Title: FORMULATIONS AND METHODS FOR ORAL DELIVERY OF PROTEINS

Complexation at low pH limits diffusion of drug through polymer matrix; provides enzymatic protection to protein

Deprotonation of hydrogen bonds occurs at high pH; ionic repulsion increases mesh size (ξ), allowing for release of drug to be absorbed by intestinal epithelium

Compositions for delivering a drug to a subject are provided, as well as associated methods of making and using such compositions. In certain embodiments, a composition is provided for delivering a drug to a subject, the composition comprising hydrogel matrix formed from a copolymer of an acrylic acid and an N-vinyl pyrrolidone, wherein the hydrogel matrix is at least partially crosslinked and a drug, wherein the drug is disposed within the hydrogel matrix. Methods of making a composition for delivering a drug to a subject, as well as methods of treating a subject by administering to the subject such compositions, are also provided. In certain embodiments, these hydrogel networks may respond to pH changes, which may comprise swelling at increased pH to release at least a portion of the drug from the hydrogel network.
FORMULATIONS AND METHODS FOR ORAL DELIVERY OF PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 60/863,847 filed November 1, 2006, which is incorporated by reference herein.

STATEMENT OF GOVERNMENT INTEREST

This disclosure was made with support under grant numbers EB-000246 awarded by the National Institutes of Health. Accordingly, the U.S. government has certain rights in the invention.

BACKGROUND

Some means for delivering therapeutic agents are often severely limited by biological, chemical, and physical barriers. Typically, these barriers are imposed by the environment through which delivery occurs, the environment of the target (organ, tissue, cell or ensemble of cells) for delivery, and/or the target itself. Biologically and chemically active agents are particularly vulnerable to such barriers.

In the delivery to animals of biologically active and chemically active pharmacological and therapeutic agents, barriers are imposed by the body. Examples of physical barriers are the skin, lipid bilayers, and various organ membranes that are relatively impermeable to certain active agents but must be traversed before reaching a target, such as the circulatory system. Physicochemical and chemical barriers include, but are not limited to, pH variations in the gastrointestinal (GI) tract and enzymes that can degrade active biologicals.

Following the oral drug delivery route, protein drugs are readily degraded by the low pH of gastric medium in the stomach. The absorption of protein drugs following oral administration is challenging due to their high molecular weight, hydrophilicity, and susceptibility to enzymatic inactivation. Protein drugs at the intestinal epithelium could not partition into the hydrophobic membrane and thus can only traverse the epithelial barrier via the paracellular pathway. However, the tight junctions present in the intestinal epithelium form a barrier that limits the paracellular diffusion of hydrophilic molecules.
These barriers are of particular significance in the design of oral delivery systems. Oral delivery of many biologically or chemically active agents would be the route of choice for administration to humans and other animals if not for biological, chemical, and physical barriers. Among the numerous agents which are not typically amenable to oral administration are biologically or chemically active peptides, such as calcitonin, insulin, erythropoietin, interferons, growth hormones, growth factors; polysaccharides, and in particular mucopolysaccharides including, but not limited to, heparin, heparinoids, antibiotics, and other organic substances. These agents may be rapidly rendered ineffective or destroyed in the gastrointestinal tract by acid hydrolysis, enzymes, and the like. In addition, the size and structure of macromolecular drugs may prohibit absorption.

SUMMARY

The present disclosure is generally related to compositions and methods for oral delivery of proteins and other drugs. In particular, the present disclosure relates to compositions comprising a hydrogel network and a drug and associated methods of making and using such compositions.

In certain embodiments, the present disclosure relates to a composition for delivering a drug to a subject, the composition comprising a hydrogel matrix formed from a copolymer of an acrylic acid and an N-vinyl pyrrolidone, wherein the hydrogel matrix is at least partially crosslinked, and a drug, wherein the drug is disposed within the hydrogel matrix.

In certain embodiments, the present disclosure relates to a method of making a composition for delivering a drug to a subject, the method comprising polymerizing an acrylic acid and an N-vinyl pyrrolidone to form a hydrogel matrix that includes a drug disposed within the hydrogel matrix.

In certain embodiments, the present disclosure relates to a method of treating a subject, the method comprising administering to the subject a composition comprising a hydrogel matrix formed from a copolymer of an acrylic acid and an N-vinyl pyrrolidone, wherein the hydrogel matrix is at least partially crosslinked, and a drug, wherein the drug is disposed within the hydrogel matrix.
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DRAWINGS

Some specific example embodiments of the disclosure may be understood by referring, in part, to the following description and the accompanying drawings.

Figure 1 shows pH dependent delivery by a hydrogel matrix according to one embodiment of the present disclosure.

Figure 2 shows an example of a scheme for forming a hydrogel matrix according to one embodiment of the present disclosure.

Figure 3 shows growth hormone release profiles from P(MAA-g-PEG). A 10 mg sample of growth hormone loaded P(MAA-g-PEG) microparticles was placed in 4 ml of PBS and 0.02% (w/v) Tween 20, pH 7.4 (■) or in 4 ml of HCl, pH 1.2 (●). Solutions were maintained at 37 °C. Samples were taken over the course of 5 hours and growth hormone concentration was determined by HPLC. n=4 ± SD.

Figure 4 shows the results of a dynamic swelling (5 min.) of P(MAA-co-NVP) 1:1 (CL=MBA, 1=0.1 M).

Figure 5 shows the dynamic swelling data for P(MAA-co-NVP), 1:1 monomer molar feed amounts, at 1% (●), 3% (■) and 5% (A) EGDMA crosslinking ratios (n=3 ± SD).

Figure 6 shows the dynamic swelling data for P(MAA-co-NVP), 1% EGDMA crosslinking ratio, at increasing molar feed amounts of N-vinyl pyrrolidone. 1:1 MAA:NVP(^), 1:2 MAA:NVP (■) and 1:4 MAA:NVP (A)(n=3 ± SD).

Figure 7 shows cytotoxicity of P(MAA-co-NVP) 1:1 with Caco-2 cells. Caco-2 cells were seeded at a density of 1.4 x 10^4 cells/cm^2 and cultured in a 96-well plate until 90% confluence. P(MAA-co-NVP) microparticles were suspended in HBSS at various concentrations and incubated with the cells for 2 hours. Microparticles were removed via a wash step and metabolic activity was determined by a colorimetric assay. No significant toxicity was observed at the concentrations tested. n=6-8 ± SD.

Figure 8 shows growth hormone release profiles from P(MAA-co-NVP). A 10 mg sample of growth hormone loaded P(MAA-co-NVP) microparticles was placed in 4 ml of PBS and 0.02% (w/v) Tween 20, pH 7.4 (■) or in 4 ml of HCl, pH 1.2 (●). Solutions were maintained at 37 °C. Samples were taken over the course of 5 hours and growth hormone concentration was determined by HPLC. n=4 ± SD.
Figure 9 shows growth hormone release profiles from P(MAA-co-NVP) and P(MAA-g-PEG). A 10 mg sample of growth hormone loaded P(MAA-co-NVP) microparticles (■) or P(MAA-g-PEG) microparticles (♦) was placed in 4 ml of PBS and 0.02% (w/v) Tween 20, pH 7.4. Solutions were maintained at 37 °C. Samples were taken over the course of 5 hours and growth hormone concentration was determined by HPLC. n=3 ± SD.

Figure 10 shows loading efficiencies of insulin and growth hormone in P(MAA-co-NVP) microparticles before (solid bars) and after (checkered bars) collapse by 0.1N HCl (n=3 ± SD).

Figure 11 shows insulin release studies in gastric and intestinal pH solutions from P(MAA-co-NVP) microparticles (n=3 ± SD).

Figure 12 shows calcitonin release studies at pH 7.4 and pH 10 for P(MAA-co-NVP) 1:1 and 4:1 microparticles (n = 3 ± SD).

Figure 13 shows the cytotoxicity profile of microparticles from copolymer P(MAA-co-NVP) 4:1 (left bars) and P(MAA-co-NVP) 1:4 (right bars) on Caco-2 cell monolayers after 2 hours of incubation as measured by MTS assay. Percent viability of Caco-2 cells is expressed relative to control cells (n=6-8). 1.4 x 10^4 cells/well in 96-well plate at 37°C atmosphere were cultured at a 80-90% of confluency and the medium in the wells was replaced with HBSS medium containing microparticles of varying concentrations.

Figure 14 shows transepithelial electrical resistance (TEER) used to evaluate the development of tight junctions.

Figure 15 shows transepithelial electrical resistance (TEER) used to evaluate the development of tight junctions.

While the present disclosure is susceptible to various modifications and alternative forms, specific example embodiments have been shown in the figures and are herein described in more detail. It should be understood, however, that the description of specific example embodiments is not intended to limit the invention to the particular forms disclosed, but on the contrary, this disclosure is to cover all modifications and equivalents as illustrated, in part, by the appended claims.

DESCRIPTION

The present disclosure provides, in certain embodiments, compositions and methods for oral delivery of drugs. In certain embodiments, such drugs require at least some protection in the
digestive system. In particular, certain drugs need protection from the acid conditions (e.g., pH 204) found in the upper stomach. In certain embodiments, the present disclosure relates to hydrogel networks for delivery of such drugs. The hydrogel network may, among other things, protect the drug while it is in transit through the stomach and improve the bioavailability of the drug (i.e. quantity of drug absorbed into the bloodstream) by increasing the drug's transport across the cellular barrier in the upper small intestine. The hydrogel networks useful in the compositions and methods of the present disclosure may comprise a variety of physical forms, including particles, tablets, capsules, caplets, gel-seals, lozenges, syrups, sprays, and other liquid dosage forms.

As used herein, the term "NVP" refers to N-vinyl pyrrolidone and its derivatives. The term "derivative" includes any compound that is made from one of the listed compounds, for example, by replacing one atom in the listed compound with another atom or group of atoms, rearranging two or more atoms in the listed compound, ionizing one of the listed compounds, or creating a salt of one of the listed compounds.

As used herein, the term "drug" refers to any substance useful in diagnosing, curing, mitigating, treating, or preventing a disease or other medical condition in a human or other animal. Although protein drugs are used as examples herein, any molecule or combination of molecules that may benefit from protection in at least a portion of the digestive system of a subject, followed by release in another portion of the digestive system of a subject, may be used.

For example, diagnostic proteins or other molecules, such as those that conventionally require injection may be delivered using hydrogels of the present disclosure. Other biological drugs and small molecule pharmaceutics may also be delivered using hydrogels. One of ordinary skill in the art, with the benefit of this disclosure, will recognize other drugs suitable for use in the compositions and methods of the present invention.

Hydrogels are mainly hydrophilic polymer networks that swell to a high degree due to a high affinity for water, yet are substantially insoluble because of the incorporation of chemical or physical crosslinks. The hydrogel networks of the present disclosure may be responsive to, among other things, pH changes. In low pH media, for example, around about pH 1-2, the hydrogel networks may be substantially hydrated like many hydrophilic copolymeric materials. But at higher pHs, for example, around about pH 6-7, the carboxylic acid groups may
deprotonate, and the polymer may attract more solvent into the network. Among other things, this may cause an increase in the size of the network as the increased solvent content allows the copolymer chains to distance themselves from each other. This increase in size, or swelling, may release at least a portion of the drug from the hydrogel network.

In certain embodiments, pH-sensitive hydrogels may be made in part from hydrophilic monomers that incorporate separable proton species into the polymer mass. These protons establish, with an aqueous solution, an equilibrium depending on the acidity of the solution. This is modeled by the Henderson-Hasselbach equation, Eq. (1).

$$\text{pH} = \text{pKa} + \log \left( \frac{[\text{A}^-]}{[\text{HA}]_{\text{J}}} \right)$$  Eq. (1)

Here the pH is the environmental pH, the pKa is that of the acid group, A\(^-\) is the concentration of deprotonated acid groups and HA is the concentration of protonated acid groups. From Eq. (1) with the knowledge of the pKa and the pH of the environment the ratio of deprotonated to protonated carboxylic acid groups can be calculated. By calculating the percent of dissociated acid, one may adjust the compositional behavior of the overall polymer system to achieve, among other things, preferential release at certain pH values. In this way the hydrogel networks of different pKa-values may be optimized for different drugs.

In certain embodiments, the pH-sensitive hydrogels useful in the compositions and methods of the present disclosure may be based on ionic networks. Anionic networks contain acidic pendant groups, such as carboxylic acid groups, with a characteristic pKa, while cationic networks contain basic pendant groups, such as amine groups, with a characteristic pKt. In the case of anionic networks, ionization of these acid groups will occur once the pH of the environment is above the acid group's characteristic pK\(_a\). As a result of deprotonation of the acid groups, the network will, among other things, have fixed charges on its chains, resulting in an electrostatic repulsion between the chains and, in addition, an increased hydrophilicity of the network. Because of these alterations in the network, water may be absorbed into the polymer, among other things, to a greater degree. Such absorption of water may cause swelling of the hydrogel network.

In certain embodiments, the present disclosure relates to hydrogel networks of N-vinyl pyrrolidone (NVP, 1-ethenylpyrrolidin-2-one) or an NVP-like molecule and a monobasic or
dibasic acid of the acrylic acid family (referred to herein as "acrylic acids" and AAs), for example, methacrylic acid (MAA). Such compositions may function as an oral drug-delivery system that administers the protein through the hydrogel network. AAs may be used, *inter alia*, for their pH-sensitivity, and a high degree of complexation due to hydrogen bonding occurs between AAs and NVP.

In certain embodiments, the AA and NVP hydrogel networks may be pH-sensitive because, as the pH of their environment increases and causes the carboxylic acid pendant groups to deprotonate, the polymer chains in the network may develop negative charges. The charges repel each other and may cause the polymer chains to move apart, thus causing an increase in the network's volume, depending on, among other things, the pH of its environment (Figure 1).

To form a stable hydrogel network, the polymer chains should be at least partially crosslinked (Figure 2). If the chains were not partially crosslinked, when the pH of the environment increases, the chains would repel one another and would separate, becoming singular chains in solution. By at least partially crosslinking the chains together, they are at least partially chemically bound together, and a balance between the ionic repulsive forces versus the physical crosslink is achieved.

The pKa of (PAA) is approximately 4.3; thus, in an environment at pH 4, approximately half of the carboxylic acid groups are deprotonated. The Henderson-Hasselbach equation predicts that above pH 4 the number of depronated groups will increase—close to 100% of the acid groups will be deprotonated around two pH units higher than the pKa. Likewise, once the pH is around 2 or lower nearly 100 % of the carboxylic acid groups will be protonated. Thus, by changing the pH from 2 to 6 nearly all of the carboxylic acid groups transition from being protonated to being deprotonated. This range of pH values encompasses the range of pH values seen, for example, in the gastrointestinal tract of a human. If a PAA network containing a drug were introduced into the stomach, the low pH would not cause significant swelling of the networks, substantially preventing or limiting release of the drug. A similar drug-containing PAA network introduced into the upper small intestine, with its more neutral pH of 6 or 7, would experience swelling due to, *inter alia*, the ionic repulsion occurring in its backbone, allowing at least a portion of the drug to be released from the hydrogel network.
For poly(methacrylic acid) (PMAA), the pKa is higher, near 4.8. This is due to the methyl pendant group in MAA. This group serves to stabilize the carboxylic acid group and hinder deprotonation. This higher pKa results in a higher pH range in which, among other things, the majority of volume increase occurs.

The AAs suitable for use in the hydrogel networks useful in the present disclosure may include, but are not limited to, acrylic acid, methacrylic acid (2-methylprop-2-enoic acid, MAA); ethacrylic acid; propacrylic acid; crotonic acid ((E)-but-2-enoic acid); methacrylate (2-methylprop-2-enoate); (Z)-3-cyclohexylbut-2-enoic acid; butylmethacrylate (butyl 2-methylprop-2-enoate); and the like. Derivatives and/or combinations of these and other AAs may also be suitable. One or ordinary skill in the art, with the benefit of this disclosure, will recognize additional AAs that may be suitable for use in the compositions and methods of the present disclosure.

The NVPs, which include a molecule having a structure similar to NVP, suitable for use in the hydrogel networks useful in the methods of the present disclosure may include, but are not limited to, NVP (1-ethenylpyrrolidin-2-one); isobetadyne (1-ethenylpyrrolidin-2-one; molecular iodine); N-vinylsuccinimide (1-ethenylpyrrolidine-2,5-dione); (1-ethenylpyrrolidin-2-one; trimethyl-[3-(2-methylprop-2-enoylamino)propyl]azanium; chloride); P(VA-co-Nvp) (ethenol; 1-ethenylpyrrolidin-2-one); and the like. Derivatives and/or combinations of these and other NVPs may also be suitable. One of ordinary skill in the art, with the benefit of this disclosure, will recognize additional NVPs that may be suitable for use in the compositions and methods of the present disclosure.

In certain embodiments, the AA and NVP are at least partially crosslinked to form a copolymeric hydrogel network. Such crosslinking may be accomplished using a number of crosslinking agents known in the art, the choice of which may depend on the particular pairing of AA and NVP. Typically, the crosslinking agent will be biocompatible. Polymerization may be initiated with a free radical initiator such as thermal initiators including organic peroxides or UV radical initiators known in the art.

While AA and NVP are exemplified in the present disclosure, in general, any polymeric material or combination of polymeric materials which is suitable for use in the delivery of a specific drug through the GI tract and has the necessary properties to form an at least partially
pH-sensitive hydrogel network may be used in the compositions and methods of the present
disclosure. In specific embodiments, the hydrogel network comprises poly-methacrylic acid-co-
N-vinyl pyrrolidone (i.e., p(MAA-co-NVP)).

In certain embodiments, the hydrogel networks of the present disclosure include a drug
which comprises a protein. The term "protein" includes natural and nonnatural (e.g.,
recombinant) proteins, polypeptides, and peptides. The proteins may, by themselves, be
incapable of passing (or which pass only a fraction of the administered dose) through the
gastrointestinal mucosa or may be susceptible to chemical cleavage by acids or enzymes in the
gastrointestinal tract or both. In addition to proteins, the hydrogel network also may include
polysaccharides, and particularly mixtures of mucopolysaccharides, carbohydrates, lipids; other
organic compounds. For therapeutic applications, the protein may be biologically active. In
certain embodiments, the protein may be a protein that has a pKa near its isoelectric point, for
example within about ± 0.5.

Examples of suitable proteins include, but are not limited to, the following, including
synthetic, natural, or recombinant sources of each: a growth hormone (e.g., a somatotropin, e.g.,
GENOTROPIN®, NUTROPIN®, NORDITROPIN®, SAIZEN®, SEROSTIM®,
HUMATROPE®), including a human growth hormone (hGH), a recombinant human growth
hormone (rhGH), a bovine growth hormone, or a porcine growth hormone; a growth hormone-
releasing hormone; an interferon (e.g., IFN-γ, IFN-α, IFN-β, IFN-ω, IFN-τ; IFN-κ); an
interleukin (e.g., IL-1; IL-2, including, e.g., PROLEUKTN®; IL-3, IL-4, IL-5, IL-6, IL-7, IL-8,
IL-9; and the like); a growth factor (e.g., REGRANEX® (beclapermin; PDGF); FIBLAST®
(tfrafermin; bFGF); STEMGEN® (ancestim; stem cell factor); a keratinocyte growth factor; an
acidic fibroblast growth factor, a stem cell factor, a basic fibroblast growth factor, a hepatocyte
growth factor; insulin, including porcine, bovine, human, and human recombinant insulin (e.g.,
Novolin, Humulin, Humalog, Lantus, Ultralente), optionally having counter ions including
sodium, zinc, calcium and ammonium; an insulin-like growth factor, including IGF-I; a heparin,
including unfractionated heparin, heparinoids, dermatans, chondroitins, low molecular weight
heparin, very low molecular weight heparin and ultra low molecular weight heparin; calcitonin,
including salmon, eel, and human calcitonin; erythropoietin (e.g., PROCRIT®, EPREX®, or
EPOGEN® (epoetin-α); ARANESP® (darbepoetin-α); NEORECORMON®, EPOGIN®
(epoetin-β; and the like); a blood factor (e.g., ACTIVASE® (alteplase) tissue plasminogen activator; NOVOSEVEN® (recombinant human factor Vila); Factor Vila; Factor VIII (e.g., KOKENATE®); Factor IX; β-globin; hemoglobin; and the like); a colony stimulating factor (e.g., NEUPOGEN® (filgrastim; G-CSF), NEULASTA® (pegfilgrastim), a granulocyte colony stimulating factor (G-CSF), a granulocyte-monocyte colony stimulating factor, a macrophage colony stimulating factor, a megakaryocyte colony stimulating factor; and the like); an antigen; an antibody (e.g., a monoclonal antibody) (e.g., RITUXAN® (rituximab); REMICADE® (infliximab); HERCEPTIN® (trastuzumab); HUMIRA™ (adalimumab); XOLAIR® (omalizumab); BEXXAR® (tositumomab); RAPTIVA™ (efalizumab); ERBITUX™ ( cetuximab); and the like), including an antigen-binding fragment of a monoclonal antibody; a soluble receptor (e.g., a TNF-α-binding soluble receptor such as ENBREL® (etanercept); a soluble VEGF receptor; a soluble interleukin receptor; a soluble γ/δ T cell receptor; and the like); an enzyme (e.g., α-glucosidase; CERAZYME® (imiglucarase; β-glucocerebrosidase; CEREDASE® (alglucerase); an enzyme activator (e.g., tissue plasminogen activator); a chemokine (e.g., IP-10; Mig; Gro/IL-8, RANTES; MIP-1α; MIP-1β; MCP-1; PF-4; and the like); an angiogenic agent (e.g., vascular endothelial growth factor (VEGF); an anti-angiogenic agent (e.g., a soluble VEGF receptor); a neuroactive peptide such as bradykinin, cholecystokinin, gastrin, secretin, oxytocin, gonadotropin-releasing hormone, beta-endorphin, enkephalin, substance P, somatostatin, prolactin, galanin, growth hormone-releasing hormone, bombesin, warfarin, dynorphin, neurotensin, motilin, thyrotropin, neuropeptide Y, luteinizing hormone, calcitonin, insulin, glucagon, vasopressin, angiotensin II, thyrotropin-releasing hormone, vasoactive intestinal peptide, a sleep peptide, and the like; a thrombolytic agent; an atrial natriuretic peptide; a bone morphogenic protein; thrombopoietin; relaxin; glial fibrillary acidic protein; a follicle stimulating hormone; a human alpha-1 antitrypsin; a leukemia inhibitory factor; a transforming growth factor; a tissue factor; a luteinizing hormone; a leukocyte inhibitory hormone-releasing hormone; a macrophage activating factor, a tumor necrosis factor, a neutrophil chemotactic factor, a nerve growth factor, a tissue inhibitor of metalloproteinases; a vasoactive intestinal peptide, angiogenin, angiotropin, fibrin; hirudin; a leukemia inhibitory factor; an IL-1 receptor antagonist (e.g., KINERET® (anakinra)); a protease inhibitor; adrenocorticotropic; a prostaglandin; cyclosporin; cromolyn sodium (sodium or disodium
chromoglycate); vancomycin; desferoxamine (DFO); parathyroid hormone (PTH), including its fragments; an antimicrobial; and an anti-fungal agent. Combinations, analogs, fragments, mimetics or polyethylene glycol (PEG)-modified derivatives of these compounds, or other derivatives of any of the above-mentioned substances may also be suitable. Also suitable for use are fusion proteins comprising all or a portion of any of the foregoing proteins. One of ordinary skill in the art, with the benefit of the present disclosure, may recognize additional drugs, including drugs other than proteins, that may be useful in the compositions and methods of the present disclosure. Such drugs are still considered to be within the spirit of the present disclosure.

In specific embodiments, the drug may be a growth hormone (GH). As a protein, GH is not suitable for oral administration by itself because it would be substantially broken down in the digestive tract. Therefore, growth hormone disorders (such as growth hormone deficiency (GHD), also called hypopituitary dwarfism or hypopituitarism), Turner syndrome, chronic renal failure, Prader-Willi Syndrome, or children born small for gestational stage) are treated by, among other means, injections of growth hormone. Suffering young children receive injections between two and four times per week. The treatment of growth hormone deficiency usually is carried out over several years, until the child achieves an acceptable adult height or maximum growth potential is reached. GH therapy has been clearly shown to improve height velocity during childhood. Specialists have found, however, that the psychological effects of repeated injections on children are very important: needles are one of the top stressors in their medical treatment.

The protein may be loaded into the hydrogel network by any suitable technique known to one of ordinary skill in the art. Examples of suitable techniques include equilibrium partitioning or by polymerizing the AA and NVP in a solution of protein. One of ordinary skill in the art, with the benefit of this disclosure, will recognize other suitable techniques for loading of the drug. Such techniques are still considered within the spirit of the present disclosure.

For therapeutic applications, two properties of hydrogel networks may be important for efficacy of the methods of the present disclosure: the rate at which the carrier swells and the size of the carrier particles. For a prolonged release, a low swelling rate is often desirable, while for quick, controlled dosages, a high swelling rate may be needed. The swelling rate is controlled at
least in part by the hydrogel network's affinity for the swelling medium. The hydrogel networks useful in the methods of the present disclosure advantageously have a high swelling rate, which may allow these hydrogel networks to, among other things, (a) deliver more protein upon swelling, and (b) deliver the protein more precisely, that is, leakage of protein from the hydrogel matrix may be minimized because the hydrogel network may be substantially collapsed at pHs generally found in the stomach.

Not all drugs need a prolonged concentration exposure to be effective. In fact, many drugs become very harmful if given for long periods of time. Proteins such as human growth hormone releasing hormone and insulin are naturally regulated by the body. Their concentrations rise and fall depending at least in part on the body's need for them. This type of controlled release may, among other things, require delivering the needed dosage of drug to the body in a timely and controlled fashion. The length of the dosage is often much shorter, but needs to have fast release to quickly administer the therapeutic agent into the desired location.

As mentioned above, the size of the hydrogel network, which may be in the form of a particle, may be an important characteristic of the network. As the size of the hydrogel network particle increases, the response time increases. The pH-sensitive hydrogel network particles of the present disclosure may require media to enter into the network before the particles respond to the media. Thus, a larger carrier particle may have a slower response time than a smaller particle because of the difference in distance the media must travel. Thus, the compositions of the present disclosure generally are of a size suitable for use in vivo and of a size tailored to provide the appropriate release characteristics. In the case of insulin, for example, the hydrogel network may be used in the form of microparticles with a size in the range of about 150 to 220 μm.

In certain embodiments, the hydrogel network may be particles larger than about 600 nm. Such particles may be advantageous because, among other things, they may not diffuse into the blood stream or the Peyer's patches (groupings of cells in the small intestine). Particles taken into the Peyer's patches are substantially lost in terms of delivery potential, as they can no longer substantially release their therapeutic agents. Additionally, uptake into Peyer's patches may be reduced for hydrophilic polymers. Thus, hydrophilic hydrogel networks may have at least a partially reduced uptake into the blood stream and the Peyer's patches. In other embodiments, the hydrogel network may be particles larger than about 200 nm.
Hydrogel network particles may be formed using methods known in the art. For example, hydrogel network particles may be formed by polymerizing comonomer solutions to form thin films, crushing these films into microparticles, and then sieving to recover the microparticle in the desired size range. Alternatively, hydrogel network particles may be formed using an emulsion polymerization technique with silicon oil as the suspending phase to prepare particles with a monodisperse size distribution. One of ordinary skill in the art, with the benefit of this disclosure, will recognize other suitable techniques for forming hydrogel network particles. Such techniques are still considered within the spirit of the present disclosure.

The compositions of the present disclosure may be administered to a subject in an amount sufficient to produce a therapeutic or prophylactic effect. Such an amount may depend upon, among other things, the drug to be delivered by the composition and the subject to whom the composition is delivered. The compositions may be administered to a subject in a conventional dosage form prepared by combining the compositions of the present disclosure with a conventional, pharmaceutically acceptable carrier, diluent, and/or excipient according to known techniques. It will be recognized by one of ordinary skill in the art that the form and character of the pharmaceutically acceptable carrier, diluent, and/or excipient is dictated by the amount of active ingredient with which it is to be combined, the route of administration, and other well-known variables.

The compositions of the present disclosure used for administration to a patient and can be in an unencapsulated form, for example, suspended or dispersed in a liquid or solid carrier, or in an encapsulated form such as a capsule for oral administration or a microparticle, for example, one having a single layer comprising the hydrogel composition and an outer skin layer.

Pharmaceutical compositions may be used for oral ingestion in the form of a tablet, a capsule, a caplet, a gel-seal, a lozenge, liquid dosage forms such as syrups, sprays, and other liquid dosage forms, or any other dosage form useful for drug delivery using the compositions of the present disclosure.

Tablets for use in accordance with this invention may be prepared by art-recognized techniques for forming compressed tablets where the unencapsulated or encapsulated forms of the compositions of the present disclosure are dispersed on a compressible solid carrier, optionally combined with any appropriate tableting aids such as a lubricant (e.g., magnesium-
stearate) and are compressed into tablets, or by other art-recognized techniques for forming compressed tablets such as chewable vitamins. Suitable solid carrier components for tableting include manitol, microcrystalline cellulose, carboxymethyl cellulose, and dibasic calcium phosphate.

Solid dosage forms for oral ingestion administration include such dosage forms as lozenges, caplets, capsules, and gel-seals. Such solid dosage forms can be prepared using standard tableting protocols and excipients to provide lozenges, caplets, capsules, or gel-seals containing unencapsulated or encapsulated forms of the hydrogel composition.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Actual dosage levels of the protein in the compositions of the present disclosure may be varied so as to obtain an amount which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

To facilitate a better understanding of the present invention, the following examples of specific embodiments are given. In no way should the following examples be read to limit or define the entire scope of the invention.
EXAMPLES

P(MAA-g-PEG) hydrogel synthesis

P(MAA-g-PEG) hydrogels were prepared by free radical solution polymerization of: methacrylic acid (MAA, Aldrich Chemical Co., Milwaukee, WI); methoxy-terminated poly(ethylene glycol) monomethacrylate; (PEGMA, Polysciences Inc., Warrington, PA). Tetraethylene glycol dimethacrylate (TEGDMA, Polysciences Inc., Warrington, PA) was added as the crosslinking agent in the amount of 1% moles of total monomers. The initiator, IRGACURE® 184 (1-hydroxy cyclohexyl phenyl ketone, Aldrich) was added in the amount of 0.1 wt% of total monomer. The solution was diluted with a 1:1 by weight mixture of ethanol and water.

Nitrogen was bubbled through the well mixed solution for 30 minutes to remove dissolved oxygen. The reaction mixture was pipetted between glass plates to form films of 0.9 mm thickness under nitrogen (in the nitrogen glove box). The reaction was initiated by exposing the monomer film to UV light at 16 mW/cm² for 30 min. The monomer film was removed from the glass plates and rinsed in deionized water twice a day for 7 days to remove unreacted monomer and the sol fraction. One week later, the gel was dried in the vacuum during 2 days. Once the polymer film was completely dry, it was pulverized and crushed into fine microparticles. The particles were ground for sufficient time to generate particles that could pass through sieves with a mesh size of 150 nm.

Growth hormone loading into P(MAA-g-PEG) hydrogel

Growth hormone loading was done by equilibrium partitioning, consisting of a 0.5 mg/ml porcine somatotropin (Sigma-Aldrich, St. Louis, MO) solution and 70 mg of polymer microparticles sized 90-150 µm. All of the glassware used for these experiments was siliconized by treatment with SIGMACOTE® (Sigma) to minimize protein and particle adsorption to the glassware.

A growth hormone stock solution was made with 1x phosphate buffered saline (PBS) pH 7.4 and 0.02% w/v Tween 20. The growth hormone was dissolved in the buffer. A 200 µl sample was taken and filtered using a low protein binding 0.22 µm PVDF filter (Millipore, Bedford, MA) and then replaced with equal amounts of 1x PBS, pH 7.4.
A 70 mg crushed microparticle sample of polymer was added to 10 ml of the growth hormone stock solution and stirred for 2 hours at room temperature. Another 200 µl sample was taken after loading in the same manner as the one before adding the microparticles. For high pH: particles are swollen, and growth hormone partitioned into the particles. The particles were collapsed using 5 ml 0.1 N HCl. A third 200 µl sample was taken. For acidic pH: particles collapsed with the growth hormone trapped inside. The loaded microparticles were then filtered with Whatman grade 4 filter paper, and washed with 20 ml of deionized water. After filtering, particles were frozen in a -80°C freezer and lyophilized at -50 °C under vacuum (LabConco Model 77500) for 24 hours. Particles were stored at -20°C until use.

Porcine growth hormone concentration was determined using HPLC (Waters 2695 Separations Module, Milford, MA) analysis to calculate the loading efficiency. Briefly, a mixture of 70% water + 0.1% trifluoroacetic acid (TFA)/30% acetonitrile + 0.08% TFA was used as the mobile phase in a Symmetry300™ C4 column. The 20 µl samples were injected and a flow rate of 0.6 ml/min was used.

Loading efficiencies (Table 1) were determined both before and after the addition of HCl, which is added to collapse the microparticles and entrap growth hormone within the network. Loading efficiency is expressed as:

\[
Loading \ Efficiency = \frac{C_o - C_f}{C_o} \times 100
\]

where \(C_o\) is the initial growth hormone concentration and \(C_f\) is the final growth hormone concentration remaining in solution.

<table>
<thead>
<tr>
<th>Polymer Sample</th>
<th>Loading Efficiency Before HCl (%)</th>
<th>Loading Efficiency After HCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(MAA-g-PEG)</td>
<td>95.95 ± 0.41</td>
<td>57.79 ± 4.10</td>
</tr>
</tbody>
</table>

Before the addition of HCl, P(MAA-g-PEG) had a loading efficiency of 95.95 ± 0.41. The final loading efficiency was 57.79 ± 4.10. It should be noted that loading efficiencies are always lower after the addition of the HCl because the collapse of the network forces some of the growth hormone out of the polymer.

**Growth hormone** solubility
Growth hormone solubility in IxPBS (pH 7.4) was noticed to be much lower than at higher pH. For the release studies, as the concentration in growth hormone in the buffer was expected to be very low, growth hormone had to be as soluble as possible. In order to have a better solubility of porcine growth hormone in the buffer, Tween 20, a detergent polysorbate known to be a solubilizing agent of proteins, was used.

0.5 mg of growth hormone was dissolved in 2 mL of IX PBS (pH 7.4). A solution of 99.5% v/v IX phosphate buffered saline (PBS) pH 7.4 and 0.5% v/v IN NaOH was prepared. 0.5 mg of growth hormone was dissolved in 2 mL of this solution. 0.5 mg of growth hormone was dissolved in 2 mL of IX phosphate buffered saline (PBS) pH 7.4 containing 0.02% w/v Tween 20. HPLC (Waters 2695 Separations Module, Milford, MA) analysis was used to determine the best solubility of porcine growth hormone. As expected, for the same concentration of our 3 samples (0.5 mg/ml), the area under the curve using HPLC analysis was much higher when growth hormone was dissolved in PBS containing Tween 20 (Table 2). Therefore, for our following release studies, the chance to detect small concentration of growth hormone will be more important.

<table>
<thead>
<tr>
<th>PBS pH 7.4</th>
<th>PBS &amp; NaOH pH 9</th>
<th>PBS &amp; Tween 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.966E+06 ± 0.007</td>
<td>2.915E±06 ± 0.007</td>
<td>3.380E+06 ± 0.005</td>
</tr>
</tbody>
</table>

**TABLE 2**: Growth Hormone area under the curve using HPLC analysis. n=2 ± SD

**Growth hormone release from P(MAA-g-PEG) hydrogel**

10 milligrams of P(MAA-g-PEG) microparticles loaded with porcine somatotropin were suspended in 4 ml phosphate buffered saline (PBS, pH 7.4) containing 0.02% (w/v) Tween 20, and placed at 37°C in shaking condition.

Over the course of 5 hours, 0.2 ml samples were taken and filtered using a 0.22 µm PVDF filter. Samples were replaced with an equal volume of the appropriate prewarmed buffer. Determination of growth hormone concentration was done using HPLC as previously described. Samples were prepared and analyzed in duplicate.

The small mesh size of the polymeric carrier obtained at the last process of the protein loading prevents the growth hormone from being released at an acidic environment. However, as the polymeric carrier passes into a higher pH, deprotonation of the carboxyl group on the MAA
(pKa ~4.9) occurs creating ionic repulsion between the polymer chains and thus increasing the mesh size. As a result, growth hormone is released.

At a pH above the pKa of MAA, growth hormone is released from the P(MAA-g-PEG) microparticles, with more than 90% of the loaded growth hormone being released after 3 hours.

Figure 3

Synthesis of P(MAA-co-NVP) Particles

Copolymers of methacrylic acid (MAA) and N-vinyl pyrrolidone (NVP) were prepared by free radical photopolymerization. MAA (ACROS Organics, Morris Plains, NJ) was purified through the use of an inhibitor removal column (Sigma-Aldrich, Milwaukee, WI) and NVP (Sigma-Aldrich) was used as received. N,N'-Methylenebisacrylamide (MBA, Sigma-Aldrich) was used as a crosslinking agent and IRGACURE® 184 (1-hydroxy cyclohexyl phenyl ketone, Sigma-Aldrich) was used as a photoinitiator.

Molar feed ratios for the MAA and NVP monomers ranging from 4:1 to 1:4, respectively, was used in the synthesis. The MBA crosslinker was added to the mixture in the amount of 1.0 mol% of the total monomer amount. The initiator was added in the amount of 1.0 wt% of the total monomers. The entire mixture was diluted to 50.0 wt% by the addition of ethanol and distilled, deionized water (ddH2O, Millipore, Bedford, MA) in a 1:1 mixture by weight. The mixture was sonicated (Bransonic® 8510, Branson Ultrasonics Corp., Danbury, CT) for 10 min. to homogenize the components. Nitrogen was bubbled through the mixture for 20 min. to remove oxygen and the mixture was pipetted between two glass plates (150 x 150 x 3 mm³) separated by a Teflon spacer (0.7 mm thick). The mixture was exposed to UV light (16-17 mW/cm² intensity) for 30 min. in a nitrogen environment to yield a polymer gel. The hydrogel was removed from the glass plates and washed in ddH2O for 7 days to remove unreacted components. Following washing, disks were cut from the film at a diameter to thickness ratio of at least 10:1. The disks were placed in a vacuum oven at 30°C overnight for drying purposes. Once the sample was dried, disks were crushed and sieved to yield particles in the 90-150 μm range.

Swelling studies

Dynamic swelling studies were carried out on the hydrogel disks to measure the response of the crosslinked network to a changing pH. For the studies, dimethylglutaric acid/sodium hydroxide buffers (DMGA/NaOH buffer (1=0.1M)) were prepared in the pH range of 3.2-7.6 to
analyze the dynamic swelling properties of the formulations and to mimic in vivo pH changes between the stomach and small intestine. Ionic strength was controlled by the addition of sodium chloride. Studies were carried out at 37°C in glass jars and repeated in triplicate.

The weight of a dried disk was measured prior to experimentation. For the study, a single disk was placed in a buffer solution and allowed to swell for 5 min. Following swelling, the disk was removed from the solution, blotted to remove superficial moisture, and its weight was recorded. The disk was then placed in a buffer solution of a higher pH for another 5 min. and the aforementioned steps were repeated. The volume of the buffer used at each pH was held constant for the study. Figure 4 shows the results of the dynamic swelling study. Swelling studies show that considerable swelling begins above the pKa of MAA (4.9) and that increasing the amount of NVP increases the swelling ratio at all pH values. This is due to the highly hydrophilic nature of NVP.

The following formula was used to approximate 100 µm particle and the diffusion coefficient of water in dry polymer:

\[
\frac{t}{D} \rightarrow \left(\frac{0.01 \text{cm}^2}{10^6 \text{cm}^2/\text{s}}\right) \approx 100 \text{s}
\]

Using this approximation, we gather that a 5-min experimentation time (i.e. the amount of time polymer is held at one pH before increase) is a good estimate for mimicking particle swelling in vivo. Figure 5 shows the weight swelling ratio, q, of P(MAA-co-NVP), 1:1 MAA:NVP feed ratio, at various EGDMA crosslinking ratios (n=3 ± 1 SD). Figure 6 shows the weight swelling ratio, q, of P(MAA-co-NVP), 1% EGDMA, at increasing amounts of fed NVP monomer (n=3 ± 1 SD).

**Growth hormone loading into P(MAA-co-NVP) hydrogel**

Growth hormone was loaded into P(MAA-co-NVP) over 40 minutes the same way as growth hormone was loaded into P(MAA-g-PEG).

Loading efficiencies (Table 3) were determined both before and after the addition of HCl, which is added to collapse the microparticles and entrap growth hormone within the network. Before the addition of HCl, P(MAA-co-NVP) had a loading efficiency of 84.51 ± 0.26. The final loading efficiency was 67.29 ± 1.21.
TABLE 3: Growth Hormone Loading Efficiencies for P(MAA-co-NVP). n=3 ± SD

<table>
<thead>
<tr>
<th>Polymer Sample</th>
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<tbody>
<tr>
<td>P(MAA-co-NVP)</td>
<td>84.51 ± 0.26</td>
<td>50.94 ± 1.81</td>
</tr>
</tbody>
</table>

A hydrogel of MAA and poly(ethylene glycol) (PEG) was created in the same fashion as previously stated for system comparison. HPLC analysis shows that more hormone was loaded into the P(MAA-g-PEG) system (57.79% ± 4.10) than the P(MAA-co-NVP) system (50.94% ± 1.81). The addition of HCl to collapse the microparticles forces some hormone out of the network, resulting in lower efficiencies than those seen prior to collapse.

**Growth hormone release from P(MAA-co-NVP) hydrogel**

10 milligrams of P(MAA-co-NVP) microparticles loaded with porcine somatotropin were suspended in 4 ml phosphate buffered saline (PBS, pH 7.4) containing 0.02% (w/v) Tween 20, and placed at 37°C in shaking condition.

Over the course of 24 hours, 0.2 ml samples were taken and filtered using a 0.22 µm PVDF filter. Samples were replaced with an equal volume of the appropriate prewarmed buffer. Determination of growth hormone concentration was done using HPLC as previously described. Samples were prepared and analyzed in triplicate.

Release studies were performed with growth hormone-loaded P(MAA-co-NVP) microparticles at both pH 1.2 and pH 7.4 in two separate experiments. At a low pH, there was a very limited release of growth hormone from the microparticle (less than 1%). At a pH above the pKa of MAA, growth hormone is released from the P(MAA-co-NVP) microparticles, with more than 90% of the loaded growth hormone being released after 80 minutes.

Indeed, at a low pH (state of the stomach in vivo), growth hormone does not release from the particles. The protein is entrapped into the hydrogel. But when the particles reach the small intestine, the pH increases, the particles swell and growth hormone is released. The results of this experiment show that the change in pH between the stomach and the small intestine can be used as a physiologic trigger to release growth hormone from the microparticles.
Growth hormone release from P(MAA-co-NVP) hydrogel vs P(MAA-g-PEG) hydrogel

The protein loading/release capabilities of the P(MAA-co-NVP) hydrogel system was compared to P(MAA-g-PEG). Table 4 shows the protein loading efficiency of P(MAA-g-PEG) and P(MAA-co-NVP), both at 1:1 molar feed ratios and 1% crosslinking ratio. Protein was loaded for two hours in PBS pH=7.4 before addition of HCl to collapse particles.

**TABLE 4**: Comparison of Growth Hormone Loading Efficiencies in P(MAA-g-PEG) and P(MAA-co-NVP) Microparticles

<table>
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<th>Polymer</th>
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Figure 8 shows the percentage of protein released in high and low pH states and Figure 9 shows the comparison of protein concentration released from two polymer samples. The release studies demonstrate that the concentration of hormone released from the P(MAA-co-NVP) system (0.06 mg/mL) was higher than that from the P(MAA-g-PEG) system (0.016 mg/mL) (Figure 9) after 1 hour. In addition to a higher concentration released, the slope of the P(MAA-co-NVP) concentration release signifies a faster release of hormone over the P(MAA-g-PEG) system.

**Cytoxicity Experiments P(MMA-co-NVP)**

Caco-2 cells were obtained from American Type Culture Collection (ATCC, Rockwell, MD). All cell types were cultured in Dulbecco’s modified Eagle medium (DMEM) (Mediatech, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum (Cambrex, East Rutherford, NJ), 1% nonessential amino acids (Mediatech), 100 U/ml penicillin, and 100 µg/ml streptomycin (Mediatech).

Cultures were maintained in T-75 flasks (Corning, Corning, NY) at 37 °C and a humidified environment of 5% CO2 in air. The medium was changed every other day. Cells were routinely passaged at 80% confluency, which occurred between 6 and 7 days after seeding. A passage operation consisted of a 2 times wash with DPBS w/o Ca²⁺ and Mg²⁺ and then the addition of 1 ml of a 0.5% Trypsin/ 0.2% EDTA solution. Cells were then incubated with the trypsin/EDTA solution for 5 minutes, after which time cells were detached from the flasks and
could then be counted and reseeded. Caco-2 cells were seeded at a density of 3.0 x 10^3 cells/cm^2 and used between passages 60 to 80.

Cytocompatibility experiments were performed in a 96-well plate (Corning) using Caco-2 cells. Caco-2 cells were seeded at a density of 1.4 x 10^4 cells/cm^2. Cells were fed every other day and cytotoxicity studies were conducted when cells reached 98% confluence (8 days).

P(MAA-co-NVP) (sized between 90 and 150 µm) were prepared as previously described. Growth medium was removed from each well and P(MAA-co-NVP) microparticles were added to wells at concentrations 0.5 mg/mL, 1 mg/mL to 2.5 mg/mL in Hank’s balanced salt solution (HBSS) (Mediatech). Microparticles were then incubated with the cells for 2 hours at 37 °C and 5% CO₂. The microparticle suspension was removed from each well and the wells were rinsed three times with HBSS.

To determine cell viability, a cellular metabolic assay was used to measure NADPH production (CellTiter 96® AQeous One Solution Cell Proliferation Assay, Promega, Madison, WI). The assay works by adding an MTS tetrazolium compound, which is then reduced to a colored formazan product by NADH or NADPH.

A 1 ml sample of the CellTiter 96® reagent was added to 5 ml of HBSS. A sample of 120 µL of the diluted CellTiter 96® reagent was added to each well and incubated with the cells for 1.5 hours at 37 °C and 5% CO₂. A UV/vis microplate reader (Bio-Tek Synergy HT, Winooski, VT) was then used to determine the absorbance in each well at 490 nm. To determine cell viability, absorbance was compared to results from control wells that were not incubated with microparticles, but only with HBSS and the CellTiter 96® reagent. Background absorbance of control wells containing only cells and HBSS was subtracted from the results.

There was no significant decrease in cell viability when incubating the various concentrations of P(MAA-co-NVP) with Caco-2 cells as shown in Figure 7. Thus, the microparticles have minimal effect on the viability of living cells. At the highest concentration tested, 94% ± 6% of cells remained viable.

**Evaluation of growth hormone and insulin encapsulation and release**

P(MAA-co-NVP) carriers were synthesized using a UV-initiated free radical solution polymerization. The polymer networks were crosslinked with ethylene glycol dimethacrylate (EGDMA) in the amount of 1-5 mol% of the total monomer content. Polymer disks were swollen
in dimethylglutaric acid/sodium hydroxide buffers at physiological ionic strength and
temperature over a range of pH values. To simulate in vivo particle swelling, the disks were
initially swollen at low pH and moved to higher pH levels after a set time. Protein loading
occurred through equilibrium partitioning into microparticle networks (size range: 90-150 µm)
from a drug stock solution at pH=7.4. Encapsulation was achieved by lowering the pH of the
solution below the pKa of MAA (4.9). Drug release studies were conducted on a dissolution
apparatus at gastric and intestinal (non-enzymatic) pH levels, and protein quantification was
achieved through HPLC measurement.

The dynamic swelling of P(MAA-co-NVP) disks demonstrated tunable properties for the
carriers through the adjustment of the crosslinking ratio or molar feed ratio. An increase in the
crosslinking ratio decreased the weight swelling ratio (q) while an increase in the amount of fed
NVP increased the weight swelling ratio. This second dependency is most likely due to the
hydrophilic nature of NVP, leading to an increase in the overall fluid content of the network. The
results are shown in Figures 5 and 6.

In separate experiments, insulin from bovine pancreas and growth hormone from porcine
pituitary were loaded into the networks for 2 hours. Results are shown prior to and following
microparticle collapse. Overall, insulin was encapsulated more efficiently than growth hormone.
Insulin is a smaller protein (5.8 kDa) than growth hormone (22 kDa) and is able to more readily
diffuse into the network. More work is needed to determine whether or not the protein is wholly
loading in the core of the network or if any amount is surface adsorbing to the polymer chains. It
is possible that this is a reason for the enhanced loading efficiency seen with the 3% crosslinked
network. The decrease in efficiency following the addition of HCl to collapse the networks is due
to the physical constraints placed on the volume of loaded protein. The results are shown in
Figure 10.

Protein release studies were conducted using USP 28 Apparatus 2 at 37°C with a paddle
speed of 100 rpm. HPLC analysis confirmed that no release of protein occurred at low pH
(simulated with 0.1N HCl), verifying the presence of complexation within the polymer network
below the pKa of MAA. This also suggests the protection of the drug from degradation in the
acidic environment, but further investigations are needed to confirm this. At high pH (simulated
with PBS IX), the protein was able to diffuse out of the network. Insulin released within a few
minutes after introduction to the release media, whereas growth hormone release was more
controlled and did not reach its maximum percentage released until after 1h. As with loading, the
size difference between the proteins is a major contributor to the release kinetics as the growth
hormone's large molecular weight will lengthen its overall diffusion time. The results are shown
in Figure 8 for the growth hormone and Figure 11 for insulin.

**Calcitonin loading of P(MAA-co-NVP) microparticles**

P(MAA-Co-NVP) hydrogels were synthesized as described above. Microparticles
prepared from these hydrogels were loaded with salmon calcitonin (sCT) by equilibrium
partitioning as described above. Briefly, 140 mg of polymer microparticles sized 90-150 µm
were added to 20 mL of a 0.1 mg/mL sCT solution with a pH of 7.4 and stirred for 3 hours at
room temperature. Then, microparticles were collapsed by the addition of 10 mL of HCl 0.1 N,
filtered, and washed with 5 mL of deionized water. Particles were then lyophilized and stored at
-20 °C until use. Loading of sCT into the particles was performed as described above for GH, the
results of which are shown below in Table 5. Release of sCT from the particles was performed
as described above for GH, except that HPLC samples were prepared and analyzed in triplicate.
The results of this release study are shown in Figure 12. Cytocompatibility studies were also
performed as described above, except that microparticle suspensions with concentrations of 10,
5, 2.5, 1.25, and 0.625 mg/mL were used, the results of which are shown in Figure 13.

**TABLE 5:** Salmon Calcitonin Loading Efficiencies in P(MAA-co-NVP) Microparticles

<table>
<thead>
<tr>
<th>MAA:NVP Ratio</th>
<th>Before HCl (%)</th>
<th>After HCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:1</td>
<td>&gt;99</td>
<td>80.48 ± 0.41</td>
</tr>
<tr>
<td>1:1</td>
<td>76.56 ± 0.81</td>
<td>57.72 ± 1.58</td>
</tr>
<tr>
<td>1:4</td>
<td>28.97 ± 6.13</td>
<td>2.65 ± 1.04</td>
</tr>
</tbody>
</table>

**Assessment of paracellular transepithelial transport**

Since, as described above, the compositions of the present invention may benefit from the
ability of the drug to be transported paracellularly across an intestinal epithelium, an assessment
of such transport may be desirable. One way of assessing such a transport mechanism is to
evaluate the formation of tight junctions, and one way of performing such an evaluation is by
measured transepithelial electrical resistance (TEER).

Caco-2 cells were obtained from American Type Culture Collection (ATCC, Rockwell,
MD) and HT29-MTX cells were a kind gift from Dr. Thecla Lesuffleur, INSERM, Paris, France.
HT29-MTX cells are a sub-population of HT29 cells that were adapted to 10^{-6} M methotrexate (MTX). All cell types were cultured in Dulbecco's modified Eagle medium (DMEM) (Mediatech, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum (Cambrex, East Rutherford, NJ), 1% non-essential amino acids (Mediatech), 100 U/ml penicillin, and 100 µg/ml streptomycin (Mediatech).

Cultures were maintained in T-75 flasks (Corning, Corning, NY) at 37 °C and a humidified environment of 5% CO₂ in air. The medium was changed every other day. Cells were routinely passaged at 80% confluency, which occurred between 6 and 7 days after seeding. A passage operation consisted of a 2 times wash with DPBS w/o Ca^{2+} and Mg^{2+} (Mediatech) and then the addition of 1 ml of a 0.5% Trypsin/0.2% EDTA solution (Sigma, St. Louis, MO). Cells were then incubated with the trypsin/EDTA solution for 5 minutes, after which time cells were detached from the flasks and could then be counted and reseeded.

Caco-2 cells were seeded at a density of 3.0 x 10^{3} cells/cm² and used between passages 60 to 80. HT29-MTX cells were seeded at a density of 2.0 x 10^{4} cells/cm² and used between passages 8 to 20.

All transport and transepithelial electrical resistance (TEER) experiments were conducted using a Costar Transwell® plate (Corning) with a polycarbonate membrane, 0.4 µm pore size, and a cell growth area of either 4.7 cm² (6 well) or 1 cm² (12 well). Cells were seeded after a passaging procedure and cultured for 21 to 24 days. Media were changed every other day and TEER values were measured with an EVOM volt-ohm meter and a chopstick electrode (World Precision Instruments, Sarasota, FL) to monitor development of tight junctions. It has been well documented that Caco-2 cells form an absorptive polarized monolayer, develop an apical brush border, and secrete enzymes after culture for 21 days.

Caco-2 and HT29-MTX cells were maintained separately in T-75 flasks as previously described. After subculturing, cells were counted and mixed together in a 1:1 ratio before seeding on to the Transwell® plate. Previous research demonstrated that a 1:1 seeding ratio produced TEER values closest to those reported in vivo for human intestinal epithelia. As with the Caco-2 cells, media was changed every other day in the co-culture and TEER was used to monitor development of the tight junctions.
TEER was used to evaluate the development of tight junctions in the Transwell®
cultures. Measurements were taken every other day two hours after changing the media. This
ensured that the content was the same in each well and was at 37 °C when the measurement was
taken. In order to determine the resistance across the cellular monolayer, $R_{true \ tissue}$, it was
important to subtract out the resistance due to the membrane and the media within the wells. A
blank resistance measurement was taken in the presence of medium without cells, $R_{blank}$, and
then subtracted from the experimental TEER value, as shown in the following equation:

$$R_{true \ tissue} = R_{experimental} - R_{blank}$$

After $R_{true \ tissue}$ was obtained, all TEER values were multiplied by the growth area, which
provided a unit area resistance. This allowed for comparison of TEER values when cells were
grown on different sized growth areas. The results of the TEER measurements are shown in
Figure 14. Additionally, relative TEER measurements are shown in Figure 15.

**In vivo assessment of drug delivery**

Methods for conducting *in vivo* assessment of drug delivery by the compositions and
methods of the present disclosure will be apparent to one of ordinary skill in the art. By way of
example, one such assessment may be performed by synthesizing P(MAA-co-NVP)
microparticles and loading the microparticles with GH, as discussed previously. In some
instances, it may be desirable to synthesize microparticles smaller than 90-150 μm. In order to
test release and bioavailability, the microparticles may be administered to a test subject
population by injection into the ileum. If the protection of the drug in the stomach is to be tested
as well, the microparticles may be orally administered to a test subject population. The route of
administration chosen may depend on, among other things, the test subject population and/or the
drug to be delivered. One of ordinary skill in the art, with the benefit of this disclosure, will be
able to choose an administration site appropriate for the given conditions. Following
administration, blood samples may be collected via an easily accessible arterial or venous site,
such as the jugular vein, and the samples may be assayed for GH content.

Therefore, the present invention is well adapted to attain the ends and advantages
mentioned as well as those that are inherent therein. While numerous changes may be made by
those skilled in the art, such changes are encompassed within the spirit of this invention as
illustrated, in part, by the appended claims.
CLAIMS

What is claimed is:

1. A composition for delivering a drug to a subject, the composition comprising:
   hydrogel matrix formed from a copolymer of an acrylic acid and an N-vinyl pyrrolidone, wherein the hydrogel matrix is at least partially crosslinked; and
   a drug, wherein the drug is disposed within the hydrogel matrix.

2. The composition of claim 1 wherein the acrylic acid comprises at least one acrylic acid selected from the group consisting of: acrylic acid; methacrylic acid; ethacrylic acid; propacrylic acid; crotonic acid; methacrylate; (Z)-3-cyclohexylbut-2-enolic acid; butylmethacrylate; any derivative thereof; and any combination thereof.

3. The composition of claim 1 wherein the N-vinyl pyrrolidone comprises at least one N-vinyl pyrrolidone selected from the group consisting of: N-vinyl pyrrolidone; isobetadyne; N-vinylsuccinimide; p(VA-co-NVP); any derivative thereof; and any combination thereof.

4. The composition of claim 1 wherein the drug comprises at least one drug selected from the group consisting of: a growth hormone; a growth hormone-releasing hormone; an interferon; an interleukin; a growth factor; an insulin; an insulin-like growth factor; a heparin; a calcitonin; an erythropoietin; a blood factor; a colony stimulating factor; an antigen; an antibody; an enzyme; an enzyme activator; a chemokine; an angiogenic agent; an anti-angiogenic agent; a neuroactive peptide; a thrombolytic agent; a bone morphogenic protein; thrombopoietin; a leukemia inhibitory factor; a transforming growth factor; a tissue factor; follicle stimulating hormone; a tumor necrosis factor; a nerve growth factor; a tissue inhibitor of metalloproteinases; a vasoactive intestinal peptide; fibrin; hirudin; a leukemia inhibitory factor; an IL-I receptor antagonist; a protease inhibitor; a prostataglandin; cyclosporin; vancomycin; parathyroid hormone, an antimicrobial; any derivative thereof; and any combination thereof.

5. The composition of claim 1 wherein the hydrogel network is pH-sensitive.

6. The composition of claim 5 wherein the hydrogel network is pH-sensitive insofar as the hydrogel network at least partially swells in response to an increase in pH.

7. The composition of claim 5 wherein the hydrogel network is pH-sensitive insofar as the hydrogel network at least partially swells at a pH above about 5.
A method of making a composition for delivering a drug to a subject, the method comprising polymerizing an acrylic acid and an N-vinyl pyrrolidone to form a hydrogel matrix that includes a drug disposed within the hydrogel matrix.

The method of claim 8 wherein the acrylic acid comprises at least one acrylic acid selected from the group consisting of: acrylic acid; methacrylic acid; ethacrylic acid; propacrylic acid; crotonic acid; methacrylate; (Z)-3-cyclohexylbut-2-enoic acid; butylmethacrylate; any derivative thereof; and any combination thereof.

The method of claim 8 wherein the N-vinyl pyrrolidone comprises at least one N-vinyl pyrrolidone selected from the group consisting of: N-vinyl pyrrolidone; isobetadyne; N-vinylsuccinimide; p(VA-co-NVP); any derivative thereof; and any combination thereof.

The method of claim 8 wherein the drug comprises at least one drug selected from the group consisting of: a growth hormone; a growth hormone-releasing hormone; an interferon; an interleukin; a growth factor; an insulin; an insulin-like growth factor; a heparin; a calcitonin; an erythropoietin; a blood factor; a colony stimulating factor; an antigen; an antibody; an enzyme; an enzyme activator; a chemokine; an angiogenic agent; an anti-angiogenic agent; a neuroactive peptide; a thrombolytic agent; a bone morphogenic protein; thrombopoietin; a leukemia inhibitory factor; a transforming growth factor; a tissue factor; follicle stimulating hormone; a tumor necrosis factor; a nerve growth factor; a tissue inhibitor of metalloproteinases; a vasoactive intestinal peptide; fibrin; hirudin; a leukemia inhibitory factor; an IL-I receptor antagonist; a protease inhibitor; a prostaglandin; cyclosporin; vancomycin; parathyroid hormone, an antimicrobial; any derivative thereof; and any combination thereof.

The method of claim 8 wherein the hydrogel network is pH-sensitive.

The method of claim 12 wherein the hydrogel network is pH-sensitive insofar as the hydrogel network at least partially swells in response to a change in pH.

The method of claim 13 wherein the hydrogel network is pH-sensitive insofar as the hydrogel network at least partially swells at a pH above about 5.
15. A method of treating a subject, the method comprising administering to the subject a composition according to claim 1.

16. The method of claim 14 wherein the acrylic acid comprises at least one acrylic acid selected from the group consisting of: acrylic acid; methacrylic acid; ethacrylic acid; propionic acid; crotonic acid; methacrylate; (Z)-3-cyclohexylbut-2-enoic acid; butylmethacrylate; any derivative thereof; and any combination thereof.

17. The method of claim 14 wherein the N-vinyl pyrrolidone comprises at least one N-vinyl pyrrolidone selected from the group consisting of: N-vinyl pyrrolidone; isobetadyne; N-vinylsuccinimide; p(VA-co-NVP); any derivative thereof; and any combination thereof.

18. The method of claim 14 wherein the drug comprises at least one drug selected from the group consisting of: a growth hormone; a growth hormone-releasing hormone; an interferon; an interleukin; a growth factor; an insulin; an insulin-like growth factor; a heparin; a calcitonin; an erythropoietin; a blood factor; a colony stimulating factor; an antigen; an antibody; an enzyme; an enzyme activator; a chemokine; an angiogenic agent; an anti-angiogenic agent; a neuroactive peptide; a thrombolytic agent; a bone morphogenic protein; thrombopoietin; a leukemia inhibitory factor; a transforming growth factor; a tissue factor; follicle stimulating hormone; a tumor necrosis factor; a nerve growth factor; a tissue inhibitor of metalloproteinases; a vasoactive intestinal peptide; fibrin; hirudin; a leukemia inhibitory factor; an IL-1 receptor antagonist; a protease inhibitor; a prostaglandin; cyclosporin; vancomycin; parathyroid hormone, an antimicrobial; any derivative thereof; and any combination thereof.

19. The method of claim 14 wherein the hydrogel network is pH-sensitive.

20. The method of claim 18 wherein the hydrogel network is pH-sensitive insofar as the hydrogel network at least partially swells in response to a change in pH.

21. The method of claim 19 wherein said change in pH is an increase in pH.

22. The method of claim 14 further comprising: allowing the hydrogel network to at least partially swell in response to a change in pH; and allowing at least a portion of the drug to be released from the hydrogel network as a result of the hydrogel network at least partially swelling.
Complexation at low pH limits diffusion of drug through polymer matrix; provides enzymatic protection to protein.

Deprotonation of hydrogen bonds occurs at high pH; ionic repulsion increases mesh size (ξ), allowing for release of drug to be absorbed by intestinal epithelium.
Figure 2

Methacrylic Acid
N-vinyl Pyrrolidone
Ethylene Glycol Dimethacrylate (EGDMA)
Monomers
Crosslinking Agent

Monomer mixture of 1:4:1:4 MAA:NVP; 1-5% EGDMA; 1 wt% initiator in 50:50 w/w water and ethanol
Mixture subjected to UV to initiate free radical polymerization; yields thin film
Film washed repeatedly to remove unreacted components; crushed into 90-150 μm particles
Figure 3
Figure 4

Dynamic Swelling (5-min) of p(MAA-co-NVP) 1:1 (CL=MBA, I=0.1M)
Figure 5
Figure 7

The graph shows the fraction of viable cells at different concentrations of a substance. The x-axis represents the concentration (mg/ml) with levels at 0.5, 1, and 2.5 mg/ml, while the y-axis shows the fraction of viable cells, ranging from 0 to 1.4. The control group shows a fraction of viable cells close to 1.0, while the higher concentrations of 0.5, 1, and 2.5 mg/ml have a slight decrease in the fraction of viable cells, with 1 mg/ml having the smallest decrease. The error bars indicate the variability in the data.
Figure 10

Protein Loading Efficiency (%)

INSULIN

1%  3%  5%  1%

GH
Figure 11

Percent Released (%) vs. Time (min)

- 1% CL (PBS)
- 3% CL (PBS)
- 5% CL (PBS)
- 1% CL (HCl)
- 3% CL (HCl)
- 5% CL (HCl)
Figure 15

Experimental Relative Transepithelial Resistance Readings

- Monoculture + Somatropin
- Monoculture + Somatropin-Loaded P(MAA-co-NVP)
- Coculture + Somatropin
- Coculture + Somatropin-Loaded P(MAA-co-NVP)

Relative Transepithelial Resistance

Time (Hours)

0 0.2 0.4 0.6 0.8 1 1.2

0 0.5 1 1.5 2 2.5 3