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RESEARCH PAPER

Effect of Poly (Ethylene Glycol) Molecular Weight and Microparticle Size on Oral Insulin Delivery from P(MAA-g-EG) Microparticles

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ABSTRACT

Five years of successful work in our lab have shown that graft copolymer networks of poly(methacrylic acid-g-ethylene) [P(MAA-g-EG)], are very promising candidates for oral drug delivery. In an acidic environment, these copolymers form interpolymer complexes, protecting the active agent from the harsh environment of the gastrointestinal tract. At high pH, these complexes dissociate, causing the polymer to swell and release the drug. Films of P(MAA-g-EG) with a monomer ratio of 1:1 (MAA:EG) were prepared by free radical solution UV-polymerization, washed in order to remove the unreacted monomer, and crushed to form microparticles with different particle size distribution. Previous studies in our lab have focused on using polymer disks in their swelling studies. The swelling properties of polymer disks vs. crushed particles were investigated via equilibrium swelling experiments in this study. Another goal in this study is to compare different PEG chain length (MW-400 and MW-1000) and different particle size (150–212 microns, 90–150 microns and 25–90 microns) in their loading and release behavior. After 6 hours of exposing the polymer with the insulin solution we achieved approximately 90% of insulin loading.

Key Words: Oral insulin delivery; pH-swelling behavior; Controlled drug release; PEG; P(MAA-g-EG) microparticles.

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INTRODUCTION

Advances in biotechnology have led to the successful treatment of diseases. Controlled drug delivery systems offer the advantage of a controlled release of the desired molecule in the target area. This release behavior achieves the desired therapeutic effect for an extended period of time. However, the challenges are immense with the delivery of macromolecules such as peptides and proteins. To be successful, peptide and protein formulations will have to simultaneously address all of the issues that result in poor bioavailability: intrinsic permeability, degradation, rapid clearance, and chemical stability.[1–5]

Diabetes is an example of a disease that requires the administration of a macromolecule, in this case, insulin. Actual insulin therapy requires several daily injections that are very inconvenient and painful for the patient. That is why, in recent years, there has been so much innovation and excitement in the search for new therapies for diabetes that would result in improved safety, efficacy, and patient compliance.

For the past few decades, a great deal of work has focused on attempts to develop noninvasive methods of insulin delivery with the oral route cflearly being the most convenient and desired.[6–8] However, the oral route confronts several barriers, such as the presence of proteolytic enzymes in the gastrointestinal tract, chemical instability, and poor permeability of proteins across biological membranes.

In our laboratory, we have successfully developed copolymer systems that have the ability to address the barriers to the oral administration of insulin. These systems consist of hydrogels with poly(ethylene glycol) (PEG) grafted on poly(methacrylic acid) (PMMA), designated as P(MAA-g-EG). These pH-sensitive hydrogels respond to the surrounding environment by protecting the incorporated protein from the harsh environment of the stomach and releasing it in the small intestine.[5,9–17]

Poly(methacrylic acid) exhibits interpolymer complexation with PEG as the protons of carboxylic acid groups on PMAA form hydrogen bonds with ether groups on the PEG chain.[3] This complexation occurs at a low pH range when the carboxylic acids are still protonated. At higher pH values, the polymer network swells because of the disruption of the polymer complexes and the ionization and electrostatic repulsion of the acid groups in the MAA backbone.

This copolymer system has been studied for years and has been demonstrated to have the desired mucoadhesive properties[18,19] and the ability to protect and stabilize proteins and inhibit proteolytic enzymes.[20] Recently, cell studies have shown that the P(MAA-g-EG) microparticles and nanospheres have nontoxic effects when in contact with the cell monolayer and the ability to enhance the transport of proteins such as insulin and calcitonin.[5,21–24] Moreover, animal studies have been carried out showing that insulin-loaded microparticles were able to sustain a hypoglycemic effect for a sustained period of time.[25]

In order to improve the desired therapeutic effect, optimization of this polymer carrier is required. In this work we investigated the effect of two PEG molecular weights (400 and 1000), and the microparticle size (25–90, 90–150, and 150–212 μm) on the equilibrium swelling behavior and insulin loading and release from P(MAA-g-EG) microparticles.

MATERIALS

Methacrylic acid (MAA), and poly(ethylene glycol) ether monomethacrylate (PEGMA) with a molecular weight of 400 and 1000 were purchased from Aldrich Chemical Company (Milwaukee, WI) and used to synthesize P(MAA-g-EG) microparticles. Tetra(ethylene glycol) dimethacrylate (TEGDMA) was purchased from Polysciences, Inc. (Warrington, PA) and used as the crosslinking agent in the amount of 0.75% mol of the total monomers. Irgacure® 184 was employed as the photoinitiator and obtained from Ciba-Geigy Co. (Hawthorne, NY); it was incorporated in 0.1% (w/w) of the monomer mixture. The P(MAA-g-EG) microparticles were loaded with bovine insulin that was purchased from Sigma Chemical Co. (St. Louis, MO).

METHODS

Preparation of P(MAA-g-EG) Microparticles

P(MAA-g-EG) hydrogels were prepared by free radical solution ultraviolet (UV)-polymerization of MAA and PEGMA. The monomers were mixed in 1:1 molar ratio of methacrylic acid-ethylene glycol units. The PEGMAs with different molecular weights (400 and 1000) of the PEG graft chains were used in the synthesis of these hydrogels. TEGDMA was used as the crosslinking agent and was added in the amount of 0.75% moles of the total amount of monomers. The photoinitiator, Irgacure 184, was added in the amount of 0.1% wt of the total amount of monomers. The monomer mixture was diluted with a mixture of 50% v/v ethanol and deionized water (Milli-Q Plus system,

Millipore, Billerica, MA). Nitrogen was bubbled through the well-mixed solution for 15 minutes to remove dissolved oxygen, which acts as a free radical scavenger.

The mixture was then carefully poured between microscope slides ($75 \times 50 \times 1$ mm) (Fisher, Pittsburgh, PA) separated by Teflon spacers with a thickness of 0.9 mm. The microscope slides were then placed in a nitrogen atmosphere under a UV light source of 11 mW/cm^2 at 365 nm for 30 minutes. The polymer films were washed in deionized water for approximately 7 days in order to remove unreacted monomers, crosslinking agent, initiator, and sol fraction. For some of the swelling studies, the films were cut into discs of approximately 1.2 cm in diameter and 0.8 mm in thickness. Both the polymer films and discs were then dried in a vacuum oven at 27°C for 3 days. The dry polymer films were then crushed by using a mortar and pestle and then sieved to the desired particle size range (25–90, 90–150, or 150–212 μm) by using the corresponding sieves (VWR Scientific; West Chester, PA).

Swelling Studies

Mellvaine buffer solutions[26] were used for the swelling behavior studies of microparticles as a function of pH in order to keep the ionic strength constant at 0.1 M. The pH was varied from 3.0 to 8.0 depending on the specific experiment. The dried discs were weighed and placed in 50-mL flaks containing buffer solutions. In the case of the polymer microparticles, a ratio of 0.01 mg of polymer/mL of buffer solution was used.

When working with polymer microparticles, all flasks were pretreated by using a silicon-containing compound, Sigmacote, (Sigma Chemical Co., St. Louis, MO) in order to avoid adsorption of the polymer microparticles to the walls of the flasks. This compound is a chlorinated organopolysiloxane in heptane and forms a tight, microscopically thin film of silicon on glass (water repellent). The disks and microparticles were kept in contact with buffer solution at 37 ± 0.5°C for a period of 24 hours when an equilibrium swollen state was achieved. The excess of water from the polymer disks was carefully removed by gently placing them in a Kim Wipe. The crushed particles were vacuum-filtered with a 0.45-μm Millipore filter paper. The weight-swelling ratio, q, was calculated by the weight ratio of the swollen to the dry polymer sample.

Insulin Loading

Drug loading was accomplished by equilibrium partitioning of insulin into the P(MAA-g-EG) microparticles. Insulin stock solutions with a concentration of bovine insulin of 0.5 mg/mL were prepared. The insulin stock solutions consisted of 10% v/v of 0.1 M HCl, 10% v/v of 0.1 M NaOH and 80% v/v phosphate buffer solution at pH of 7.4. The ionic strength of the final insulin stock solution was 0.1 M. Polymer microparticles were kept in contact with the insulin stock solution in a ratio of 7 mg of polymer/mL of insulin stock solution. The closed vials containing the polymer and the insulin stock solutions were agitated for 6 hours at room temperature. Then, the microparticles were precipitated by adding 0.1 M HCl, and vacuum-filtered with a 0.45-μm Millipore filter paper. The loaded microparticles were dried by using a freeze dryer.

The amount of insulin loaded in the polymer carrier was calculated by a comparison of the amount of insulin left in the insulin solution at time t with the amount of insulin in the loading solution at the beginning of the experiment. Samples were analyzed by using a reversed-phase high performance liquid chromatography unit (Waters Associates, Bedford, MA).

Insulin Release Studies

The insulin release studies were carried out in a USP II dissolution apparatus (Distel model 2100B dissolution apparatus, Inc., North Brunswick, NJ) by using the paddle method. Dried microparticles of P(MAA-g-EG) were placed into contact with phosphate buffer solution at pH of 7.4 and ionic strength of 0.1 M in a ratio of 2.8 mg of loaded polymer/mL of buffer solution. These solutions were placed in 100-mL dissolution cells in the dissolution apparatus. Again, all the vials that were used in this experiment were previously coated with Sigmacote. The samples were maintained at 37°C. The paddles were rotated at a velocity of 100 rpm. Samples of 0.5 mL were taken at different time intervals and then analyzed by using reverse-phase HPLC.

RESULTS AND DISCUSSION

Equilibrium Swelling Behavior

Successful studies in our laboratory have shown that graft copolymer network gels of P(MAA-g-EG) exhibit a pH-dependent swelling behavior due to the formation of interpolymer complexes.[11,27] Our previous swelling studies were done using polymer discs of 1.2 cm in diameter and 0.8 mm in thickness. It is generally believed that discs, microparticles, and nanospheres of this system show the same swelling

Table 1. Effect of the PEG tethered chains' molecular weight on the equilibrium weight swelling ratio, q, of P(MAA-g-EG) microparticles and discs.

P(MAA-g-EG) system	Molecular weight of the PEG graft chain	Equilibrium weight swelling ratio, q
Microparticles	400	27.9±3.2
	1000	29.7±4.2
Discs	400	29.1±0.3
	1000	30.6±0.9

Microparticles and discs were prepared with an initial monomer ratio MAA:EG of 1:1. The microparticles size was 150–212 μm. Swelling studies were carried out at 37°C in a phosphate buffer solution at a pH 7.4 and ionic strength of 0.1 M. Each data entry represents an average of three independent studies and the error represents one standard deviation.

behavior. Therefore, although this has not been proven, the swelling studies in this work were carried out in order to compare the swelling behavior of polymer discs and microparticles. Results from Table 1 show that there was no significant difference in the equilibrium weight ratio of both systems. Moreover, the molecular weight of the PEG tethered chains did not have an effect on the equilibrium swelling ratio of these systems.

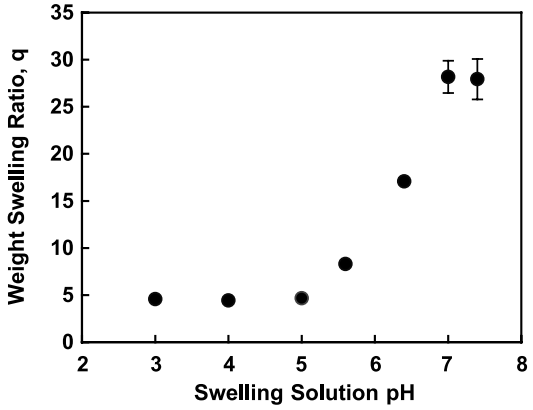

Figure 1. Equilibrium swelling behavior of P(MAA-g-EG) microparticles as a function of the pH on the swelling solution. Microparticles had tethered PEG chains with a molecular weight of 1000, prepared with an initial monomer ratio MAA:EG of 1:1, and crushed and sieved to a particle size of 150–212 μm. Swelling studies were carried out at 37°C. The ionic strength of the swelling solutions was 0.1 M. Each data point represents an average of three independent experiments and the error bars represent one standard deviation. (*View this art in color at www.dekker.com.*)

The swelling behavior of P(MAA-g-EG) microparticles (150–212 μm and PEG 1000) as a function of pH at I = 0.1 M is shown in Fig. 1. This figure indicates the transition pH where the interpolymer complexes started to dissociate. This transition occurred at a very narrow pH range. Torres-Lugo and Peppas[28] observed the same transition pH range when using P(MAA-g-EG) discs (monomer ratio 1:1, PEG 1000).

Insulin Loading Behavior

Loading of insulin was accomplished by equilibrium partitioning of insulin into the P(MAA-g-EG) microparticles. The amount of insulin loaded into the polymer microparticles was determined by calculating the partitioning efficiency of insulin:

$$\text{Partitioning Efficiency} : \frac{C_i - C_f}{C_i} \quad (1)$$

where, C_i=the initial insulin concentration before the 6 hours of stirring and C_f=the final insulin concentration in the solution. This final concentration was determined for three different steps in the loading procedure: after the 6 hours of stirring when the microparticles were still in their swollen state, after collapsing the microparticles, and after filtering the microparticles.

Results from Table 2 show partitioning efficiencies as high as 99% after the microparticles were in contact with the insulin stock solution for 6 hours. In this stage, the microparticles were still in their swollen state. However, once the microparticles were collapsed,

Table 2. Calculations of the amount of insulin loaded into P(MAA-g-EG) microparticles at different stages in the loading procedure.

Step in the insulin loading procedure	pH of the loading solution	Insulin partitioning efficiency (%)
Before complexation of the microparticles	6.7±0.5	98.4±0.8
After complexation of the microparticles	3.0±0.6	38.9±2.8
After filtration of the microparticles	2.7±0.2	32.7±10.9

Microparticles had tethered PEG chains with a molecular weight of 1000, prepared with an initial monomer ratio MAA:EG of 1:1, and crushed and sieved to a particle size of 150–212 μm. Each result represents an average of six independent experiments, and the error bars represent one standard deviation.

Table 3. Effect of the PEG tethered chains' molecular weight on the amount of insulin loaded into P(MAA-g-EG) microparticles.

Molecular weight of PEG tethered chains	Insulin partitioning efficiency (%)
400	38.2±27.8
1000	32.7±10.9

Microparticles were prepared with an initial monomer ratio MAA:EG of 1:1 and crushed and sieved to a particle size of 150–212 μm. Each result represents an average of six independent experiments, and the error bars represent one standard deviation.

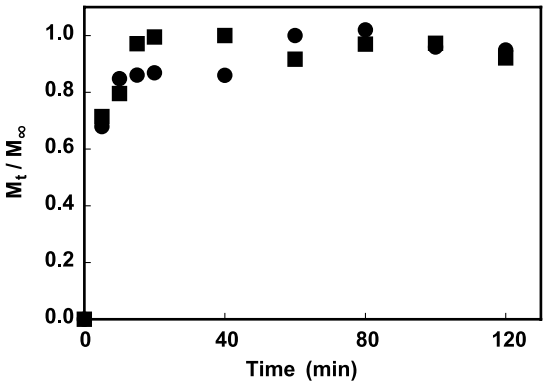

Figure 2. Effect of the PEG tethered chains' molecular weight on the insulin released behavior from P(MAA-g-EG) microparticles. Microparticles were prepared with an initial monomer ratio MAA:EG of 1:1 and a PEG molecular weight of (●) 400 and (■) 1000; then they were crushed and sieved to a particle size of 150–212 μm. Released studies were carried out at 37°C in a phosphate buffer solution of pH 7.4 and ionic strength of 0.1 M.

the partitioning efficiencies dropped to around 40%. There was also no significant difference in determining the partitioning efficiency after collapsing the microparticles and after filtering the microparticles. Therefore, all the calculations were based on the calculation of the insulin partitioning efficiencies after the filtration of the microparticles.

There could be several explanations for the decrease in partitioning efficiencies. One explanation is a possible ''burst effect'' or rapid release of the insulin that was accumulated on the surface of the polymer network.[29–34] Another possibility is that the sudden change in pH in the loading solution and, consequently, significant change in polymer configuration, led to the rapid diffusion of insulin from the polymer network.[35–40] However, additional studies should be done in order to make conclusions about this aspect.

Neither the molecular weight of the PEG tethered chains nor the microparticle size had a statistical effect on the amount of insulin that was loaded into the P(MAA-g-EG) microparticles (Tables 3 and 4). Each data entry represents an average of six independent studies within one standard deviation.

Insulin Release Studies

Loaded P(MAA-g-EG) microparticles with a MAA:EG monomer ratio of 1:1 were used for the release studies. We studied the effect of the molecular weight of the PEG chains on the release of insulin (Fig. 2). The curves obtained show a controlled release of insulin. The insulin release behavior was described by the ratio M_t/M_∞, where M_t is defined as the total mass of insulin released from the microparticles at time t and M_∞ is defined as the total maximum amount of insulin that was

Table 4. Effect of varying particle sizes on the amount of insulin loaded on P(MAA-g-EG) microparticles.

Dry microparticle size (μm)	Insulin partitioning efficiency (%)
25–90	85.7±16.1
90–150	73.7±37.0
150–212	63.9±32.3

Microparticles had tethered PEG chains with a molecular weight of 1000 and were prepared with an initial monomer ratio MAA:EG of 1:1. Each result represents an average of six independent experiments, and the error bars represent one standard deviation.

Table 5. Effect of the PEG tethered chains' molecular weight on the maximum amount of insulin released from P(MAA-g-EG) microparticles during the release experiments.

Molecular weight of PEG	Insulin released (%)
400	41.2±4.6
1000	39.7±11.4

Microparticles were prepared with an initial monomer ratio MAA:EG of 1:1, then crushed and sieved to a particle size of 150–212 μm. Released studies were carried out at 37°C in a phosphate buffer solution of pH 7.4 and ionic strength of 0.1 M. Each result represents an average of three independent experiments, and the error bars represent one standard deviation.

released during the duration of the release experiment. These results show that there was no difference in the insulin release behavior between the microparticles containing PEG chains of molecular weight of 400 and those containing a molecular weight of 1000. The maximum amount of insulin was released within the first 60 to 80 minutes. Rapid but controlled release of insulin is desired for drug delivery applications because the drug will then rapidly reach the blood stream and have the desired therapeutic effect.[41–46]

The results from Table 5 show the maximum percentage of insulin that was released from the polymer microparticles. Each datum point represents the average of three independent studies and the error represents one standard deviation. Both P(MAA-g-EG) microparticles containing PEG chains with molecular weight of 400 and 1000 released around 40% of the insulin that was initially trapped in the polymer network. There was no significant difference in the percentage of insulin released from both microparticle systems.

The effect of the P(MAA-g-EG) microparticle size on the insulin release behavior was also studied. These microparticles also contained a MAA:EG monomer ratio of 1:1. Figure 3 shows that there was no difference in the insulin release behavior of three different sizes (25–90 μm, 90–150 μm, and 150–212 μm) of the polymer microparticles. The maximum amount of insulin was released within the first 60 and 80 minutes of the experiment (Table 6). The results

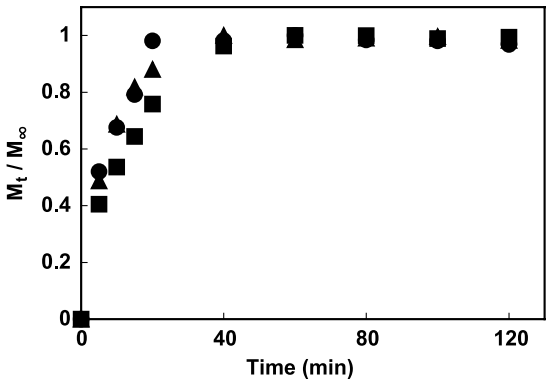

Figure 3. Effect of varying particle sizes on the amount of insulin released from P(MAA-g-EG) microparticles. Microparticles had tethered PEG chains with a molecular weight of 1000 and were prepared with an initial monomer ratio MAA:EG of 1:1. Released studies were carried out at 37°C in a phosphate buffer solution of pH 7.4 and ionic strength of 0.1 M. Microparticles with a size of 150–212 μm and (●) 25–90 μm, (■) 90–150 μm, and (▲) 25–90 μm were used in this study.

Table 6. Effect of varying particle sizes on the maximum amount of insulin released from P(MAA-g-EG) microparticles during the release studies.

Dry microparticle size (μm)	Insulin released (%)
25–90	56.61 ± 15.1
90–150	50.57 ± 1.01
150–212	39.70 ± 11.42

Microparticles had tethered PEG chains with a molecular weight of 1000 and were prepared with an initial monomer ratio MAA:EG of 1:1. Release studies were carried out at 37°C in a phosphate buffer solution at a pH of 7.4 and ionic strength of 0.1 M.

show that there were no statistical differences in the amount of insulin released from the studied P(MAA-g-EG) microparticles.

CONCLUSIONS

Both P(MAA-g-EG) discs and microparticles showed the same equilibrium weight swelling behavior. Neither the molecular weight of the PEG chains tethered to the polymer miroparticles nor the microparticle size had a significant effect on their equilibrium weight swelling behavior. All microparticle systems studied showed the desired pH-dependent behavior for drug delivery systems.

Neither the difference in molecular weight of the PEG tethered chains nor the microparticle size caused a significant effect on either the insulin loading or release behavior. All microparticle systems studied were found to have promising results in terms of their release profiles.

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REFERENCES

1. Fix, J.A. Oral controlled release technology for peptides: status and future prospects. Pharm. Res. **1996**, *13*, 1760–1764.
2. Bures, P.; Huang, Y.; Oral, E.; Peppas, N.A. Surface modifications and molecular imprinting of

polymers in medical and pharmaceutical applications. J. Control. Release **2001**, *72*, 25–33.
3. Peppas, N.A.; Kim, B.S.; Donini, C.; Sipahigil, O.; Leobandung, W. Stimuli-sensitive polymers for oral and parenteral administration. In *New Trends in Polymers for Oral and Parenteral Administration: From Design to Receptors*; Barratt, G., Duchêne, D., Fattal, F., Legendre, J.Y., Eds.; Editions de Santé: Paris, 2001; 32–41.
4. Ichikawa, H.; Peppas, N.A. Synthesis and characterization of pH-responsive nanosized hydrogels of poly(methacrylic acid-g-ethylene glycol) for oral peptide delivery. In *New Trends in Polymers for Oral and Parenteral Administration: From Design to Receptors*; Barratt, G., Duchêne, D., Fattal, F., Legendre, J.Y., Eds.; Editions de Santé: Paris, 2001; 261–264.
5. Torres-Lugo, M.; Garcia, M.; Record, R.; Peppas, N.A. Physicochemical behavior and cytotoxic effects of P(MAA-g-EG) nanospheres for oral delivery of proteins. J. Control. Release **2002**, *80*, 197–205.
6. Carino, G.P.; Mathiowitz, E. Oral insulin delivery. Adv. Drug Deliv. Rev. **1999**, *35*, 249–257.
7. Parker, R.S.; Doyle, F.J., III; Peppas, N.A. A model-based algorithm for blood glucose control in type I diabetic patients. IEEE Trans. Biomed. Eng. **1999**, *46*, 148–157.
8. Parker, R.S.; Ward, J.H.; Doyle, F.J., III; Peppas, N.A. Robust control in diabetes using a physiological model. AIChE J. **2000**, *46*, 2537–2549.
9. Bell, C.L.; Peppas, N.A. Modulation of drug permeation through interpolymer complexed hydrogels for drug delivery applications. J. Control. Release **1996**, *39*, 201–207.
10. Lowman, A.M.; Peppas, N.A. Analysis of the complexation/decomplexation phenomena in graft copolymer networks. Macromolecules **1997**, *30*, 4959–4965.
11. Lowman, A.M.; Peppas, N.A. Molecular analysis of interpolymer complexation in graft copolymer networks. Polymer **2000**, *41*, 73–80.
12. Peppas, N.A.; Ichikawa, H.; Torres-Lugo, M. In *Cytotoxicity and Transport Enhancement of Proteins Through Cell Monolayers Using Novel pH-Sensitive Hydrogels*, Proceed. World Meeting APV/APGI, 2000; 201–202.
13. Garcia, M.; Torres-Lugo, M.; Alonso, M.J.; Peppas, N.A. Biointeractions of pH-sensitive poly(methacrylic acid-g-ethylene glycol) hydrogel microspheres with the Caco-2 model cell line. In *New Trends in Polymers for Oral and Parenteral Administration: from Design to Receptors*; Barratt, G., Duchêne, D., Fattal, F., Legendre, J.Y., Eds.; Editions de Santé: Paris, 2001; 386–389.
14. Morishita, M.; Lowman, A.M.; Takayama, K.; Nagai, T.; Peppas, N.A. Elucidation of the mechanism of incorporation of insulin in controlled release systems based on complexation polymers. J. Control. Release **2002**, *81*, 25–32.
15. Leobandung, W.; Ichikawa, H.; Fukumori, Y.; Peppas, N.A. Preparation of stable insulin-loaded nanospheres of poly(ethylene glycol) macromers and N-isopropyl acrylamide. J. Control. Release **2002**, *80*, 357–363.
16. Robinson, D.N.; Peppas, N.A. Preparation and characterization of pH-responsive poly(methacrylic acid-g-poly(ethylene glycol) nanospheres. Macromolecules **2002**, *35*, 3668–3674.
17. Torres-Lugo, M.; Peppas, N.A. Preparation and characterization of P(MAA-g-EG) nanospheres for protein delivery applications. J. Nanopart. Res. **2002**, *4*, 73–81.
18. Huang, Y.; Leobandung, W.; Foss, A.; Peppas, N.A. Molecular aspects of muco- and bioadhesion: tethered structures and site-specific surfaces. J. Control. Release **2000**, *65*, 63–71.
19. Huang, Y.; Efremova, N.; Peppas, N.A.; Leckband, D.E. Direct measurement of interactions between tethered PEG chains and adsorbed mucin layers. Langmuir **2002**, *18*, 836–845.
20. Madsen, F.; Peppas, N.A. Complexation graft copolymers networks: swelling properties calcium binding and proteolytic enzyme inhibition. Biomaterials **1999**, *20*, 1701–1708.
21. Foss, A.; Peppas, N.A. Investigation of the cytotoxicity and insulin transport of acrylic-based copolymer protein delivery systems in contact with Caco-2 cultures. Eur. J. Pharm. Biopharm. **2004**, *57*, 530–537 .
22. Foss, A.C.; Goto, T.; Morishita, M.; Peppas, N.A. Development of acrylic-based copolymers for oral insulin delivery. J. Control. Release., Europ. J. Pharm. Biopharm. **2004**, *57*, 163–169.
23. Kim, B.; Peppas, N.A. Synthesis and characterization of pH-sensitive glycopolymers for oral drug delivery systems. J. Biomater. Sci., Polym. Ed. **2002**, *13*, 1271–1281.
24. Kim, B.; Peppas, N.A. Complexation phenomena in pH-responsive copolymer networks with pendent saccharides. Macromolecules **2002**, *35*, 9545–9550.
25. Lowman, A.L.; Morishita, M.; Kajita, M.; Nagai, T.; Peppas, N.A. Oral delivery of insulin using

pH-responsive complexation gels. J. Pharm. Sci. **1999**, *88*, 933–937.
26. Elving, P.J.; Markowitz, J.M.; Rosenthal, I. Preparation of buffer systems of constant ionic strength. Anal. Chem. **1956**, *28*, 1179–1180.
27. Lowman, A.M.; Peppas, N.A. Pulsatile drug delivery based on a complexation/decomplexation mechanism. In *Intelligent Materials for Controlled Release*; Dinh, S.M., DeNuzzio, J.D., Comfort, A.R., Eds.; ACS Symposium Series: Washington, D.C., American Chemical Society (ACS), 1999; Vol. 728, 30–42.
28. Torres-Lugo, M.; Peppas, N.A. Molecular design and in vitro studies of novel pH-sensitive hydrogels for the oral delivery of calcitonin. Macromolecules **1999**, *32*, 6646–6651.
29. Leobandung, W.; Ichikawa, H.; Fukumori, Y.; Peppas, N.A. Monodisperse nanoparticles of poly(ethylene glycol) macromers and N-isopropyl acrylamide for biomedical applications. J. Appl. Polym. Sci. **2003**, *87*, 1678–1684.
30. Kavimandan, N.J.; Peppas, N.A. Insulin transport across cell monolayers in the presence of complexation hydrogels. Trans. Soc. Biomater. **2003**, *29*, 248.
31. Blanchette, J.; Park, K.; Peppas, N.A. Use of complexation hydrogels for oral administration of chemotherapeutic agents. Trans. Soc. Biomater. **2003**, *29*, 246.
32. Kim, B.; La Flamme, K.; Peppas, N.A. Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery. J. Appl. Polym. Sci. **2003**, *89*, 1606–1613.
33. Kavimandan, N.J.; Peppas, N.A.; Morishita, M.; Goto, T.; Nagai, T.; Takayama, K. Experimental investigation of the effect of complexation hydrogels on insulin transport across model intestinal cell monolayers. Drug Deliv. Syst. **2003**, *18*, 283.
34. Morishita, M.; Goto, T.; Nagai, T.; Peppas, N.A.; Takayama, K. Development of oral insulin delivery systems and evaluation as pharmaceutical dosage forms in diabetic rats. Drug Deliv. Syst. **2003**, *18*, 282.
35. Kim, B.; Peppas, N.A. Analysis of molecular interactions in P(MAA-g-EG) hydrogels. Polymer **2003**, *44*, 3701–3707.
36. Kim, B.; Peppas, N.A. PEG-containing hydrogel microparticles for oral protein delivery applications. Biomed. Microdevices **2003**, *5*, 333–341.
37. Kim, B.; Peppas, N.A. In vitro release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers. Int. J. Pharm. **2003**, *266*, 29–37.
38. Ichikawa, H.; Peppas, N.A. Novel complexation hydrogels for oral peptide delivery: in vitro evaluation of their cytocompatibility and insulin-transport enhancing effects using Caco-2 cell monolayers. J. Biomed. Mater. Res. **2003**, *67*, 609–617.
39. Langer, R.; Peppas, N.A. Advances in biomaterials, drug delivery, and bionanotechnology. AIChE J. **2003**, *49*, 2990–3006.
40. Berger, J.; Reist, M.; Mayer, J.M.; Felt, O.; Peppas, N.A.; Gurny, R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur. J. Pharm. Biopharm. **2004**, *57*, 19–34.
41. Oral, E.; Peppas, N.A. Responsive and recognitive hydrogels using star polymers. J. Biomed. Mater. Res. **2004**, *68A*, 439–447.
42. López, J.E.; Peppas, N.A. Cellular evaluation of insulin transmucosal delivery. J. Biomater. Sci., Polym. Ed. **2004**, *15*, 385–396.
43. Peppas, N.A. Kinetics of smart hydrogels. In *Reflexive Polymers and Hydrogels: Understanding and Designing Fast-Responsive Polymeric Systems*; Yui, N., Mrsny, R., Park, K., Eds.; CRC Press, 2004; 99–114.
44. Nakamura, K.; Murray, R.J.; Joseph, J.I.; Peppas, N.A.; Morishita, M.; Lowman, A.M. Oral insulin delivery using P(MAA-g-EG) hydrogels: effects of network morphology on insulin delivery characteristics. J. Control. Release, **2004**, *95*, 589–599.
45. Lowman, A.M.; Dziubla, T.D.; Bures, P.; Peppas, N.A. Structural and dynamic response of neutral and intelligent networks in biomedical environments. In *Molecular and Cellular Foundations of Biomaterials*; Peppas, N.A., Sefton, M.V., Eds.; Academic Press: New York, 2004; 75–130.
46. Morishita, M.; Goto, T.; Peppas, N.A.; Joseph, J.I.; Torjman, M.C.; Munsick, C.; Nakamura, K.; Takayama, K.; Lowman, A.M. Mucosal insulin delivery systems based on complexation polymer hydrogels: effect of particle size on insulin enteral absorption. J. Control. Release, **2004**, *in press*.

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