



METHOD FOR ORAL DELIVERY OF PROTEINS

Field of the Invention

The present invention relates to a composition comprising a swellable
5 hydrogel matrix and a protein contained therein, and the use of such a composition for
oral delivery of bioactive compounds in an active form to the intestines of a
vertebrate.

Background of the Invention

10 Two major problems exist in developing oral delivery systems for
proteinaceous compounds such as insulin. The first problem is the inactivation of
many proteins by digestive enzymes in the gastrointestinal (GI) system, mainly in the
stomach. This can be overcome by designing carriers which would protect the protein
from the harsh environments of the stomach before releasing the drug into more
15 favorable regions of the GI tract, specifically the lower regions of the intestine.
Additionally, protease inhibitors can be used to retard the action of enzymes present in
the GI system which could degrade orally administered proteins. The other problem is
the slow transport of intact large peptides, across the lining of the intestine into the
blood stream. Researchers have attempted to bypass this hurdle with the addition of
20 absorption enhancers which aid the transport of macromolecules across boundaries.
However, currently available delivery vehicles suffer from a lack of effectiveness.
Accordingly, an oral delivery system is desired that is effective and can be prepared at
relatively low cost.

25 Summary of the Invention

The present invention is directed to a composition comprising a
hydrogel matrix carrier and a bioactive compound, and the use of that composition to
deliver the compound in an active form to the intestines. One preferred hydrogel
matrix comprises a copolymer network of poly(methacrylic acid-g-ethylene glycol)
30 crosslinked with tetraethylene glycol dimethacrylate, "P(MAA-g-EG) hydrogels", that
exhibit pH dependent swelling behavior due to the presence of acidic pendant groups
and the formation of interpolymer complexes between the etheric groups on the graft

chains and protonated pendant groups. In acidic media, such systems are relatively unswollen due to the formation of the interpolymer complexes. In basic solutions, the pendant groups ionize and the complexes dissociate. The pH dependent swelling of these hydrogels together with their bioadhesive properties make these hydrogels ideal for oral delivery of proteins.

Brief Description of the Drawings

Fig. 1 Reversible complexation in P(MAA-g-EG) hydrogels. C represents the entrapped bioactive compound.

Fig. 2 Equilibrium polymer volume fraction as a function of pH for samples containing PEG grafts of MW 1000 and a MAA:EG molar ratio of 1:1 at 37°C.

Fig. 3 Equilibrium mesh size as a function of pH for samples containing PEG grafts of MW 1000 and a MAA:EG molar ratio of 1:1 at 37°C.

Fig. 4 Controlled release of proxyphylline in solutions of pH of 3.2 (●) and 7.4 (■) and vitamin B₁₂ at pH of 3.2 (□) and 7.4 (○) in buffered saline solutions at 37°C.

Fig. 5a Pulsatile release of theophyllin *in-vitro* from P(MAA-g-EG) hydrogels at 37°C.

Fig. 5b Pulsatile release of vancomycin *in-vitro* from P(MAA-g-EG) hydrogels at 37°C.

Fig. 5c Pulsatile release of insulin *in-vitro* from P(MAA-g-EG) hydrogels at 37°C.

Fig. 6 Adhesive behavior of P(MAA-g-EG) hydrogels containing a 1:1 MAA/EG ratio and graft PEG chains of molecular weight 1000 at pH values of 3.2 and 7.4 in contact with bovine submaxillary gland mucin.

Fig. 7 Blood glucose concentration in rats after oral administration of insulin loaded P(MAA-g-EG) hydrogels at 25 U/kg (○) and 50 U/kg (●) and 50 U/kg insulin solution (■) (N = 5).

Fig. 8 Blood glucose concentration in rats after oral administration of insulin loaded P(MAA-g-EG) hydrogels at (25 U/kg dose) without aprotinin (○) and with aprotinin (●) and 50 U/kg insulin solution (■) (N = 5).

Fig. 9 Blood glucose response in healthy (●) and diabetic (o) male Wistar rats after the oral administration of P(MAA-g-EG) microspheres (25 IU/kg body weight insulin doses) using gelatin capsules.

Fig. 10 Blood glucose response in diabetic male rats after oral administration of P(MAA-g-EG) microspheres (25 IU/kg body weight) using Eudragit capsules.

Fig. 11 Blood glucose response in healthy dogs (25kg) after the oral administration of P(MAA-g-EG) microspheres (10 IU/kg body weight insulin doses) using gelatin capsules.

Fig. 12 Blood glucose response in diabetic dogs (25 kg) oral administration of P(MAA-g-EG) microspheres (10 IU/kg body weight insulin doses) using gelatin capsules.

Detailed Description of the Invention

The present invention is directed to compositions for delivering biologically active proteins and pharmaceuticals to vertebrates via oral administration. The term bioactive compound as used herein refers to any compound that has an effect on living cells, for example a compound that induces a biochemical effect in a cell. In accordance with one embodiment the orally administered composition comprises a swellable hydrogel matrix, and a labile protein contained within the swellable hydrogel matrix. A labile protein as used herein includes any protein whose biological activity is destroyed or diminished by exposure to low pH or exposure to enzymes present in the digestive tract of warm blooded species.

Hydrogels are water swellable, cross-linked polymer matrices that are well known to those of ordinary skill in the art. See, for example, Dresback, U.S. Patent No. 4,220,152, issued September 2, 1980, the disclosure of which is expressly incorporated herein by reference. Hydrogels have been found to be an effective delivery vehicle for orally delivering proteins to vertebrate species. The swellable properties of hydrogels can be utilized, first to protect the hydrogel contents from the harsh environments of the stomach as the composition passes through the digestive tract, and then to release the hydrogel contents into the more favorable regions of the GI tract, specifically the lower regions of the intestine. The hydrogel compositions of

the present invention have been found to pass through the stomach without substantial swelling and become localized in the small intestine, where they swell and concomitantly release their contents.

Hydrogels can be impregnated or loaded with a variety of bioactive compounds, including but not limited to pharmaceuticals, growth hormones, vaccine compositions, vitamins, steroids and peptides, and used as a delivery vehicle for orally administering such bioactive compounds. Compounds loaded into the hydrogel are released in a controlled manner as the hydrogel becomes hydrated within the animal's digestive system. In one embodiment, the present hydrogel matrix is in a pelletized form comprised of polymethacrylic acid, and the polymethacrylic acid polymers are grafted with an ionic long chain polymer such as polyethylene glycol (PEG).

The hydrogel pellets are preferably synthesized by polymerizing methacrylic acid, in the presence of a crosslinking agent. The crosslinking agent can be selected from a wide variety of biocompatible crosslinking agents known to those skilled in the art including tetraethylene glycol dimethacrylate, ethylene dimethacrylate, diethylene dimethacrylate, triethylene dimethacrylate, tetraethylene dimethacrylate, pentaethylene dimethacrylate, the corresponding diacrylates, or a star polymer comprising methacrylate, acrylate or methylene bis-acrylamido groups. Polymerization is initiated with a free radical initiator such as thermal initiators including organic peroxides or UV radical initiators known to those skilled in the art.

In one embodiment the hydrogel matrix comprises a co-polymer of methacrylic acid and a poly(alkylene glycol) monomethacrylate (or monoacrylate) crosslinked with a biocompatible crosslinking agents. "Poly(alkylene glycol) monomethacrylate" as used herein includes poly(ethylene glycol) monomethacrylate, poly(propylene glycol) monomethacrylate and poly(ethylene/propylene glycol) monomethacrylate, wherein poly(ethylene/propylene glycol) monomethacrylate is the polymer formed by hydroxy functional methacrylate initiated polymerization of a mixture of ethylene oxide and propylene oxide. The resulting pendant poly(alkylene glycol) groups have a molecular weight ranging from about 200 to about 4000, more typically about 200 to about 2000, and in one embodiment about 200 to about 1200. The molar ratio of methacrylic acid and poly(alkylene glycol) monomethacrylate (or monoacrylate) monomers is about 4:1 to about 1:4.

In one preferred embodiment the hydrogel matrix comprises a polymer of methacrylic acid and poly(ethylene glycol) monomethacrylate crosslinked with tetraethylene glycol dimethacrylate, "P(MAA-g-EG) hydrogels". In preparation of that polymer, poly(ethylene glycol) monomethacrylate having a molecular weight of about
5 200 to about 2000, more typically about 200 to about 1200 is co-polymerized with methacrylic acid and tetraethylene glycol dimethacrylate. The molar ratio of methacrylic acid and poly(ethylene glycol) monomethacrylate monomers ranges from about 4:1 to about 1:4. In one embodiment, the molar ratio of methacrylic acid and poly(ethylene glycol) monomethacrylate monomers is about 1:1. The crosslinking
10 agent is added in an amount of about 0.25 to about 10.00 mol%, more preferably at about 0.25 to about 1.00 mol% and in one embodiment, about 0.75 mol%.

The hydrogels can be loaded with the desired compounds using standard techniques known to those skilled in the art. In one embodiment the P(MAA-g-EG) hydrogels are formed as microparticles ranging in size from about 50 μm to
15 about 500 μm in diameter, more preferably about 100-200 μm in diameter. The hydrogel microparticles are formed in accordance with one embodiment by forming a polymerized matrix and grinding the matrix to form a hydrogel particulate having the desired average particle size. The hydrogel microparticles can be loaded with the desired compound and packaged in standard tablet or capsule forms using standard
20 techniques known to those skilled in the art. In one embodiment the hydrogel particles are packaged in a gelatin capsule.

In accordance with one embodiment the hydrogels are loaded with bioactive compounds by equilibrium portioning. More particularly, the hydrogels are hydrated in a solution having a $\text{pH} > 5.8$ and containing the composition to be loaded.
25 The hydrogels are then recovered and washed with a solution having a pH of < 5.8 and the loaded hydrogels are then dried and stored at 4°C . Another method of loading the hydrogels of the present invention comprises the steps of adding an aqueous solution of the desired compound to a solution of monomers and a cross-linker, and initiating polymerization of the mixture.

30 The ability of a compound to diffuse through a crosslinked polymer network is dependent on the degree to which the gel swells and the size of compound. As a hydrogel swells, the polymer chains between crosslink points are elongated and

the network mesh size or correlation length, ξ , is increased allowing for greater solute permeation in the material. (See Fig. 1) In equilibrium swollen complexation networks, the degree of network swelling is dependent on the number of chemical and physical crosslinks present in the system. In P(MAA-g-EG) networks, the equilibrium swelling ratio is strongly dependent on the pH of the surrounding environment.

The P(MAA-g-EG) hydrogels of the present invention, form temporary physical crosslinks when exposed to acidic conditions (typically $\text{pH} < 5.8$) due to hydrogen bonding between the polymethacrylate groups and the pendant poly(alkylene glycol) groups. These physical crosslinks are reversible in nature and dependent on the pH and ionic strength of the environment. Thus, the degree of crosslinking and network mesh size, ξ , are strongly dependent on the pH and ionic strength of the surrounding environment. In acidic media, such systems are relatively unswollen due to the formation of the intermacromolecular complexes. In basic solutions, the pendant groups ionize and the complexes dissociate. The equilibrium swelling of P(MAA-g-EG) hydrogels is shown in Fig. 2. The data is presented as the polymer volume fraction of the hydrogels (PEG grafts of 1000MW and MAA:EG molar ratio of 1:1) as a function of pH. For the case of equimolar amounts of MAA and EG at low pH values, the degree of complexation was high and the polymer volume fraction in the gel in the swollen state, $v_{2,s}$, was almost 0.70. However, as the pH of the swelling solution increased above $\text{pH} = 4.6$, the complexes began to dissociate and the backbone chains extended resulting in a significant decrease in the equilibrium polymer volume fraction in the gel. The highly swollen, non-complexed hydrogels contained less than 5% polymer as more water was incorporated into the structure.

Because of the complexation/decomplexation phenomena in the P(MAA-g-EG) gels, the mesh size of the networks will vary significantly over the pH range of interest. Additionally, the moduli of the hydrogels for small deformations (less than 10%) were obtained in solutions of differing pH. Using these data, the mesh sizes were calculated as a function of pH by determining the end-to-end distance of the polymer chains between the crosslinks, both covalent and physical. The average network mesh size or correlation length was dramatically affected by the pH of the swelling solution (Fig. 3). In low pH solutions in which complexation will occur, the network mesh sizes for P(MAA-g-EG) hydrogels were as low as 70 Å. However, as

the pH was increased the physical crosslinks dissociated and the polymer chains elongated resulting in an increase in the network mesh size by a factor of 3, to almost 210 Å. More importantly, assuming ideal networks, the available area for diffusion is equal to the square of the mesh size. Thus, there exists 9 times greater area for
5 diffusion in the non-complexed hydrogels (pH greater than 5.2) than the complexed hydrogels (pH less than 5.2). Because of the reversible nature of the complexation phenomena, the P(MAA-g-EG) hydrogels are ideal for the oscillatory release of drugs. Additionally, due to the large changes in the network structure over a small pH change, these materials function well as carriers for peptides and proteins. In
10 particular, the P(MAA-g-EG) hydrogels of the present invention can serve as a delivery vehicle for compounds having a molecular weight ranging from about 1,000 to about 100,000, more preferably ranging from about 1,000 to about 20,000

The important parameter in evaluating the potential of a gel to serve as a carrier for a particular drug is the ratio of the effective molecular size (hydrodynamic
15 diameter, d_h) to the network mesh size. In order to study the size-exclusion characteristics of these networks, the release of two solutes of differing molecular size, proxyphylline (molecular weight 238 and $d_h = 4.3$ Å) and vitamin B₁₂ (molecular weight 1355 and $d_h = 17$ Å), from complexed and non-complexed hydrogels was studied (Fig. 4). In solutions of pH = 3.2, the hydrogel polymers were highly
20 complexed, and the transport of drug was significantly hindered. Less than 10% of the vitamin B₁₂ diffused out of the network in two hours. However, due to its smaller size, almost 30% of the proxyphylline was released from the gel in the same time period. When the hydrogels were contacted with a solution of pH = 7.4, the interchain complexes of the hydrogel disassociated due to ionization of the pendant acid groups.
25 As a result, the hydrogels swelled to a large degree allowing for substantial diffusion of vitamin B₁₂ and proxyphylline from the polymers.

The release data were fit to the short time approximation for the solution of the classical Fickian expression for planar systems and the diffusion coefficients were calculated for the diffusion of proxyphylline and vitamin B₁₂ through
30 complexed and non-complexed P(MAA-g-EG) hydrogels (Table 1). The transport of the larger molecular weight solute, vitamin B₁₂, was more significantly affected by complexation than proxyphylline due to the increased ratio of solute diameter to the

network mesh size. The diffusion coefficient for vitamin B₁₂ from the non-complexed hydrogels was two orders of magnitude higher than that for the complexed hydrogels, while the proxiphylline diffusion coefficient was only one order of magnitude higher for the non-complexed hydrogels relative to the complexed hydrogels.

5

Table 1. Diffusion coefficients for proxiphylline and vitamin B₁₂ in complexed and non-complexed P(MAA-g-EG) hydrogels.

	Solute	pH	$\xi(\text{\AA})$	d_p/ξ	$D_{3,12} \times 10^8$ (cm ² /s)
10	proxiphylline	3.2	70.8	0.060	0.403
	proxiphylline	7.4	194.4	0.022	9.38
	vitamin B ₁₂	3.2	70.8	0.240	0.0168
	vitamin B ₁₂	7.4	194.4	0.087	6.75

15

To further investigate the ability of P(MAA-g-EG) hydrogels to function as oral delivery vehicles for vitamins, pharmaceuticals and other bioactive compounds, the pulsatile release of various compounds was determined under simulated gastrointestinal conditions. *In vitro* release experiments were performed using theophylline (MW=180.2) vancomycin (MW=1485.7) and insulin (MW=5733.2).

20

See Example 1. Each of the compounds was loaded into P(MAA-g-EG) hydrogels by equilibrium partitioning and then the loaded hydrogels were soaked in 200 ml of pH = 1.2, simulated gastric fluid for 2 hours. The polymer microparticles were then transferred to pH = 6.8 phosphate buffer solutions. The insulin concentration released into the surrounding solution was monitored using HPLC and the results for

25

theophylline, vancomycin and insulin are shown in Fig. 5a-5c respectively.

Release of the compounds from the hydrogel matrix is reduced in the acidic solution (see Fig. 2, first two hours of exposure). However, a rapid release of the compound is observed in the pH 6.8 buffer solution. This trend became more pronounced as the drug molecular weight increased. For example the P(MAA-g-EG) hydrogels are effective delivery vehicles for insulin (MW = 5733.2): Less than 10% of the insulin was released from the polymer in the simulated gastric fluid (pH - 1.3) during the first phase of the experiment. However, after the particles were placed in pH = 7.4 buffer solution, the hydrogels swelled rapidly allowing for a rapid release of

30

insulin. These results indicate that the graft copolymers P(MAA-g-EG) are useful for development of oral insulin delivery system. As used herein the term insulin is intended to include purified human and animal natural insulin as well as derivatives thereof, such as insulin lispro and recombinant forms of insulin, and mono or divalent salts of insulin or insulin derivatives.

Additionally, P(MAA-g-EG) hydrogels exhibit strong mucoadhesive characteristics due to the presence of the grafted PEG chains, which serve as adhesion promoters. Furthermore, the mucoadhesive characteristics of P(MAA-g-EG) hydrogels is strongly dependent on the pH of the environmental fluid (See Fig. 6).

The adhesion between the gel and the mucous is significantly greater in conditions simulating the intestinal pH (pH = 7.4) relative to conditions simulating the stomach environment. However, to truly compare the mucoadhesive characteristics of the gels, the work of adhesion was normalized to account for the polymer gel fraction. The normalized work of adhesion was two-orders of magnitude greater for hydrogels in the non-complexed state. Accordingly, the mucoadhesive properties of the P(MAA-g-EG) hydrogels will be relatively low as they pass through the stomach and remain in a complexed state. After reaching the intestines the interchain complexes will dissociate, thus enhancing the adhesion to the hydrogels to the mucosa of the intestine relative to stomach mucosa. Therefore, the residence time of insulin carriers is much greater in regions where the insulin could be absorbed (i.e. in the intestine) after oral administration to a vertebrate.

The differences in the adhesive characteristics of the hydrogels at different pH values are due to mobility of the PEG chains in each material. In the highly swollen, non-complexed state, the pendant PEG chains are free and readily penetrated the mucosa to serve as anchors for adhesion. In the complexed state, the pendant PEG chains in the P(MAA-g-EG) form complexes with the backbone chains and are unavailable for interactions with mucosal surfaces.

In accordance with the present invention the hydrogel compositions can be utilized to administer a therapeutically effective amount of a protein to a vertebrate.

The method comprises the step of orally administering to a vertebrate, a composition comprising the protein contained within a hydrogel carrier. The composition contained within the hydrogel matrix may further comprise protease inhibitors, pharmaceutically

acceptable carriers, stabilizing agents and biocompatible fillers known to those of ordinary skill in the art.

One preferred hydrogel carrier is P(MAA-g-EG), and in one embodiment the P(MAA-g-EG) hydrogel matrix contains a pharmaceutically acceptable composition comprising insulin. Furthermore, in accordance with one embodiment the insulin composition further comprises a protease inhibitor or an absorption enhancer. Compositions comprising insulin contained within a P(MAA-g-EG) hydrogel have been shown to be surprisingly effective in delivering insulin to the blood stream of animals (see Examples 3 and 4). The hydrogel matrix is typically prepared in particulate form and packaged within a suitable oral delivery vehicle (i.e. tablet, capsule, etc.) using techniques known to those skilled in the art.

In one embodiment, the delivery system consists of microparticles of crosslinked copolymers of poly(methacrylic acid) and poly(ethylene glycol) and contains insulin. This system is particularly effective because the structure of the copolymers exhibits pH sensitive swelling behavior that allows for protection of the insulin while the composition passes through the harsh environment of the stomach. The pendant PEG chains also serve as adhesive promoters to increase the resident time of the hydrogel carrier at the intended delivery site. As noted in Example 2 the mucoadhesive properties of the hydrogels is strongly influenced by pH thus favoring adhesion to intestinal surfaces over the surface of the stomach. Additionally, the presence of the pendant PEG polymers serve as peptide stabilizers and help maintain the biological activity of bioactive compounds such as insulin.

The inter-chain complex formation in the hydrogel copolymers is sensitive to the nature and pH of the surrounding fluid as well as the copolymer composition and graft chain length. In the acidic environment of the stomach, the hydrogels are in the complexed state due to the formation of interpolymer complexes stabilized by hydrogen bonding between the carboxylic acid protons and the etheric groups on the grafted chains. In these conditions compounds having a molecular weight size of at least 1000 (insulin, for example) cannot readily diffuse through the membrane because of the small pore size, ξ , and thus these compounds are protected from the harsh environment of the stomach. As the particles pass through the stomach and into the intestine, the environmental pH increases above the transition pH of the

gel. The complexes immediately dissociate and the network pore size rapidly increases leading to the release of compounds having a molecular weight size of less than 100,000. Accordingly the P(MAA-g-EG) hydrogels can be used as an effective oral delivery vehicle for compounds having a molecular weight ranging from about 1,000 to about 100,000.

Example 1

pH Dependent Release of P(MAA-g-EG) Hydrogel Contents

The ability of P(MAA-g-EG) hydrogels to function as delivery vehicles was investigated for three compounds of different sizes: theophylline (MW 180.2), vancomycin (MW 1485.7) and insulin (MW 5733.2).

P(MAA-g-EG) hydrogels were prepared at 37° C by free-radical solution polymerization of methacrylic acid and poly(ethylene glycol) monomethacrylate, and the oligomer chains were crosslinked with tetraethylene glycol dimethacrylate. The ensuing hydrogels were rinsed for a week in deionized water to remove unreacted monomer and non-crosslinked oligomer chains, dried under vacuum and ground into a powder having an average particulate diameter ranging from 100-150 μ m.

Drug incorporation experiments were performed using theophylline (MW 180.2), vancomycin (MW 1485.7) and insulin (MW 5733.2). Each drug was dissolved in a pH 7.4 phosphate buffer solution and P(MAA-g-EG) hydrogels were added to the drug solution to load the hydrogels by equilibrium partitioning. The hydrogel matrix was then contacted with an acid solution to induce the formation of interpolymer complexes, and thus reduce the pore size of the hydrogel matrix. The hydrogel microspheres were then collected by filtration, and dried under vacuum. Incorporation efficiencies were calculated from the residual drug amount of the concentrations of the initial solutions and the filtrate obtained from the washings of the isolated hydrogels, as determined from HPLC analysis.

Drug release experiments were performed following the Japanese pharmacopoeia (JP) paddle method. The compositions were stirred with a paddle at 100 rpm and 37° C in a first (pH 1.2) and a second (pH 6.8) fluid of JP. After 2 hours of treatment with the first fluid, the polymer samples were collected by filtration and

transferred to the second fluid of pH 6.8. The drug concentration was monitored by HPLC.

The mean insulin incorporation efficiency into the hydrogel matrix reached 94% at 30 min after starting the experiment, thus the polymer is thought to be a suitable carrier for insulin. The results of the release experiments for theophylline, vancomycin and insulin from P(MAA-g-EG) hydrogels are shown in Fig. 5a, 5b and 5c, respectively. Release of the compounds from the hydrogel matrix is reduced in the acidic solution (see first two hours of exposure). However, a rapid release of the compound is observed in pH 6.8 buffer solution. This trend became more pronounced as the drug molecular weight increased; less than 10% of the insulin was released from the polymer in the simulated gastric fluid (pH = 1.3) during the first phase of the experiment. However, after the particles were placed in pH = 7.4 buffer solution, the hydrogels swelled rapidly allowing for a rapid release of insulin. These results indicate that the graft copolymers P(MAA-g-EG) are useful for development of oral insulin delivery system.

Example 2

In vitro Mucoadhesion Studies

P(MAA-g-EG) hydrogels were prepared in thin films by a solution polymerization technique. The hydrogels were swollen to equilibrium in DMGA buffered saline solutions of pH = 3.2 and 7.4. The swollen hydrogels were cut into disks with diameters of 20 cm and placed in a tensile tester at 25° C and 90% RH. The polymer samples were adhered to the upper holder of the tester using cyanacrylate medical adhesive, whereas a sample of gelled bovine submaxillary mucin was affixed on the lower jaws using the adhesive. The two jaws were brought together for 15 min and then separated at 1 mm/min. The detachment force was measured as a function of displacement. The work of fracture, equivalent to the work of bioadhesion was calculated as the area under the curve.

P(MAA-g-EG) hydrogels function well as oral insulin devices because they are able retard the action of protease inhibitors and also because they adhere to the mucosa of the intestinal wall, allowing for intimate contact and thus aiding in absorption of the drug.

When the insulin containing hydrogels were placed in intestinal fluid, the hydrogels swelled rapidly allowing insulin to be released. Insulin containing, P(MAA-g-EG) microparticles were swollen for 1 hour phosphate buffered saline solutions and then transferred into intestinal fluid. The proteolysis of the insulin in the intestinal fluid was monitored using an insulin EIA kit. Greater than 50% of the biological activity of the insulin was maintained for over 1 hour in the presence of proteolytic enzymes. In comparison, when insulin is dissolved in intestinal fluid, the biological activity is rapidly lost. P(MAA-g-EG) hydrogels serve to protect the insulin by binding calcium to the ionized pendant groups which in turn retards the action of proteolytic enzymes.

Additionally, P(MAA-g-EG) hydrogels exhibit mucoadhesive characteristics due to the presence of the graft PEG chains which serve as adhesion promoters. The mucoadhesive characteristics of P(MAA-g-EG) hydrogels were strongly dependent on the pH of the environmental fluid (Fig. 6). The area under the curves of Fig. 6 is equivalent to the adhesive force between the gel and the mucosa. In conditions simulating the intestinal pH (pH = 7.4) the adhesion between the gel and the mucous was significantly greater. However, to truly compare the mucoadhesive characteristics of the hydrogels, the work of adhesion was normalized to account for the polymer gel fraction (See Table 2). The normalized work of adhesion was two orders of magnitude greater for hydrogels in the non-complexed state relative to complexed hydrogels. Accordingly, the hydrogels adhere to the mucosa of the intestine to a much greater extent than the stomach. Therefore, the residence time of insulin carriers is much greater in regions where the insulin could be absorbed.

Table 2. Work of adhesion for P(MAA-g-EG) hydrogels containing a 1:1 MAA/EG ratio and graft PEG chains for molecular weight 1000.

pH	Work of Adhesion $W \cdot 10^6$ (J)	Polymer Volume Fraction, O_{2s}	Normalized Work of Adhesion $W/(O_{2s})^{2/3} \cdot 10^6$ (J)
3.2	5.38	0.693	62.1
7.4	9.34	0.049	6720

The differences in the adhesive characteristics of the hydrogels at different pH values were due to mobility of the PEG chains in each material. In the highly swollen, non-complexed state, the graft PEG chains were free and readily penetrated the mucosa to serve as anchors for adhesion. In the complexed state, the graft PEG chains in the P(MAA-g-EG) formed complexes with the backbone chains and were unable to penetrate the gel/mucosa interface and form temporary anchors.

Example 3

In vivo Administration of Insulin to Rats

The graft copolymers were prepared by free radical solution polymerization of methacrylic acid and poly(ethylene glycol) monomethacrylate. The ensuing hydrogels were rinsed for 7 days in deionized water to remove unreacted monomer and uncrosslinked oligomer chains. The hydrogels were dried under vacuum and ground into powders. The powders were filtered to obtain particles with diameters of 100 - 150 μm . Crystalline porcine insulin (26.9 U/mg) was loaded by equilibrium partitioning. The drug loaded particles were filtered and washed to remove surface drug and dried under vacuum.

Male Wistar rats (200 g) were fasted for 24 hours. The rats were restrained in the supine position and administered insulin loaded polymer microparticles using a gelatin capsule, which dissolves instantly in the stomach. Serum glucose was monitored by collecting 0.2 ml aliquot blood samples from the jugular vein prior to the experiment and at 0.25, 0.5, 1, 2, 4, 6 and 8 hours after dosing. Serum was separated by centrifugation at 3000 rpm for 3 minutes and frozen until analysis. The serum insulin levels were determined by enzyme immunoassay using an insulin EIA kit. The serum glucose levels were determined by the glucose oxidase method using a glucose B-Test kit.

Fig. 7 summarizes the blood glucose response of rats receiving insulin doses contained in P(MAA-g-EG) microparticles. Within 2 hours of receiving the polymeric dosage form, a strong hypoglycemic effect (lowering of the blood glucose level) was observed. The reduction of the blood glucose levels depends strongly on the insulin dose. No response was observed in rats receiving the insulin solutions.

The effects of administering a composition comprising a P(MAA-g-EG) hydrogel containing insulin and the protease inhibitor, aprotinin, are shown in Fig. 8. Control groups of rats were administered hydrogels containing insulin without a protease (for comparison) and a group was administered a 50U/Kg insulin solution (to serve as a control). The two groups receiving polymeric dosage forms of insulin had a large decrease in blood glucose concentration within two hours of administration. Those rats receiving a combination of insulin and the protease inhibitor, aprotinin, showed the greatest reduction in blood glucose levels. Aprotinin retards the action of the degradative enzymes in the intestine and allows the insulin released locally to remain active longer. Thus, the amount of insulin transported into the bloodstream is highest in the rats receiving the hydrogel insulin and protease inhibitor composition (encapsulated within the P(MAA-g-EG) hydrogel), resulting in a greater reduction in the blood glucose concentration.

15 Example 4

In Vivo Studies in Diabetic Rats and Dogs

Diabetes was induced in healthy, male Wistar rats by administration of streptozotocin. Healthy dogs were made diabetic by administration of alloxan. P(MAA-g-EG) microspheres were prepared by a free-radical bulk, suspension polymerization of methacrylic acid and poly(ethylene glycol) dimethacrylate (PEG MW = 1000). Tetraethylene glycol dimethacrylate was added as the crosslinking agent. 2,2'-Azobisisobutyronitrile (AIBN) was added in the amount of 0.5% of the total monomers as the thermal reaction initiator.

Drug loading was accomplished by equilibrium portioning of the insulin into the P(MAA-g-EG) microparticles. Bovine pancreatic insulin was dissolved in 200 μ l of 1 N NaOH. The insulin solution was diluted with 20 ml of phosphate buffer solution (pH = 7.4) and normalized with 200 μ l of 0.1 N NaOH. Loading was accomplished by swelling initially dry, P(MAA-g-EG) for 24 hours in the insulin solution. The particles were then filtered and washed with 100 ml of 0.1 N HCl solution to collapse the microparticles and "squeeze out" the remaining buffer solution. The drug loaded microspheres were dried under vacuum and stored at 4° C. The

degree of loading was determined from HPLC analysis of the insulin concentrations of the initial solutions and the filtrate from the washings.

Prior to administration of the insulin loaded P(MAA-g-EG) hydrogels, the male Wistar rats (250 g) were fasted for 24 hours. The rats were restrained in the supine position and administered the insulin loaded P(MAA-g-EG) microparticles and the control solutions via the mouth using gelatin capsules and capsules prepared using Eudragit L100. The gelatin capsules dissolved readily in the stomach while the Eudragit capsules dissolved at significantly slower rate.

During the experiment, the rats were separated (4 animals per cage) and allowed to drink water. A 0.2 ml aliquot of blood was collected from the jugular vein at 0.25, 0.5, 1, 2, 4, 6 and 8 hours following dosing. The blood serum was separated by centrifugation at 3000 rpm for 3 minutes. The serum glucose levels were determined by the glucose oxidase method using a glucose B-test kit.

The diabetic dogs (25 kg) were fasted for 24 hours prior to administration of the formulations. The polymer dosage forms were administered orally using gelatin capsules. The dogs were fed at the time of administration.

During the experiment, the dogs were caged and allowed to drink water. Blood samples were taken from an in-dwelling catheter. Serum glucose levels were determined using a portable glucose analyzer.

While a number of systems have been effective in lowering the blood glucose levels of healthy animals following oral administration of polymeric carriers containing insulin, similar results have not been observed in diabetic animals. The blood glucose response of diabetic and healthy rats after oral administration of insulin containing P(MAA-g-EG) microparticles using gelatin capsules (25 IU/kg doses) is shown in Fig. 9. The blood glucose levels of the diabetic rats were lowered by up to 40% of the initial level. The reduction in blood glucose levels lasted for greater than 8 hours, and the degree to which the glucose levels were suppressed was in fact greater for the diabetic animals than the healthy animals. Additionally, the strong hypoglycemic effects were observed to last longer in the diabetic animals.

The blood glucose response of diabetic rats following oral administration of Eudragit capsules containing insulin loaded P(MAA-g-EG) microparticles (25 IU/kg doses) is shown in Fig. 10. The glucose levels of rats

receiving these dosages were reduced by greater than 50% for at least eight hours following a single administration. The microparticles encapsulated in Eudragit were more effective than gelatin capsules presumably because the microparticles contained in the Eudragit capsules were exposed to the harsh environment of the upper GI tract for shorter periods of time due to the slow dissolution of the Eudragit capsules.

The blood glucose of healthy dogs was significantly lowered following the oral administration of a single polymeric dosage from (10 IU/kg). At time zero, the dogs were fed and the normal response of the body would be to maintain the basal level. After feeding, the blood glucose levels increased, however, within two hours of dosing, the blood glucose levels were reduced by greater than 20% due to uptake of insulin in the upper small intestine. Additionally, a second decrease occurred around the eight hour point, consistent to what was previously see in rats, probably due to colonic absorption of insulin. Additionally, the blood glucose levels steadily decline after eight hours, probably due to colonic absorption of the insulin.

The glucose response of diabetic dogs also verifies the uptake of insulin following oral administration. The blood glucose levels of diabetic dogs was controlled by the oral administration of insulin containing P(MAA-g-EG) microparticles using gelatin capsules (10 IU/kg doses). Following feeding and administration of the polymer dosage form, the glucose levels of the dogs rose rapidly, initially. However, after one hour the glucose levels began to stabilize for the next three hours as the insulin was absorbed. The blood glucose levels of the diabetic dogs which received the polymer dosage forms was 40% less than dogs that had not received any insulin.

Oral insulin delivery systems must be able to protect the drug from the harsh environment of the stomach and deliver the insulin in an biologically active conformation for extended period of time to more favorable regions for absorption along the GI tract such as the upper small intestine. Because of their nature, complexing P(MAA-g-EG) hydrogels are ideal for this application.

P(MAA-g-EG) hydrogels are able to effectively deliver biologically active insulin via the oral route. These materials have been shown to reduce the blood glucose levels in diabetic rats and dogs and maintain the blood glucose at near normal levels for greater than eight hours. These materials function well because the majority

of the insulin contained in the hydrogels is not released until the materials reach the upper small intestine. While in the intestine, the hydrogels adhere strongly to the mucosa allowing for intimate contact between the carrier and the absorption site. Additionally, the polymers serve to retard the activity of proteolytic enzymes in the

5 intestine allowing the insulin to remain active for longer periods of time prior to absorption. The inhibitory effect of the polymers on enzyme function is believed to be derived from the polymers' ability to form complexes with cations, such as calcium, necessary for enzymatic function.



THIS SHEET HAS BEEN
REPLACED

Claims:

1. A pharmaceutical composition for oral administration, said composition comprising a swellable hydrogel matrix and a labile protein contained
5 therein, said hydrogel matrix comprising a crosslinked co-polymer of methacrylic acid and poly(alkylene glycol) monomethacrylate.
2. The composition of claim 1 wherein the poly(alkylene glycol) monomethacrylate is poly(ethylene glycol) monomethacrylate.
3. The composition of claim 1 wherein the hydrogel matrix is
10 crosslinked with tetraethylene glycol dimethacrylate.
4. The composition of claim 2 wherein the molar ratio of methacrylic acid and poly(ethylene glycol) monomethacrylate is about 1:1.
5. The composition of claim 4 wherein the protein has a molecular weight from about 1,000 to about 20,000.
- 15 6. The composition of claim 4 wherein the protein is insulin.
7. The composition of claim 4 wherein the poly(ethylene glycol) monomethacrylate has a molecular weight from about 200 to about 4000.
8. The composition of claim 4 wherein the hydrogel is in particulate form and contained within a capsule
- 20 9. The composition of claim 8 wherein the capsule is a gelatin capsule.
10. The composition of claim 4 further comprising a protease inhibitor.
11. A composition for oral delivery of insulin to a vertebrate, said
25 composition comprising insulin contained within a P(MAA-g-EG) hydrogel.
12. The composition of claim 11 further comprising a protease inhibitor.
13. The composition of claim 11 wherein the hydrogel is in particulate form and contained within a capsule
- 30 14. The composition of claim 13 wherein the capsule is a gelatin capsule.

This Sheet Has Been Replaced



15. A method of administering a therapeutically effective amount of a protein to a vertebrate, said method comprising the step of orally administering to said vertebrate the composition of claim 1.

5 16. A method of forming the composition of claim 1 comprising the steps of

polymerizing methacrylic acid and poly(alkylene glycol) dimethacrylate and a crosslinking agent to form a hydrogel matrix;

contacting said hydrogel matrix with an aqueous solution of the protein, wherein the solution has a pH of greater than about 5.4; and

10 adjusting the pH of the solution to less than about 5.4; and isolating the protein-containing hydrogel matrix.

17. The method of claim 16 wherein the poly(alkylene glycol) monomethacrylate is poly(ethylene glycol) monomethacrylate.



This Sheet Has Been Replaced

