

Expert Opinion

1. Introduction
2. Strategies for oral insulin delivery
3. Expert opinion and conclusions

Delivery

Hydrogels for oral delivery of therapeutic proteins

Nicholas A Peppas, Kristy M Wood & James O Blanchette
*Laboratory for Biomaterials, Drug Delivery, Bionanotechnology and Molecular Recognition,
Departments of Chemical and Biomedical Engineering and Division of Pharmaceutics, University of
Texas at Austin, Austin, TX 78712, USA*

In recent years there has been significant new interest in the development of transmucosal (mostly oral) pharmaceutical formulations for the delivery of therapeutic proteins. Emphasis has been given to the molecular design of new carriers for the delivery of insulin, calcitonin and various types of interferons for the treatment of diabetes, osteoporosis, multiple sclerosis and cancer. Most popular carriers include advanced designs of swollen hydrogels prepared from neutral or intelligent polymeric networks. In this review, the most successful of such systems is presented and their promise in the field described.

Keywords: bioavailability, drug delivery, hydrogel, insulin, intelligent biomaterial, long-term stability, protein

Expert Opin. Biol. Ther. (2004) 4(6):

1. Introduction

Oral delivery of peptides and proteins has long been dubbed the 'Holy Grail' of drug delivery [1,2]. This route of administration is preferred because it increases patient compliance and comfort over injection, mimics physiological delivery of proteins, provides a simple administration, reduces the costs and potentially improves efficacy [3].

The proper carrier for the oral delivery of a protein drug depends on where the drug is intended to act, how the gastrointestinal (GI) tract acts on the drug and how the drug acts on the GI tract before reaching this site of action. If a protein drug is intended to be absorbed in the upper small intestine, then a carrier may be necessary. The carrier could be used to ensure that the drug does not cause toxicity to the lining of the digestive tract prior to reaching the colon, does not become sequestered along the way in an organ like the stomach and therefore not reach its target and does not become inactivated or altered in some way by the environment of the GI tract [1,3-5].

As most proteinic drugs are given by intravenous injection, a drug-loaded carrier that can be ingested and will promote absorption of the drug from the intestinal lumen to the bloodstream would be of significant clinical importance. Using a carrier to promote drug absorption from the GI tract has similar concerns as for drugs acting on the GI tract. Two of the major carriers used to deliver compounds to the small intestine are carriers with enteric coatings and hydrogels [1,6-8].

An enteric coating is a polymer layer placed over a capsule, tablet or particle for the purpose of shielding it from the upper portions of the digestive tract. The two major types of enteric coatings are cellulose- and acrylic-based coatings. Some examples of cellulose-based coatings are cellulose acetate phthalate (CAP) coatings, such as Aquacoat® (FMC BioPolymer). Aquacoat CPD is a 30% by weight aqueous dispersion of CAP, with a plasticiser added to allow the coating to be moulded to the appropriate shape. The coating dissolves when it reaches the neutral pH of the upper small intestine, allowing the material inside to release into the intestinal lumen.

Ashley Publications
www.ashley-pub.com



Eudragit® (Röhm Pharma Polymersis), the most prominent acrylic-based enteric coating and numerous types have been developed for specific applications. Variances in the average values for different regions of the small intestine can be exploited to target breakdown of the enteric coating to a specific region of the GI tract as discussed above. Eudragit is an aqueous, anionic polymer composed of methacrylic acid and methacrylates. The exact composition can be varied to target breakdown of the coating at a specific pH. Eudragit L100-55 is designed to dissolve at pH 5.5 for release in the duodenum. Eudragit L100 is designed to dissolve at pH 6.0 for release in the jejunum. Eudragit S100 dissolves at pH values in the range 6.0 – 7.5 for release in the ileum. Eudragit FS30D is designed to dissolve at pH values > 7.0 for release in the colon [8]. These values assume that the patient has typical pH values for these regions of the GI tract and modifications may be necessary for certain patients. The variations in pH that are common when considering a large patient population would cause release at an unintended site for delivery systems triggered by such a precise pH value.

A hydrogel is a three-dimensional, water-swollen structure composed of hydrophilic polymers. This network attains physical integrity and is made insoluble due to the presence of chemical and/or physical crosslinks [1,9,10]. Hydrogels can be classified as neutral and ionic. Ionic hydrogels contain pendant groups that can become ionised in response to changes in the environment, causing the hydrogel network to swell as it becomes more hydrophilic. Hydrogels that are capable of responding to their surroundings are termed physiologically-responsive hydrogels. Some of the physiological stimuli that these hydrogels can respond to are changes in temperature, variations in ionic strength of their environment and a change in pH such as that seen when passing through the pyloric sphincter [1,11,12].

As hydrogels are highly biocompatible, they are appropriate for a number of clinical applications. In addition to drug delivery carriers, hydrogels are used as contact lenses and scaffolds for tissue engineering applications. The polymer network can be homopolymers or copolymers, with the chemical structure determining the properties of the hydrogel. Some of the most common monomers used to form hydrogels for protein delivery are 2-hydroxyethyl methacrylate, ethylene glycol dimethacrylate, *N*-isopropyl acrylamide, acrylic acid and methacrylic acid. Poly(ethylene glycol) (PEG) and poly(vinyl alcohol) are two other polymers that have been used to form hydrogels.

The network structure of the hydrogel can be characterised by a number of parameters. One parameter of particular concern to this work is the mesh size. The mesh size is the term used to define the distance between crosslinks in the hydrogel network. A change in mesh size will alter the diffusion of a therapeutic protein from a hydrogel carrier. The value for this parameter can be determined experimentally or by theoretical calculations [13-16].

The polymer fraction in the swollen state is a measure of how much water the hydrogel can imbibe when placed in an

aqueous environment. The ability of hydrogels to retain large amounts of water makes them similar to natural tissue and may contribute to their high biocompatibility. Both the molecular weight and distance between consecutive crosslinks give an indication of the crosslinking density of the network. Due to the randomness involved with polymer formation, these parameters can only be given as average values throughout the hydrogel. These parameters can indicate the mesh size available for protein diffusion through a hydrogel. This value, along with the size of the protein to be delivered, will be important in determining the release kinetics of the agent from the hydrogel in drug delivery applications. The degree of swelling affects the mesh size. Therefore, a physiologically responsive hydrogel that swells when presented with certain stimuli can have different release kinetics at different sites in the body.

Environmentally or physiologically responsive hydrogels, based on complexation due to hydrogen bonding, are composed of ionic components and swell in response to pH changes. This swelling behaviour can be controlled by decomplexation and ionisation of the pendent groups in the network. Decomplexation refers to the breakdown of hydrogen bonds between polymer chains within the hydrogel network. The loss of these bonds can allow the network to expand and reformation of these bonds at higher pH is involved in collapsing the network. Ionic pendent groups exhibit electrostatic repulsion that leads to imbibition of water and increased mesh size. This also depends on the level of crosslinking present in the hydrogel. Highly crosslinked materials will not be able to swell to as high a degree as materials with lower crosslinking ratios due to decreased chain mobility. The degree to which a hydrogel network swells is also dependent upon the ability to imbibe water. Hydrogels with hydrophilic groups can imbibe more water than those with hydrophobic groups and can therefore swell to a greater extent. The hydrophobicity/hydrophilicity of the network will therefore also have an impact on the diffusion of any compound embedded within a hydrogel network.

An example of a monomer that can polymerise to form an ionic hydrogel with pH-responsive swelling behaviour is methacrylic acid (MAA). When the pH of the environment is greater than the pK_a of the carboxylic acid groups in PMAA, the latter become ionised and causes interchain repulsion. The charged groups are also hydrophilic and allow water to enter the network and continue the swelling process. The process of ionisation is reversible and depends on the pH of the environment [17-21].

If the MAA is grafted with another polymer capable of forming hydrogen bonds, such as PEG, then hydrogen bonds can form between the chains when in the protonated state at low pH. Hydrogels that are capable of forming hydrogen bonds are called complexation hydrogels. This pH-dependent formation of hydrogen bonds provides another means by which the network exists in a compact state at a low pH and a more open state at the elevated pH.

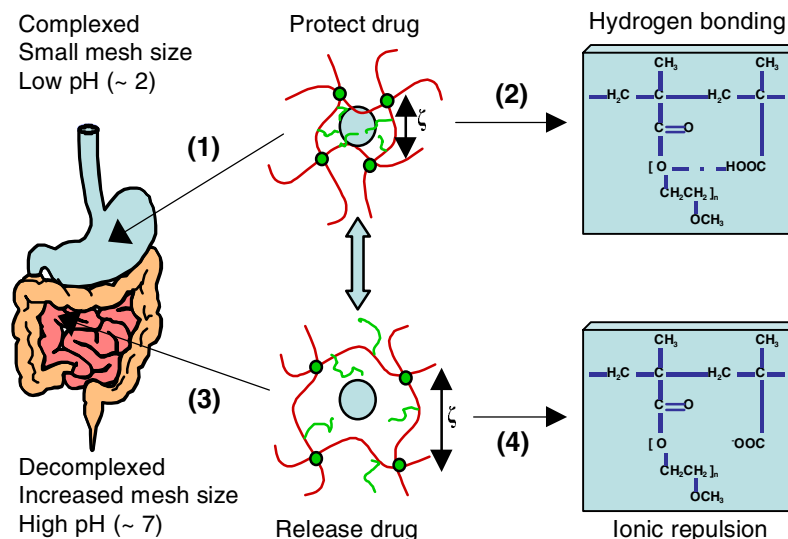


Figure 1. (1) Protection of the drug in a low pH environment. (2) Complexation of the carrier occurs due to hydrogen bonding between polymer chains. (3) Release of the drug in the upper small intestine. (4) Decomplexation and an increase in mesh size occurs due to ionic repulsion and swelling of the polymer at a high pH.

More specifically, hydrogels whose complexation states are based on the pH of the environment are termed pH-responsive complexation hydrogels (Figure 1).

Through the use of monomers with side chains containing groups with pK_a values in the range desired, a pH-responsive hydrogel could be designed much in the same manner as enteric coatings. Hydrogels can exhibit swelling to different degrees based on the intensity of the stimulus and this could be used to target release of multiple compounds at different sites. For example, a hydrogel could be loaded with two different chemotherapeutic agents of different sizes. If the smaller compound is able to diffuse from the hydrogel with only a moderate amount of swelling, it could be released at a lower pH than the larger agent. This could allow for targeted delivery of two agents to distinct sites in the GI tract. The variability of the hydrogel delivery system in what it will respond to and how it will respond makes it an attractive candidate for numerous clinical applications including targeted drug delivery.

2. Strategies for oral insulin delivery

Strategies of oral protein delivery include:

- use of protease inhibitors
- use of permeation enhancers
- protein microencapsulation using micro or nanospheres
- covalent and non-covalent drug modification

Protease inhibitors interact with digestive enzymes to reduce inactivation of the delivered drug. They include sodium glycocholate, camostat mesilate, bacitracin and other drugs effective in the large intestine [22]. Additionally,

chymostatin and elastinal can be covalently linked and complexed with EDTA [19]. Yet potential effects include toxicity and reduction in normal enzyme function.

Permeation enhancers usually increase the permeability of proteins by enhancing transcellular and paracellular transport. They include fatty acids, bile acids, zonula occludens toxin and EDTA [23,24]. Typically, there is concern that they open the tight junctions and can increase transport of toxins and biological pathogens. Additionally, continuous opening of tight junctions may not be reversible.

The use of carriers in the form of polymer or hydrogel micro- and nano-spheres is preferred because of their efficacy. Such systems include complexation and pH-sensitive hydrogels, coated liposomes and chitosan [25,26]. Microspheres can be modified by muco- or cyto-adhesion to increase the residence time in the GI tract [27-29]. However, their poorly understood immune response is a major problem.

Covalent and non-covalent drug modifications are two methods for oral insulin delivery employed by NOBEX Corporation and Emisphere Technologies, respectively. The goal of covalent modification is to increase both drug solubility and stability, thus allowing for improved transport of the drug across the epithelial layer. NOBEX have modified recombinant human insulin at the lysine-29 site on the α -chain by covalently binding a hydrophilic PEG chain and a lipophilic alkyl chain. Clinical trials with Type 1 and Type 2 diabetic patients demonstrate initial efficacy but low bioavailability (estimated at 5%) continues to be a problem [30,31]. In addition, covalent modification may affect pharmacological activity of the protein.

Eligen™ (Emisphere Technologies, Inc.) utilises small organic compounds to non-covalently interact with a drug to

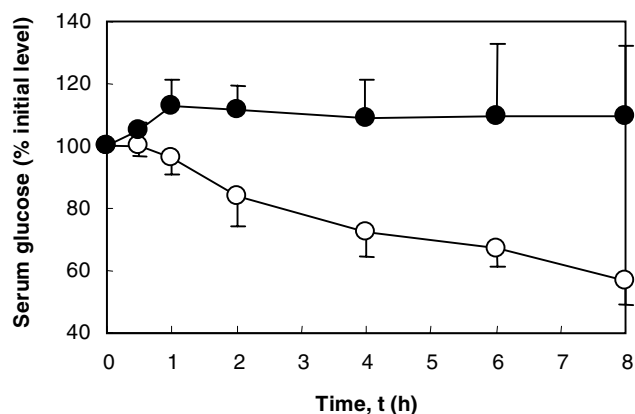


Figure 2. Blood glucose response in diabetic, male Wistar rats following oral administration of 25 IU/kg body weight doses contained in P(MAA-g-EG) (open circles) microspheres and insulin control solutions (closed circles) (n = 5).

IU: International units; P(MAA-g-EG): poly(methacrylic acid) grafted with poly(ethylene glycol)

enhance absorption. The hydrophobic organic compounds are mainly composed of amino acids, with a molecular weight range of 200 – 400. Through non-covalent modification, Emisphere is seeking to increase lipophilicity of the drug and thus enhance drug transport. Binding of the organic compounds to the protein is reversible and dissociation occurs in the bloodstream after absorption. While bound, the organic molecule causes a reversible conformational change in the protein structure to increase lipophilicity. Bioavailability of 13% after 1 h and 2% after 6 h in initial studies with Type 2 diabetes patients demonstrate the potential use of the eligen technology for protein delivery [2,32].

2.1 Oral delivery based on complexation hydrogels

Polymeric carriers for insulin and other proteins must be designed to minimise the affects of proteolytic enzymes throughout the GI tract, but still effectively deliver the drug to the upper small intestine and allow for absorption across the intestinal epithelium into the bloodstream. Complexation hydrogels composed of methacrylic acid or acrylic acid grafted with ethylene glycol chains (P[AA-g-EG] or P[MAA-g-EG]) exhibit these desired properties [3- 7,11-17,27]. The polymeric carriers are prepared through UV-initiated free radical polymerisation and the resulting polymer film can be crushed and sieved into microparticles of varying size.

Temporary physical crosslinks formed from the reversible complexation of the polymer chains and the ability of the polymer carrier to respond to pH changes in the GI tract [12] make this class of hydrogels a suitable vehicle for oral delivery of proteins (Figure 1). Complexation of the polymeric carrier is due to the hydrogen bonding between the carboxyl group of the MAA and the oxygen of the PEG chains, which protects the drug in the low pH environment of the stomach. As the environmentally sensitive polymeric carrier passes into the small intestine, a pH shift to ~ 7 occurs, causing

deprotonation of the carboxyl group on the MAA (pK_a 4.9) and thus creating ionic repulsion between the polymer chains. The increased mesh size, due to the ionic repulsion and the uptake of water in the decomplexed state, allows for release of the drug at the targeted site of absorption [7,15]. By varying MAA:EG feed ratios, the mesh size has been shown to increase to 70 – 210 Å upon swelling of the hydrogel. The P(MAA-g-EG) carrier not only protects and delivers the drug to a targeted site but the carrier also increases residence time at the site of absorption through mucoadhesion. Tethered PEG chains diffuse out of the polymer carrier upon swelling and entangle with the mucosal layer to promote carrier adhesion [33] at the site of absorption.

In addition to the complexation and adhesive behaviour, these gels are promising candidates for oral protein delivery because they have the capability to entrap and release insulin efficiently, as well as to enhance transport across the intestinal mucosa. It has previously been reported that the insulin incorporation efficiency into these hydrogels is > 90% [12,17] and that the insulin could be protected in simulated gastric fluid (pH 1.2) and released in simulated intestinal fluid (pH 7.4). The oral administration of insulin-loaded complexation polymers provided significant insulin absorption and glucose reduction in healthy and diabetic rats [12]. Extensive studies with Wistar rats showed insulin bioavailability based on insulin as high as 12.8% with a fairly rapid insulin release from the polymer microparticles (Figure 2) [10,29,33,34]. Moreover, P(MAA-g-EG) copolymers induced the decrease in mucosal membrane resistance in Caco-2 monolayers but cytotoxicity did not appear after the exposure of hydrogels to the Caco-2 monolayer (Figure 3) [10,15]. This previous work clearly demonstrates the potential of P(MAA-g-EG) carriers for oral insulin delivery.

Another interesting study using ionic hydrogels was reported by Platé *et al.* [35]. Although promising, this study does not report bioavailability data and requires the use of protease inhibitors.

3. Expert opinion and conclusions

Promising techniques currently exist for the oral delivery of therapeutic proteins but many problems regarding adequate bioavailability and potential immune response need to be answered to ensure oral protein delivery will be a reality. Promising oral protein delivery systems consist of insulin incorporated in carrier microparticles able to:

- protect insulin degradation in the lumen of the stomach
- adhere to the mucus of the intestinal wall and release insulin, increasing the residence time of the protein in the small intestine
- protect the released insulin to a certain extent by inhibiting the activity of Ca^{2+} -dependent proteolytic enzymes

Receptor-mediated transcytosis has been considered an effective approach for achieving specific delivery of proteins and

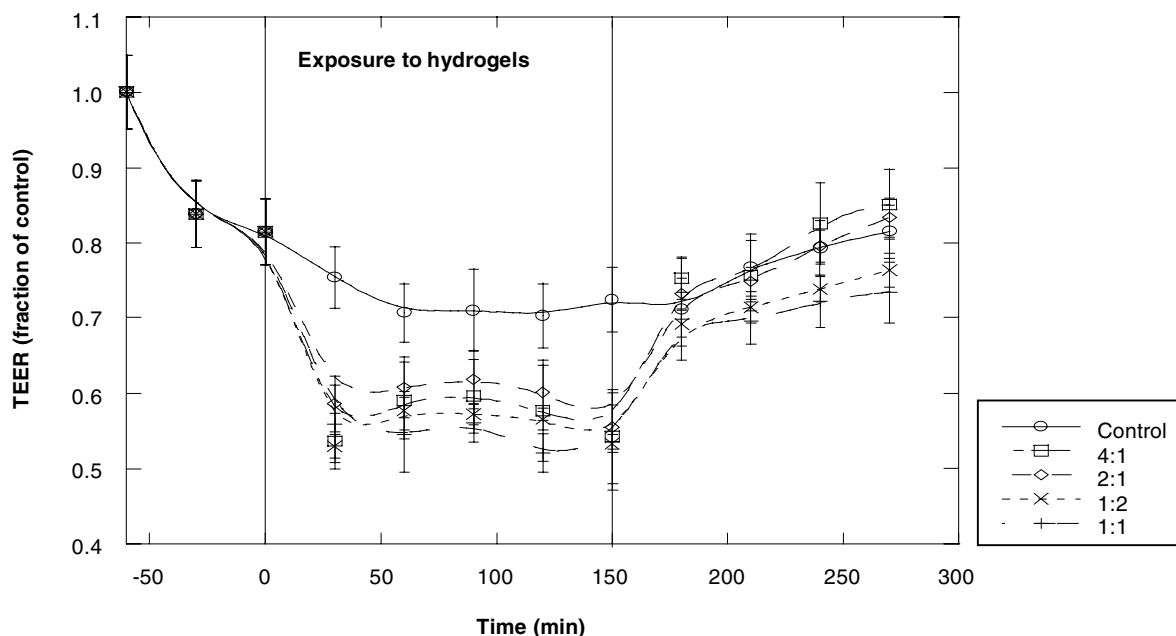


Figure 3. Fraction of the TEER as a function of time for monolayers exposed to P(MAA-g-EG) hydrogel nanospheres with different monomer ratios with a concentration 10 mg/ml in Caco-2 cell monolayers at 37°C. Each datum point represents an average of $n = 3$ for control and experimental wells \pm one s.d.

s.d.: Standard deviation; TEER: ;P(MAA-g-EG): poly(methacrylic acid) grafted with poly(ethylene glycol).

peptides across cellular barriers such as endothelium and epithelium. As this transport process across the epithelia takes place without opening of the tight junctions between the cells, it eliminates the possibility of the toxic components crossing the epithelium. Naturally occurring proteins such as transferrin (protein of molecular weight of $\sim 80,000$ involved in iron transport) have been investigated for targeted delivery of the drug molecules since these proteins are biodegradable and non-toxic.

Furthermore, site-specific conjugation of PEG to proteins results in increased stability of the protein because of the shielding of protein domains susceptible to proteolytic

attack. In addition to this, the PEG conjugation almost completely eliminates the resultant conjugate's immunogenicity and allergenicity. We believe that the use of PEG-based microparticles as delivery vehicles for PEG protein and protein conjugates will constitute superior transmucosal delivery systems for proteins.

These systems are advantageous over other designs because we have been able to obtain strong, dose-dependent hypoglycaemic effects without the addition of absorption enhancers or protease inhibitors. Due to the decrease in costs of insulin production, a reasonable target for insulin bioavailability has been estimated as on the order of 35%.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

- PEPPAS NA, BURES P, LEOBANDUNG W, ICHIKAWA H: Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.* (2000) 50:27-46.
- GOLDBERG M, GOMEZ-ORELLANA I: Challenges for the oral delivery of macromolecules. *Nature Rev. Drug Disc.* (2003) 2:289-295.
- TORRES-LUGO M, PEPPAS NA: Transmucosal delivery systems for calcitonin: a review. *Biomaterials* (2000) 21:1191-1196.
- LOWMAN AM, PEPPAS NA: Molecular analysis of interpolymer complexation in graft copolymer networks. *Polymer* (2000) 41:73-80.
- FOSS AC, PEPPAS NA: Acrylic-based copolymers for oral insulin delivery systems. *Polym. Prepr.* (2001) 42(2):94-95.
- GARCIA M, TORRES-LUGO M, ALONSO MJ, PEPPAS NA: Biointeractions of pH-sensitive poly(methacrylic acid-g-ethylene glycol) hydrogel microspheres with the Caco-2 model cell line. In: *New Trends in Polymers for Oral and Parenteral Administration: From Design to Receptors*. Barratt G, Duchêne D, Fattal F, Legendre JY (Eds), Editions de Santé, Paris, France (2001):386-389.
- TORRES-LUGO M, PEPPAS NA: Preparation and characterization of

- P(MAA-g-EG) nanospheres for protein delivery applications. *J. Nanoparticle Res.* (2002) 4:73-81.
- **Detailed analysis of molecular structure of intelligent hydrogels for protein delivery.**
8. BLANCHETTE J, PARK K, PEPPAS NA: Oral administration of chemotherapeutic agents using complexation hydrogels. In: *Biological and Biomimetic Materials – Properties to Function*. McKittrick J, Aizenberg J, Orme C, Vekilov P (Eds), MRS, Pittsburgh, PA, USA (2002):218-222.
 9. KIM B, PEPPAS NA: PEG-containing hydrogel microparticles for oral protein delivery applications. *Biomed. Microdevices* (2003) 5:333-341.
 10. ICHIKAWA H, PEPPAS NA: Novel complexation hydrogels for oral peptide delivery: *in vitro* evaluation of their cytocompatibility and insulin-transport enhancing effects using Caco-2 cell monolayers. *J. Biomed. Mater. Res.* (2003) 67:609-617.
 11. ICHIKAWA H, PEPPAS NA: Synthesis and characterization of pH-responsive nanosized hydrogels of poly(methacrylic acid-g-ethylene glycol) for oral peptide delivery. *New Trends in Polymers for Oral and Parenteral Administration: From Design to Receptors*. Barratt G, Duchène D, F, Legendre JY (Eds), Editions de Santé, Paris, France (2001):261-264.
 12. LOWMAN AM, MORISHITA M, KAJITA M, NAGAI T, PEPPAS NA: Oral delivery of insulin using pH-responsive complexation gels. *J. Pharm. Sci.* (1999) 88:933-937.
 - **Pioneering work in the field; first study with substantial insulin bioavailability in Wistar rats.** 13. MORISHITA M, LOWMAN AM, TAKAYAMA K, NAGAI T, PEPPAS NA: Elucidation of the mechanism of incorporation of insulin in controlled release systems based on complexation polymers. *J. Control. Release* (2002) 81:25-32.
 14. TORRES-LUGO M, GARCIA M, RECORD R, PEPPAS NA: Physicochemical behavior and cytotoxic effects of P(MAA-g-EG) nanospheres for oral delivery of proteins. *J. Control. Release* (2002) 80:197-205.
 15. TORRES-LUGO M, GARCÍA M, RECORD R, PEPPAS NA: pH-Sensitive hydrogels as gastrointestinal tract absorption enhancers: transport mechanisms of salmon calcitonin and other model molecules using the Caco-2 cell model. *Biotechnol. Prog.* (2002) 18:612-616.
 16. ROBINSON DN, PEPPAS NA: Preparation and characterization of pH-responsive poly(methacrylic acid-g-poly(ethylene glycol) nanospheres. *Macromolecules* (2002) 35:3668-3674.
 17. LOWMAN AM, COWANS BA, PEPPAS NA: Investigation of interpolymer complexation in swollen polyelectrolyte networks by solid state NMR spectroscopy. *J. Polym. Sci. Polym. Phys.* (2000) 38:2823-2831.
 18. SCOTT R, WARD JH, PEPPAS NA: Development of acrylate and methacrylate polymer networks for controlled release by photopolymerization technology. In: *Handbook of Pharmaceutical Controlled Release Technology*. Wise DL, Brannon-Peppas L, Klibanov AM, Langer R, Mikos AG, Peppas NA, Trantolo DJ, Wnek GE, Yaszemski MJ (Eds), Dekker, New York, NY, USA (2000):47-64.
 19. BERNKOP-SCHNURCH A, SCERBE-SAIKO A: Synthesis and *in-vitro* evaluation of chitosan-EDTA-protease-inhibitor conjugates which might be useful in oral delivery of peptides and proteins. *Pharm. Res.* (1998) 15:263-269.
 - **Earliest in a series of interesting studies by this Austrian group.** 20. PEPPAS NA; Gels for drug delivery. In: *Encyclopedia of Materials: Science and Technology*. Elsevier, Amsterdam, The Netherlands (2001):3492-3495.
 21. PEPPAS NA, BRANNON-PEPPAS L: Drug delivery biomaterials. In: *Encyclopedia of Materials: Science and Technology*. Elsevier, Amsterdam, The Netherlands (2001):2351-2355.
 22. YAMAMOTO A, TANIGUCHI T, RIKYUU K *et al.*: Effects of various protease inhibitors on the intestinal absorption and degradation of insulin in rats. *Pharm. Res.* (1994) 11:1496-1500.
 23. MESIHA M, PLAKOGIANNIS F, VEJOSOTH S: Enhanced oral absorption of insulin from desolvated fatty acid-sodium glycocholate emulsions. *Int. J. Pharm.* (1994) 111:213-216.
 24. FASANO A, UZZAU S: Modulation of intestinal tight junctions by zona occludens toxin permits enteral administration of insulin and other macromolecules in an animal model. *J. Clin. Invest.* (1997) 99:1158-1164.
 - **Pioneer work on understanding protein transport by disruption of cell tight junctions.** 25. BERGER J, REIST M, MAYER JM, FELT O, PEPPAS NA, GURNY R: Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *Eur. J. Pharm. Biopharm.* (2004) 57:19-34.
 - **Exceptional review of the importance of chitosans in the field.** 26. BERGER J, REIST M, MAYER JM, FELT O, GURNY R: Structure and interaction in chitosan hydrogels formed by complexation of aggregation for biomedical applications. *Eur. J. Pharm. Biopharm.* (2004) 57:35-52.
 27. DONINI C, ROBINSON DN, COLOMBO P, GIORDANO F, PEPPAS NA: Preparation of P(MAA-g-EG) nanospheres for pharmaceutical applications. *Int. J. Pharm.* (2002) 245:83-91.
 28. SIPAHIGIL O, TORRES-LUGO M, PEPPAS NA: FTIR spectroscopic analysis of protein/carrier interactions in novel protein delivery systems. *STP Pharma* (2002) 12:345-350.
 29. NAKAMURA K, MURRAY RJ, JOSEPH JI, PEPPAS NA, MORISHITA M, LOWMAN AM: Oral insulin delivery using P(MAA-g-EG) hydrogels: effects of network morphology on insulin delivery characteristics. *J. Control. Release* (2004) 95(3):589-599.
 30. STILL JG: Development of oral insulin: progress and current status. *Diabetes Metab. Res. Rev.* (2002) 18(Suppl. 1):S29-S37.
 31. KIPNES M, DANDONA P, TRIPATHY D, STILL JG, KOSUTIC G: Control of postprandial plasma glucose by an oral insulin product (HIM2) in patients with type 2 diabetes. *Diabetes Care* (2003) 26:421-426.
 - **Pioneering work in the field. First clinical study of elevated bioavailability in patients.** 32. KAPITZA C, ARBIT E, ABBAS R *et al.*: Oral insulin: proof of concept in type 2 diabetic patients. (glucose monitoring and insulin delivery). *Diabetes* (2003) 52(Suppl. 1):A37.
 33. HUANG Y, EFREMOVA N, PEPPAS NA, LECKBAND DE: Direct measurement of interactions between tethered PEG chains and adsorbed mucin layers. *Langmuir* (2002) 18:836-845.

34. MORISHITA M, GOTO T, PEPPAS NA
et al.: Mucosal insulin delivery systems
based on complexation polymer hydrogels:
effect of particle size on insulin enteral
absorption. *J. Control. Release*
(2004) (In Press).
35. PLATE NA, VALUEV IL, SYTOV GA,
VALUEV LI; Mucoadhesive polymers with
immobilized proteinase inhibitors for oral
administration of protein drugs.
Biomaterials (2002) **23**:1673-1677.

Affiliation

Nicholas A Peppas, Kristy M Wood &
James O Blanchette

[†]Author for correspondence

Laboratory for Biomaterials, Drug Delivery,
Bionanotechnology and Molecular Recognition,
Departments of Chemical and Biomedical
Engineering and Division of Pharmaceutics,
University of Texas at Austin, Austin,
TX, 78712, USA

Author proof