2.2 and 7.0 indicated that the swelling mechanism at high pH value was closer to Case II transport than that at low pH value.

3.3. In vitro release behavior of the P(MAA-g-EG) hydrogels

To investigate the pH-responsive release of insulin from the microparticles, the initially dry, insulin-incorporated particles were placed in pH 2.2 solution for 1 hour and the pH of the medium was changed to pH 6.5. The fractional release of insulin, defined here as the ratio of

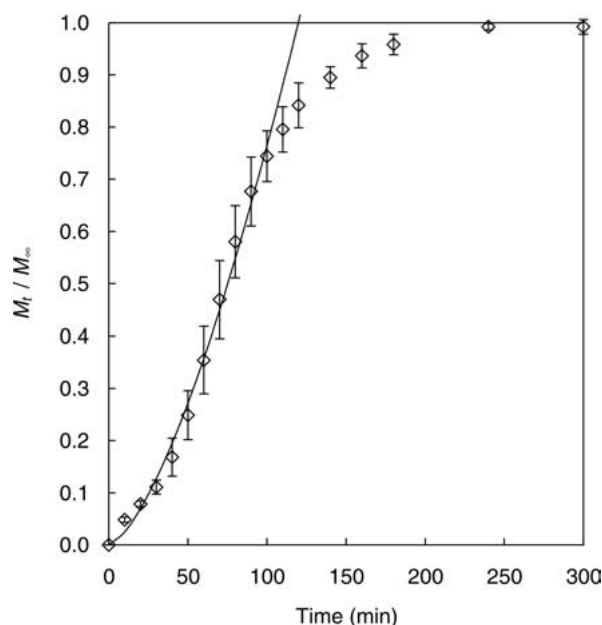


Fig. 9. Fractional water uptake, M_t/M_∞ , of P(MAA-g-EG) hydrogels with PEGMA2000 (1:1 EG:MAA) swollen in pH 7.0 buffer solution at 37°C; experimental data (\diamond) and equation (1) (—) (average \pm S.D., $n=3$).

the amount released at any time, I_t , to the total amount released after 3 hour, I , is shown in Figure 11. For the initial treatment in an acidic medium (pH 2.2), the microparticles were complexed and 10 and 47% of the insulin was released from the P(MAA-g-EG) hydrogels with PEGMA2000 and PEGMA1000, respectively. This implies that these carriers had the desired property for oral delivery of insulin since a significant fraction of insulin remained in the microparticles as they passed through the low pH environment of the stomach. When the pH of the medium was changed to 6.5, the remaining insulin was released rapidly.

It was observed that P(MAA-g-EG) hydrogels with PEGMA2000 exhibited decreased insulin release at both low and high pH. At an incorporation pH of 7.0, the carboxylic acid groups in the hydrogels as well as the insulin, which has pI of 5, were negatively charged resulting in repelling each other. Thus, the negatively charged insulin was mainly distributed in the neutral PEG chain domains. Moriyama and Albertsson (Albertsson et al., 1987; Moriyama et al., 1996, 1999) reported that that insulin appeared to partition into the PEG phase in PEG and negatively charged component systems. When these insulin-incorporated P(MAA-g-EG) microparticles were placed in the media, it was assumed that since the hydrogel with PEGMA2000 had more PEG chain entanglements than the hydrogel with PEGMA1000, it rendered the release of insulin entrapped in the PEG chains slower.

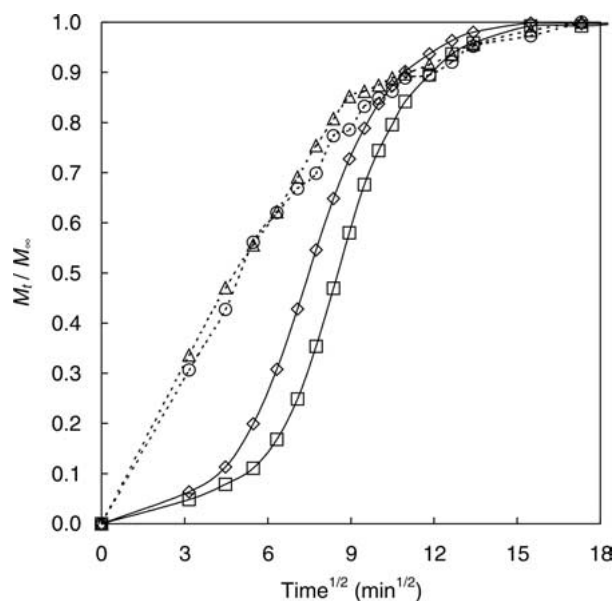


Fig. 10. Fractional water uptake, M_t/M_∞ , of P(MAA-g-EG) hydrogels (1:1 EG:MAA) as a function of the square root of time swollen in buffer solutions at 37°C; PEGMA1000/pH 2.2 (Δ), PEGMA2000/pH 2.2 (\circ), PEGMA1000/pH 7.0 (\diamond), and PEGMA2000/pH 7.0 (\square).

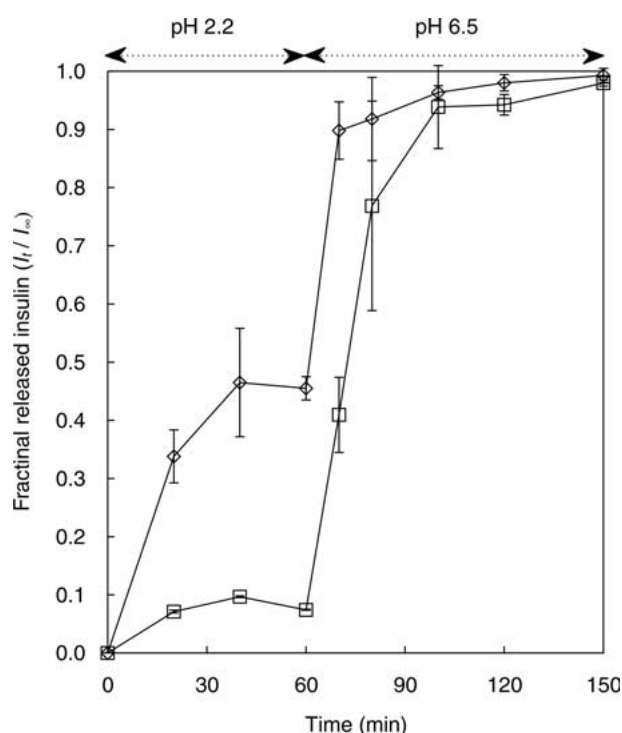


Fig. 11. Effect of the environmental pH change on the release of insulin from the insulin-incorporated P(MAA-g-EG) microparticles; PEGMA1000 (\diamond) and PEGMA2000 (\square). The microparticles were first placed in pH 2.2 buffer solution and then the pH of medium was changed to 6.5 after 1 hour. Temperature was maintained at 37°C (average \pm S.D., $n=3$).

4. Conclusions

P(MAA-g-EG) hydrogels containing PEG tethered chains with 1,000 or 2,000 molecular weight were prepared. P(MAA-g-EG) hydrogels showed a drastic change of the equilibrium swelling ratio of between pH 2.2 and 7.0. At pH 2.2, there was no significant difference of swelling ratio according to the grafted PEG molecular weight. However, at pH 7.0, hydrogels with PEGMA2000 showed higher swelling ratios than hydrogels with PEGMA1000.

For both hydrogels with PEGMA1000 and PEGMA2000, the swelling mechanism became more relaxation controlled as the environmental pH changed from 2.2 to 7.0 due to the ionization of the functional groups in polymer networks. At pH 2.2, the values of the exponent n were approximately 0.5, which indicated a Fickian diffusion, regardless of molecular weight of the grafted PEG while at pH 7.0 the values of n were about 1.5.

P(MAA-g-EG) hydrogels showed the greatest change in insulin release rate from the low to high pH. Insulin release from P(MAA-g-EG) hydrogel with PEGMA2000 was slower than the hydrogel with PEGMA1000 at both low and high pH.

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