Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 266 (2003) 29–37
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

In vitro release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers

Bumsang Kim[1], Nicholas A. Peppas*

Biomaterials, Drug Delivery and Molecular Recognition Laboratories, Departments of Chemical Engineering, Biomedical Engineering, and Pharmaceutics, 1 University Station Code C 0400, The University of Texas, Austin, TX 78712-0231, USA

Received 2 December 2002; received in revised form 4 June 2003; accepted 9 June 2003

Abstract

Novel pH-responsive complexation hydrogels containing pendent glucose (P(MAA-co-MEG)) or grafted PEG chains (P(MAA-g-EG)) were synthesized by photopolymerization. The feasibility of these hydrogels as oral protein delivery carriers was evaluated. The pH-responsive release behavior of insulin was analyzed from both P(MAA-co-MEG) and P(MAA-g-EG) hydrogels. In acidic media (pH 2.2), insulin release from the hydrogels was very slow. However, as the pH of the medium was changed to 6.5, a rapid release of insulin occurred. In both cases, the biological activity of insulin was retained. For P(MAA-co-MEG) hydrogels, the biological activity of insulin decreased when the pendent glucose content increased. In P(MAA-g-EG) hydrogels, when the grafted PEG molecular weight increased, the insulin biological activity decreased. Finally, hydrogels of P(MAA-co-MEG) prepared with an initial ratio of 1:4 MEG:MAA and P(MAA-g-EG) hydrogels containing PEG chains of molecular weights of 200 showed the greatest change in insulin release rate from acidic to basic pH solutions and the greatest protective effect for insulin in simulated GI tract conditions.

Keywords: Hydrogels; pH-responsive; Insulin; Oral drug delivery; Protein-release; Protein-stability

1. Introduction

Oral delivery of drugs, especially therapeutic proteins, is the preferred route of administration because it offers advantages over injection, which is the presently accepted route of therapeutic protein administration. The oral delivery route is more natural and less invasive. The protein drug can be self-administered and the method is less expensive. However, there exist several problems for the development of oral protein delivery systems. One major problem is the degradation of proteins by proteolytic enzymes and the acidic environment of the stomach. Another problem is the low penetration of proteins across the lining of the intestine into the blood stream (Lee and Yamamoto, 1990; Woodley, 1994).

Among the various methods that have been developed to assist to these problems (Shichiri et al., 1974; Arrieta-Molero et al., 1982; Patel et al., 1982; Couvreur and Puisieux, 1993; Rubinstein et al., 1997), use of environmentally sensitive hydrogels, especially methacrylic acid (MAA)-based complexation and pH-sensitive hydrogels, is the most promising method. In general, MAA-based hydrogels can form polymer complexes in response to the environmental

* Corresponding author.
E-mail address: peppas@che.utexas.edu (N.A. Peppas).
[1] Present address: Department of Chemical Engineering, Pennsylvania State University, College Station, PA, USA.

0378-5173/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0378-5173(03)00378-8

pH. In the acidic environment of the stomach, these hydrogels are in a collapsed state due to hydrogen bonding, which can protect proteins by not allowing them to diffuse out from the hydrogel. In the intestine, as the environmental pH increases, the complexes dissociate and the pore size of the hydrogels increases leading to protein (Bell and Peppas, 1996; Lowman and Peppas, 1999; Peppas et al., 1999, 2000; Kim and Peppas, 2002a,b; Robinson and Peppas, 2002; Blanchette et al., 2003). Additionally, the ionized carboxylic acid groups of PMAA have the ability to bind calcium ions in the extracellular medium. Therefore, they can help to minimize the proteolytic activity of calcium-dependent enzymes like trypsin (Lueßen et al., 1995; Madsen and Peppas, 1999) and increase the paracellular permeability of epithelial cell monolayers by opening of tight junctions between two epithelial cells (Bochard et al., 1996; Kriwet and Kissel, 1996).

In our studies, insulin was used as a model protein because it is one of the well known therapeutic proteins and it has become the standard treatment for diabetes. Diabetes mellitus is a disorder caused by decreased production of insulin or by decreased ability to use insulin, leading to increase glucose levels in the blood. Diabetes affects 20 million people in the US, approximately 10% being treated with insulin (Lowman et al., 1999). Usually, insulin is injected subcutaneously two to four times a day. Therefore, there has been significant interest in the development of oral delivery systems for insulin (Saffran et al., 1997; Lowman et al., 1999; Torres-Lugo and Peppas, 1999, 2002; Vauthier et al., 1999).

In this study, the feasibility of MAA-based hydrogels containing various functional groups as oral delivery carriers for proteins was evaluated by investigating the pH-responsive release behavior of insulin in the physiological pH range and protective ability of hydrogels for insulin in simulated gastric solutions.

2. Experimental

2.1. Preparation of microparticles of complexation hydrogels

MAA-based copolymers of MAA and 2-methacryloxyethyl glucoside (MEG), henceforth designated as P(MAA-co-MEG), were prepared by free-radical photopolymerization. In addition, MAA and poly (ethylene glycol) monomethyl ether monomethacrylate (PEGMA), henceforth designated as P(MAA-g-EG), were prepared also by free-radical photopolymerization. MAA (Polysciences, Warrington, PA) was distilled under vacuum prior to use in order to remove an inhibitor, while MEG (Polysciences, Warrington, PA) and PEGMA (Polysciences, Warrington, PA) were used as received. Tetra(ethylene glycol) dimethacrylate (TEGDMA, Polysciences, Warrington, PA) was used as a crosslinking agent without further purification (Kim and Peppas, 2003).

Monomers with feed compositions (molar ratio) of 1:1, 1:2, 1:4, and 1:0 MEG:MAA for P(MAA-co-MEG) hydrogels and of 1:1 EG:MAA for P(MAA-g-EG) hydrogels using PEGMA with various PEG molecular weights (200, 400, and 1000) were mixed. In each set of the monomer mixtures, the TEGDMA was added in the amount of 1.2 mol% of total monomers. 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. See also Torres-Lugo et al. (2002a,b).

The initiator was added in the amount of 0.1% by weight of the total monomers and then these mixtures were diluted to 60% by weight of the total monomers with a 1:1 by weight mixture of ethanol and water. Following complete dissolution of monomers, crosslinking agent, and initiator, nitrogen was bubbled through the mixture for 15 min to remove dissolved oxygen that would act as an inhibitor for the reaction. The mixture was cast between glass slides to form films. The mixture was exposed to UV light (intensity $15.0 \pm 0.5\,mW/cm^2$) for 30 min in nitrogen environment. The kinetics of such polymerization has been discussed extensively (Scott and Peppas, 1999; Scott et al., 2000; Ward and Peppas, 2000).

Hydrogel films were placed in deionized water for 7 days with the water being changed every 12 h in order to remove any unreacted monomers, crosslinking agent, and initiator. Then, the films 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 h. For incorporation, in vitro release, and stability studies of proteins, dried hydrogels were ground into particles. These particles were

passed through sieves in the range between 150 and 212 μm.

2.2. Insulin incorporation

A sample of 50 mg of insulin (from bovine pancreas, 28.1 U/mg, Sigma, St. Louis, MO) was first dissolved in 10 ml of 0.1N HCl and this solution was neutralized with 10 ml of 0.1N NaOH. This insulin solution was then diluted with 80 ml of pH 7.4 phosphate buffer solution to make a 50 mg/ml of insulin stock solution. Incorporation of insulin was accomplished by soaking 140 mg of each set of the dried microparticles of P(MAA-co-MEG) and P(MAA-g-EG) in 20 ml of the insulin stock solution. This loading solution was placed on a microplate rotator. At specific time intervals, 0.3 ml samples were withdrawn from the solution with a syringe (Morishita et al., 2002).

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

2.3. In vitro release and stability studies of insulin

Release experiments were performed using a dissolution test system (model 2100B, Distek, North Brunswick, NJ) and the pH of the external medium was changed from 2.2 to 6.5 to mimic the physiological conditions of the GI tract. Insulin-incorporated microparticles prepared previously were placed in the dissolution test system with 50 ml of pH 2.2 phosphate-citrate buffer solution. After 1 h, the pH of the solution was changed to 6.5 by adding 1N 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 samples were removed (Kim and Peppas, in press; Kim et al., 2003).

To determine the protective ability of the hydrogel for insulin in conditions simulating the human stomach environment (Donini et al., 2002a,b), insulin and insulin-incorporated microparticles were placed in the vessel of the dissolution test system with 50 ml of simulated gastric fluid, test solution. The gastric fluid was prepared by dissolving 2.0 g of sodium chloride and 3.2 g of pepsin in 7.0 ml of HCl and water to make 1000 ml. This test solution had a pH of 1.2. After 1 h, the microparticles were collected by filtration and transferred to 50 ml of pH 7.0 phosphate buffer solution. During the experiment, the temperature and stirring speed were maintained at 37 °C and 100 rpm, respectively. At specific time intervals, 0.3 ml samples were withdrawn.

2.4. Characterization of insulin

In this study, the concentration and stability of insulin were analyzed 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, C_{18}, 25 cm × 0.46 cm, 300 Å, 5 μm particle size, Vydac, Hesperia, CA). The mobile phase consisted of two solutions; solution A was water with 0.1% (v/v) trifluoroacetic acid (TFA) and solution B was acetonitrile with 0.085% (v/v) TFA. The flow gradient was from 72.5 to 64.2% of solution A over 10 min and the flow rate was 1.5 ml/min. UV detection was performed at dual wavelength of 215 and 276 nm.

A bovine insulin ELISA kit (ALPCO, Windham, NH) was used to determine the biological activity of insulin. The results were obtained by reading the optical density with microplate reader (ELx 800NB Bio-Tek Instruments, Winooski, Vermont) at 450 and 650 nm as a reference.

3. Results and discussion

3.1. Insulin incorporation in P(MAA-co-MEG) and P(MAA-g-EG) microparticles

The results of incorporation of insulin into initially dry microparticles of P(MAA-co-MEG) and P(MAA-g-EG) are shown in Figs. 1 and 2, respectively.

We incorporated a large amount of insulin from the insulin stock solution in all the formulations of P(MAA-co-MEG) that contained MAA. Nearly 90% of the insulin was incorporated into the gels within the

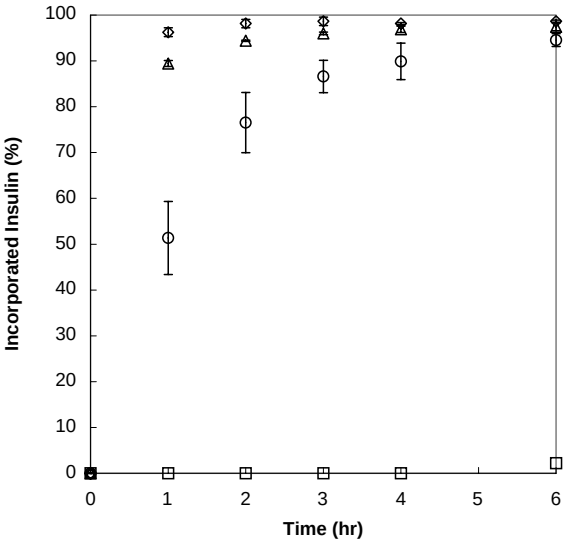

Fig. 1. Incorporation of insulin into P(MAA-co-MEG) microparticles containing different ratios of MEG:MAA at pH 7.4. Molar ratio of MEG:MAA 1:0 (□), 1:1 (○), 1:2 (△), and 1:4 (◇) (average ± S.D., $n = 3$).

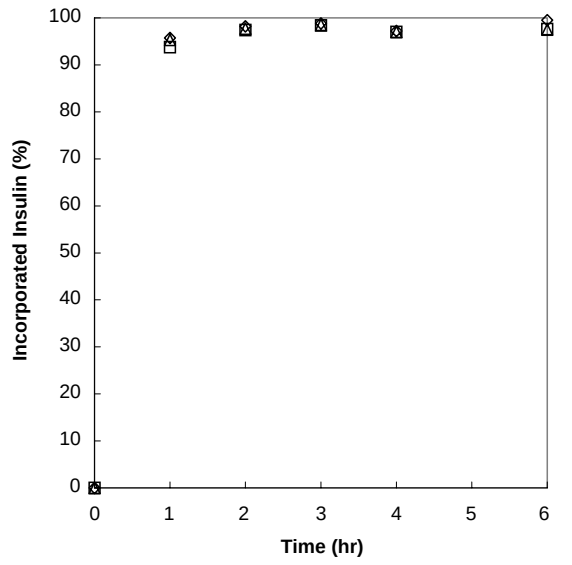

Fig. 2. Incorporation of insulin into P(MAA-g-EG) microparticles containing different grafted PEG chain molecular weight at pH 7.4; PEGMA200 (□), PEGMA400 (△), and PEGMA1000 (◇) (average ± S.D., $n = 3$).

first 1 h except for P(MAA-co-MEG) particles with a 1:1 ratio of MEG:MAA. For the P(MAA-co-MEG) with 1:1 MEG:MAA, the insulin was almost completely incorporated after 6 h. The rate of incorporation was slower when the glucose content increased in the copolymer networks.

When insulin was incorporated at pH 7.0, due to the electrostatic repulsion between ionized carboxylic acid groups of PMAA, the pore size of the networks increased to allow insulin to diffuse readily into the networks by a concentration gradient. However, for P(MAA-co-MEG) networks, the effective mesh size was probably smaller than the actual mesh size due to the presence of the pendent glucose groups. In addition, it has been shown (Miyata et al., 1994; Nakamae et al., 1994; Ichikawa and Peppas, in press) that the presence of glucose decreased protein absorption in similar gels. Therefore, it was difficult for pure PMEG networks to absorb insulin.

For P(MAA-g-EG) microparticles, all the formulations could incorporate more than 90% of the insulin within the first 1 h. It was assumed that the pore size of the network was less affected by the presence of grafted PEG chains compared to the pendent glucose groups of P(MAA-co-MEG) networks. Therefore, there was no significant difference in the rate of incorporation of insulin between gels with varying grafted PEG molecular weights (see also López and Peppas, 2003a,b).

As the pH decreased, the networks collapse and the effective pore size of the networks decreased. The insulin was entrapped inside the gels due to the small pore size of the collapsed networks, producing insulin-incorporated microparticles.

3.2. In vitro release of insulin from P(MAA-co-MEG) and P(MAA-g-EG) microparticles

The pH-responsive release behavior of insulin from the microparticles was studied by placing the initially dry, insulin-incorporated particles in pH 2.2 buffer solutions for 1 h followed by a change of the pH of the medium to 6.5. The fractional release of insulin, defined as the ratio of the amount released at any time, M_t, to the total amount released after 3 h, M_∞, is shown in Figs. 3 and 4, respectively.

In acidic media (pH 2.2), between 20 and 44% of the insulin was released from all formulations ex-

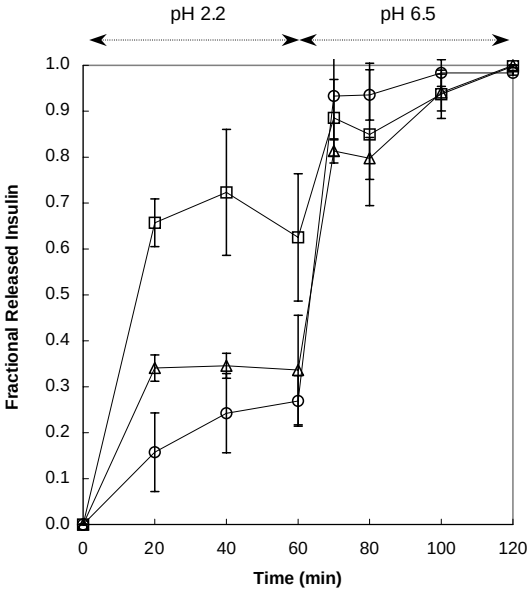

Fig. 3. Effect of the environmental pH change on the release of insulin from the insulin-incorporated P(MAA-co-MEG) microparticles containing different ratios of MEG:MAA. Molar ratio of MEG:MAA 1:1 (□), 1:2 (△), and 1:4 (○) (average±S.D., $n = 3$).

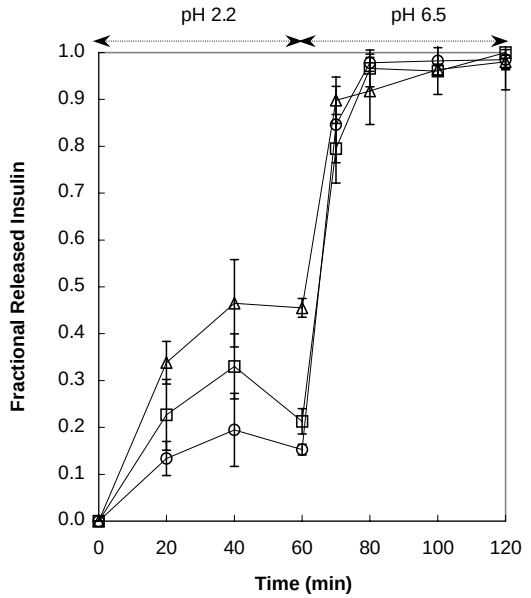

Fig. 4. Effect of the environmental pH change on the release of insulin from the insulin-incorporated P(MAA-g-EG) microparticles containing different grafted PEG chain molecular weight; PEGMA200 (○), PEGMA400 (□), and PEGMA1000 (△) (average ± S.D., $n = 3$).

cept P(MAA-co-MEG) with 1:1 MEG:MAA which released around 70% of its total load. This implies that most of the P(MAA-co-MEG) and P(MAA-g-EG) networks have the desired property for oral delivery of insulin since a significant fraction of insulin remains in the polymer in the low-pH environment of the stomach. When the pH of medium was changed to 6.5, the particles swelled rapidly and a rapid release of insulin occurred.

When the glucose content in the gel increased for P(MAA-co-MEG) networks, there was an increase in release of insulin in the acid media. The increase of glucose content resulted in less collapsed networks at low pH. This led to a relatively large pore size of the networks. Thus, insulin could diffuse readily from the gel at low pH.

For P(MAA-g-EG) networks, the released insulin in the acid media increased with the molecular weight of the grafted PEG in the network. At the incorporating pH of 7.0, the carboxylic acid groups in the networks, as well as the insulin, (p*I* of 5), were negatively charged resulting in repulsion. Thus, the negatively charged insulin was mainly distributed in the neutral PEG chain domains. Researchers such as Moriyama and Yui (1996), Moriyama et al. (1999), and Albertsson et al. (1987) reported that that insulin appeared to partition into the PEG phase in hydrogels containing PEG and a negatively charged component.

When insulin-incorporated P(MAA-g-EG) microparticles were placed in acidic media, particles with longer PEG chains, where more insulin was distributed, had more chance to contact the outer aqueous environment, and as a result insulin was released by a concentration gradient at low pH (Kavimandan and Peppas, 2003). However, there was no significant difference of insulin release at high pHs from systems with different PEG molecular weights.

The P(MAA-co-MEG) with 1:4 MEG:MAA and P(MAA-g-EG) with PEGMA200 hydrogels showed the greatest change in insulin release rate moving from the low-pH to high-pH solutions.

3.3. Protective effect of P(MAA-co-MEG) and P(MAA-g-EG) network for insulin

To investigate the protective ability of the hydrogel for insulin in the harsh environment of the stomach, insulin and insulin-incorporated microparticles were

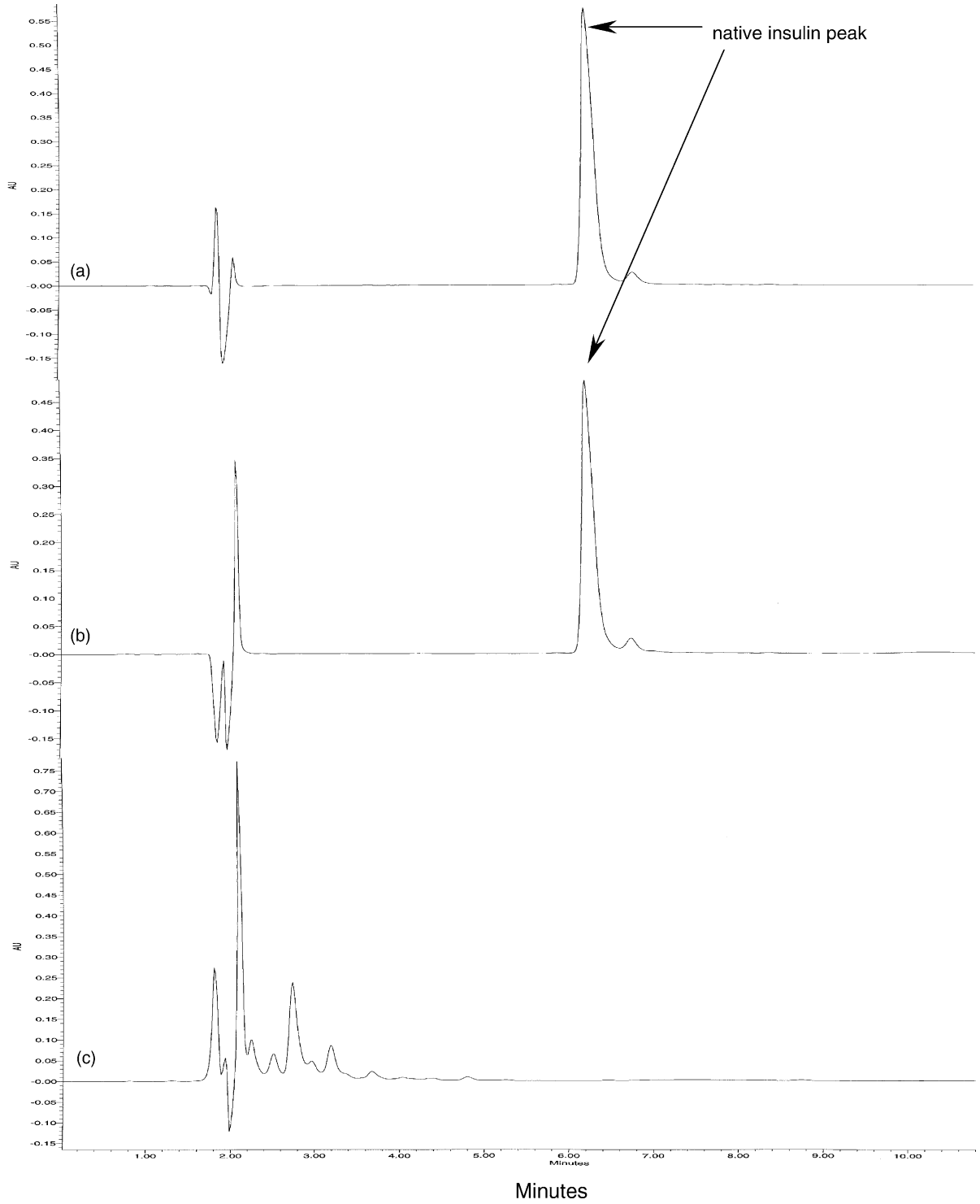

Fig. 5. HPLC chromatograms of insulin solutions; (a) after 2 h dissolved in phosphate buffer solution at pH 7.0, (b) after 2 h dissolved in 0.1N HCl solution at pH 1.2, and (c) right after dissolved in simulated gastric solution at pH 1.2 (temperature = 37 °C).

treated with a simulated gastric solution that contained endopesidase pepsin. After the treatment in gastric solution, the biological activity of insulin was determined with HPLC and an insulin ELISA kit.

Chromatograms of insulin dissolved in phosphate buffer solution (pH 7.0), 0.1N HCl (pH 1.2), and gastric solution (pH 1.2) are presented in Fig. 5. There was little difference between the chromatograms of insulin in phosphate buffer solution (Fig. 5a) and HCl solution (Fig. 5b) after 2 h. This implies that, for a short period of time, the low-pH condition did not significantly affect the biological activity of insulin. However, there was no peak at around 6 min in Fig. 5c that is the HPLC chromatogram of insulin right after dissolved in simulated gastric solution. These results indicated that all insulin was degraded immediately after insulin was in contact with gastric fluid and the main cause of degradation was the proteolytic enzyme, pepsin.

After being treated with gastric fluid, both P(MAA-co-MEG) and P(MAA-g-EG) hydrogels demonstrated a protective effect on insulin (Sipahigil et al., 2002). The biological activity remained after the treatment with gastric fluid of P(MAA-co-MEG) and P(MAA-g-EG) particles (Tables 1 and 2). The biological activity is defined as the ratio of the amount of native insulin released in pH 7.0 buffer solutions for 2 h after 1 h treatment with the gastric fluid to the amount of native insulin released in the pH 7.0 buffer solutions for 3 h. Studies with P(MAA-co-MEG) hydrogel showed that when the glucose content increased, degradation of insulin increased. P(MAA-g-EG) systems when the grafted PEG molecular weight increased, the biological activity decreased, because as previously discussed, some insulin was released from the microparticles to the surrounding medium under these conditions.

Table 1
Biological activity of insulin remained after being treated with the simulated gastric solution (pH 1.2) from the insulin-incorporated P(MAA-co-MEG) microparticles with various molar ratios of MEG and MAA

MEG:MAA (molar ratio)	Biological activity of insulin remained after being treated with simulated gastric solution (%)
1:1	15.3 ± 7.5
1:2	49.4 ± 13.3
1:4	69.4 ± 9.6

The microparticles were transferred to pH 7.0 buffer solution to release the incorporated insulin after being placed in the gastric fluid for 1 h (average ± S.D., $n = 3$).

Table 2
Biological activity of insulin remained after being treated with the simulated gastric solution (pH 1.2) from the insulin-incorporated P(MAA-g-EG) microparticles with various grafted PEG molecular weights

Grafted PEG MW	Biological activity of insulin remained after being treated with simulated gastric solution (%)
200	75.3 ± 11.8
400	63.6 ± 8.2
1000	49.5 ± 9.2

The microparticles were transferred to pH 7.0 buffer solution to release the incorporated insulin after being placed in the gastric fluid for 1 h (average ± S.D., $n = 3$).

The P(MAA-co-MEG) formulation with 1:4 MEG:MAA and the P(MAA-g-EG) formulation with PEGMA200 showed the greatest protective effect for insulin in the simulated stomach conditions.

4. Conclusions

In all the formulations of P(MAA-co-MEG) and P(MAA-g-EG) networks, we could incorporate nearly 90% of insulin from the insulin stock solution after 6 h. However, insulin was not incorporated into pure PMEG networks. For P(MAA-co-MEG) microparticles, the rate of incorporation was slower when the glucose content increased in the copolymer networks. For P(MAA-g-EG) microparticles, there was no significant difference in the rate of incorporated insulin between the grafted PEG molecular weights.

The pH-responsive release behavior of insulin was observed from both P(MAA-co-MEG) and P(MAA-g-EG) microparticles. For the initial treatment in acidic media (pH 2.2), between 20 and 44% of the insulin was released from all the formulations except P(MAA-co-MEG) with 1:1 MEG:MAA which released over 70%. Then, as the pH of the medium was changed into high (pH 6.5), the particles swelled immediately and a rapid release of insulin occurred.

After treatment with a simulated gastric solution, both P(MAA-co-EG) and P(MAA-g-EG) hydrogels demonstrated the desired protective effect of insulin. P(MAA-co-MEG) hydrogels showed when the pendent glucose content increased degradation of insulin

increased and P(MAA-g-EG) demonstrated when the grafted PEG molecular weight increased the biological activity decreased.

P(MAA-co-MEG) with 1:4 MEG:MAA and P(MAA-g-EG) with PEGMA200 hydrogels showed the greatest change in insulin release rate as the pH changed from low to high values and created the greatest protective effect for insulin in the simulated stomach condition.

Acknowledgements

This work was supported by a grant from the National Institutes of Health (No. EB 00246-11). This work is dedicated to Prof. Hans Junginger of the University of Leiden on the occasion of his 60th birthday. His work has provided significant insight to our work.

References

Albertsson, P.-Å., Cajarville, A., Brooks, D.E., Tjerneld, F., 1987. Partition of proteins in aqueous two-phase systems and the effect of molecular weight of the polymer. Biochim. Biophys. Acta 926, 87–93.

Arrieta-Molero, J.F., Aleck, K., Shina, M.K., Brownschneidle, C.M., Shapiro, L.J., Sperling, M.A., 1982. Orally administered liposome-entrapped insulin in diabetic animals. A critical assessment. Horm. Res. 16, 249–256.

Bell, C.L., Peppas, N.A., 1996. Water, solute and protein diffusion in physiologically responsive hydrogels of poly(methacrylic acid-g-ethylene glycol). Biomaterials 17, 1203–1218.

Blanchette, J., Park, K., Peppas, N.A., 2003. Use of complexation hydrogels for oral administration of chemotherapeutic agents. Trans. Soc. Biomat. 29, 246.

Bochard, G., Lueßen, H.L., de Boer, A.G., Verhoef, J.C., Lehr, C.-M., Junginger, H.E., 1996. The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption. III: effects of chitosan-glutamate and carbomer on epithelial tight junctions in vitro. J. Control. Release 39, 131–138.

Couvreur, P., Puisieux, F., 1993. Nano-and microparticles for the delivery of polypeptides and proteins. Adv. Drug Deliv. Rev. 10, 141–162.

Donini, C., Giordano, F., Colombo, P., Peppas, N.A., 2002a. Characterization and optimization of P(MAA-g-EG) insulin-loaded nanospheres. Proceedings of the World Meeting on APGI/APV 4, 695–696.

Donini, C., Robinson, D.N., Colombo, P., Giordano, F., Peppas, N.A., 2002b. Preparation of P(MAA-g-EG) nanospheres for pharmaceutical applications. Int. J. Pharm. 245, 83–91.

Ichikawa, H., Peppas, N.A., in press. 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.

Kavimandan, N.J., Peppas, N.A., 2003. Insulin transport across cell monolayers in the presence of complexation hydrogels. Trans. Soc. Biomater. 29, 248.

Kim, B., Peppas, N.A., 2002a. Synthesis and characterization of pH-sensitive glycopolymers for oral drug delivery systems. J. Biomater. Sci. Polym. Ed. 13, 1271–1281.

Kim, B., Peppas, N.A., 2002b. Complexation phenomena in pH-responsive copolymer networks with pendent saccharides. Macromolecules 35, 9545–9550.

Kim, B., Peppas, N.A., 2003. Analysis of molecular interactions in P(MAA-g-EG) hydrogels. Polymer 44, 3701–3707.

Kim, B., Peppas, N.A., in press. PEG-containing hydrogel microparticles for oral protein delivery applications. Biomed. Microdevices.

Kim, B., La Flamme, K., Peppas, N.A., 2003. Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery. J. Appl. Polym. Sci. 89, 1606–1613.

Kriwet, B., Kissel, T., 1996. Poly(acrylic acid) microparticles widen the intercellular spaces of Caco-2 cell monolayers: examination by confocal laser scanning microscopy. Eur. J. Pharm. Biopharm. 42, 233–240.

Lee, V.H.L., Yamamoto, A., 1990. Penetration and enzymatic barriers to peptide and protein absorption. Adv. Drug Deliv. Rev. 4, 171–207.

López, J.E., Peppas, N.A., 2003a. Cellular evaluation of insulin transmucosal delivery. J. Biomater. Sci. Polym. Ed. 15, 18–31.

López, J.E., Peppas, N.A., 2003b. Effect of PEG molecular weight and microparticle size on oral insulin delivery from P(MAA-g-EG) microparticles. Eur. J. Pharm. Biopharm 57, 111–118.

Lowman, A.M., Peppas, N.A., 1999. Solute transport analysis in pH-sensitive, complexing hydrogels of poly(methacrylic acid-g-ethylene glycol). J. Biomat. Sci. Polym. Ed. 10, 999–1009.

Lowman, A.M., Morishita, M., Kajita, M., Nagai, T., Peppas, N.A., 1999. Oral delivery of insulin using pH-responsive complexation gels. J. Pharm. Sci. 88, 933–937.

Lueßen, H.L., Bochard, G., Verhoef, J.C., Lehr, C.-M., de Boer, A.G., Junginger, H.E., 1995. Mucoadhesive polymers in peroral peptide drug delivery. II. Carbomer and polycarbophil are potent inhibitors of the intestinal proteolytic enzyme trypsin. Pharm. Res. 12, 1293–1298.

Madsen, F., Peppas, N.A., 1999. Complexation graft copolymer networks: swelling properties, calcium binding and proteolytic enzyme inhibition. Biomaterials 20, 1701–1908.

Miyata, T., Ootsuki, N., Nakamae, K., Okumura, M., Kinomura, K., 1994. Protein adsorption on a copolymer having pendent monosaccharide groups: relationship between surface free energy and protein adsorption. Macromol. Chem. Phys. 195, 3597–3607.

Morishita, M., Lowman, A.M., Takayama, K., Nagai, T., Peppas, N.A., 2002. Elucidation of the mechanism of incorporation of insulin in controlled release systems based on complexation polymers. J. Control. Release 81, 25–32.

Moriyama, K., Yui, N., 1996. Regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol). J. Control. Release 42, 237–248.

Moriyama, K., Ooya, T., Yui, N., 1999. Hyaluronic acid grafted with poly(ethylene glycol) as a novel peptide formulation. J. Control. Release 59, 77–86.

Nakamae, K., Miyata, T., Ootsuki, N., Okumura, M., Kinomura, K., 1994. Surface characterizations of copolymer films with pendent monosaccharides. Macromol. Chem. Phys. 195, 1953–1963.

Patel, H.M., Stevenson, R.W., Parsons, J.A., Ryman, B.E., 1982. Use of liposomes to aid intestinal absorption of entrapped insulin in normal and diabetic dogs. Biochim. Biophys. Acta 716, 188–193.

Peppas, N.A., Keys, K.B., Torres-Lugo, M., Lowman, A.M., 1999. Poly(ethylene glycol)-containing hydrogels in drug delivery. J. Control. Release 62, 81–87.

Peppas, N.A., Bures, P., Leobandung, W., Ichikawa, H., 2000. Hydrogels in pharmaceutical formulation. Eur. J. Pharm. Biopharm. 50, 27–46.

Robinson, D.N., Peppas, N.A., 2002. Preparation and characterization of pH-responsive poly(methacrylic acid-g-ethylene glycol) nanospheres. Macromolecules 35, 3668–3674.

Rubinstein, A., Tirosh, B., Baluom, M., Nassar, T., David, A., Radai, R., Irit-Kabir, I., Friedman, M., 1997. The rational for peptide drug delivery to the colon and the potential of polymeric carriers as effective tools. J. Control. Release 46, 59–73.

Saffran, M., Pansky, B., Colin Budd, G., Williams, F.E., 1997. Insulin and the gastrointestinal tract. J. Control. Release 46, 89–98.

Scott, R.A., Peppas, N.A., 1999. Kinetics of copolymerization of PEG-containing multiacrylates with acrylic acid. Macromolecules 32, 6149–6158.

Scott, R., Ward, J.H., Peppas, N.A., 2000. Development of acrylate and methacrylate polymer networks for controlled release by photopolymerization technology. In: Wise, D.L., Brannon-Peppas, L., Klibanov, A.M., Langer, R., Mikos, A.G., Peppas, N.A., Trantolo, D.J., Wnek, G.E., Yaszemski, M.J. (Eds.), Handbook of Pharmaceutical Controlled Release Technology. Dekker, New York, pp. 47–64.

Shichiri, M., Shimizu, Y., Yoshida, Y., Kawamori, R., Fukychi, M., Shigeta, Y., Abe, H., 1974. Enteral absorption of water-in-oil-in-water insulin emulsions in rabbit. Diabetologia 10, 317–321.

Sipahigil, O., Torres-Lugo, M., Peppas, N.A., 2002. FTIR spectroscopic analysis of protein/carrier interactions in novel protein delivery systems. S.T.P. Pharma Sci 12, 345–350.

Torres-Lugo, M., Peppas, N.A., 1999. Molecular design and in vitro studies of novel pH-sensitive hydrogels for the oral delivery of calcitonin. Macromolecules 32, 6646–6651.

Torres-Lugo, M., Peppas, N.A., 2002. Preparation and characterization of P(MAA-g-EG) nanospheres for protein delivery applications. J. Nanopar. Res. 4, 73–81.

Torres-Lugo, M., Garcia, M., Record, R., Peppas, N.A., 2002a. Physicochemical behavior and cytotoxic effects of P(MAA-g-EG) nanospheres for oral delivery of proteins. J. Control. Release 80, 197–205.

Torres-Lugo, M., Garcia, M., Record, R., Peppas, N.A., 2002b. pH-sensitive hydrogels as gastrointestinal tract absorption enhancers: transport mechanisms of salmon calcitonin and other model molecules using the Caco-2 cell model. Biotechnol. Prog. 18, 612–616.

Vauthier, C., Seiller, M., Weingarten, C., Couvreur, P., 1999. Contribution to the development of oral dosage form for insulin delivery. S.T.P. Pharma Sci. 9, 391–396.

Ward, J.H., Peppas, N.A., 2000. Kinetic gelation modeling of controlled radical polymerization. Macromolecules 33, 5137–5142.

Woodley, J.F., 1994. Enzymatic barriers for GI peptide and protein delivery. Crit. Rev. Ther. Drug 11, 61–95.