# Development of a pH-responsive hydrogel network for the oral delivery of human growth hormone

Stephanie Steichen[1], and Nicholas A. Peppas[1, 2, 3]

[1]Department of Biomedical Engineering, [2]Department of Chemical Engineering, and
[3]Division of Pharmaceutics, University of Texas at Austin, Austin, TX-78712-1062, USA

**Statement of Purpose:** Complexation hydrogels composed of methacrylic acid (MAA) and N-vinyl pyrrolidone (NVP), henceforth designated as P(MAA-co-NVP) are excellent candidates for transmucosal delivery of a number of therapeutics including insulin and growth hormone. These hydrogels are ideal carriers for proteins and peptides due to their ability to respond to changes in the pH of the gastrointestinal (GI) tract and protect the therapeutic payload from the harsh conditions present in the GI tract. At low pH, such as in the stomach, the hydrogel network remains collapsed due to the presence of interpolymer complexes in the form of hydrogen bonds[1]. At increasing pH, such as in the small intestine, the complexes disassociate and the hydrogels swell due to a combination of electrostatic repulsion and water imbibition. These hydrogel systems are capable of effectively delivering therapeutic proteins, which are typically limited to intravenous administration due to their fragile nature and large size. The protein can be loaded into the network, protected from the low pH and enzymes present in the upper GI tract, then released into the upper small intestine. P(MAA-co-NVP) has been optimized for the delivery of insulin and shown success in *in vitro* cellular models[2]. The structure of these gels must now be optimized for the delivery of other therapeutic proteins. The specific properties of each protein will determine its interactions with the hydrogel carrier and can affect loading, release and subsequent bioavailability. This work focuses on the optimization of a P(MAA-co-NVP) based hydrogel system for the oral delivery of human growth hormone (hGH). This protein was selected not only for its therapeutic relevance, treating conditions such as Prader-Willi Syndrome and growth hormone deficiency, but also for its large size, approximately 4 times greater than previously delivered proteins.

**Methods:** *Hydrogel Synthesis.* The network used in this work is P((MAA-co-NVP)-g-EG), which is comprised of a poly(methacyrlic acid) and N-vinyl pyrrolidone backbone and grafted poly(ethylene glycol) tethers. The hydrogels were synthesized at a 2:1:1 molar ratio with different crosslinking type and density utilizing a UV-initiated free radical solution polymerization. The polymer film was then crushed into microparticle carriers approximately 50-75 µm in size. *Polymer Characterization.* Equilibrium and dynamic swelling studies were completed to determine the swelling profile of the hydrogel network and to determine behavior at gastric and intestinal pH conditions. Fourier transform infrared spectroscopy (FTIR) was performed to examine the surface characteristics of each gel and to ensure presence of PEG. SEM was also used to compare the surface morphology of the particles. *Protein loading and release.* Protein loading was accomplished by incubating the hydrogel microparticles in a protein-PBS solution (pH 7.4) and then adding 1N HCl to the solution to lower the pH and collapse the microparticles. Release of hGH from the microparticles was studied under dynamic pH conditions designed to mimic transit through the GI tract. Growth hormone loading and release was quantified with HPLC and protein-specific ELISAs, respectively. *In vitro viability assays.* Particle cytotoxicity was measured on three separate cell lines, L929 fibroblasts, CaCo2 colon adenocarcinomas and HT29 mucus-secreting cells. Particles at varying concentrations were introduced to the cells in a 96-well plate for 2 hours. An MTS assay was used to measure cell viability.

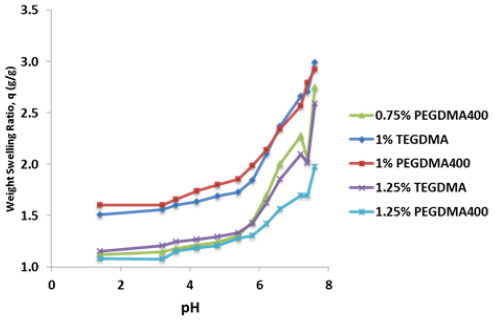


**Figure 1.** Dynamic pH swelling studies of hydrogel discs comparing P((MAA-co-NVP)-g-EG) gels of various crosslinker composition (0.75, 1, or 1.25% of PEGDMA-400 and TEGMDA) in DMGA/NaOH buffers.

**Results:** Figure 1 shows the dynamic swelling behaviour of the synthesized P((MAA-co-NVP)-g-EG) hydrogels. All of the formulations remain collapsed at low pH and begin to swell as the pH is increased. Gels with 1% crosslinker exhibited the greatest swelling at neutral pH but were not as tightly collapsed at low pH. Conversely, the gels with 1.25% crosslinker swelled less at neutral pH but remained tightly collapsed at low pH. The gel with 0.75% crosslinker, however, was tightly collapsed at low pH and swelling to almost the same amount as the 1% crosslinked gels. Different length and hydrophilicity of crosslinkers also had some effect. Figure 2 shows the Caco2 cell viability after exposure to the microparticle formulations at concentrations ranging from 1.25 – 5 mg/mL. At concentrations less than 5 mg/mL there is minimal to no cytotoxic effects from the particles.

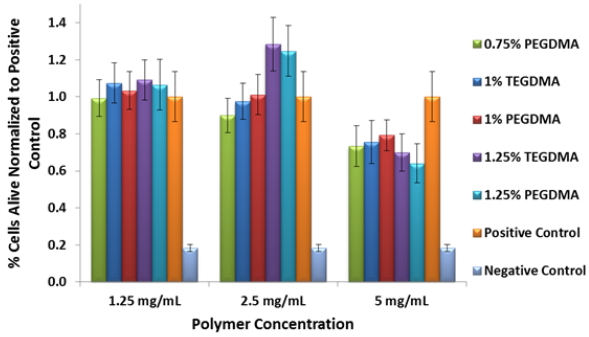


**Figure 2**. Cell viability measured with an MTS assay after introduction of P((MAA-co-NVP)-g-EG) microparticles. The positive control was media while the negative control was a bleach/media solution.

**Conclusions:** In this study, P(MAA-co-NVP) hydrogels were optimized for the delivery of a high molecular weight protein, growth hormone. Varying crosslinking type and density changed the swelling profiles and cytotoxicity of the carriers. Characterizing the polymer system through swelling, loading/release and cytotoxicity, we can select optimum polymer composition for oral protein delivery.

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**References:**

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[2] Carr, D. A. and N.A. Peppas, *J Biomed Mater Res A.* (2010) 92, 504-12.