Published in final edited form as:

J Drug Deliv Sci Technol. 2015 December 1; 30(Pt B): 352–359. doi:10.1016/j.jddst.2015.07.023.

Hybrid responsive hydrogel carriers for oral delivery of low molecular weight therapeutic agents

M. Caldorera-Moore^{a,b,c,*}, K. Maass^b, R. Hegab^a, G. Fletcher^c, and N. Peppas^{b,c,d,e,**}

^aDepartment of Biomedical Engineering, Louisiana Tech University, Ruston, LA 71272, USA

^bDepartment of Chemical Engineering, The University of Texas at Austin, Austin, TX 78712, USA

^cDepartment of Biomedical Engineering, The University of Texas at Austin, Austin, TX 78712, USA

^dDivision of Pharmaceutics, The University of Texas at Austin, Austin, TX 78712, USA

^eInstitute for Biomaterials, Drug Delivery and Regenerative Medicine, The University of Texas at Austin, Austin, TX 78712, USA

Abstract

Hydrogels have been influential in the development of controlled release systems for a wide variety of therapeutic agents. These materials are attractive as carriers for transmucosal and intracellular drug delivery because of their inherent biocompatibility, tunable physicochemical properties, basic synthesis, and ability to be physiologically responsive. Due to their hydrophilic nature, hydrogel-based carrier systems are not always the best systems for delivery of small molecular weight, hydrophobic therapeutic agents. In this work, versatile hydrogel-based carriers composed of copolymers of methyl methacrylate (MMA) and acrylic acid (AA) were designed and synthesized to create formulations for oral delivery of small molecular weight therapeutic agents. Through practical material selection and careful design of copolymer composition and molecular architecture, we engineered systems capable of responding to physiological changes, with tunable physicochemical properties that are optimized to load, protect, and deliver their payloads to their intended site of action. The synthesized carriers' ability to respond to changes in pH, to load and release small molecular weight drugs, and biocompatibility were investigated. Our results suggest these hydrophilic networks have great potential for controlled delivery of small-molecular weight, hydrophobic and hydrophilic agents.

Graphical abstract

*Corresponding author. Department of Biomedical Engineering, Louisiana Tech University, Ruston, LA 71272, USA. mcmoore@latech.edu (M. Caldorera-Moore). **Corresponding author. Department of Chemical Engineering, The University of Texas at Austin, Austin, TX 78712, USA. peppas@che.utexas.edu (N. Peppas).

Keywords

Responsive hydrogels; Oral delivery; pH responsive delivery; Poly(methacrylic acid); Poly(acrylic acid)

1. Introduction

Drug delivery systems (DDS) are of utmost importance to the development of new pharmaceutical formulations. With the advent of new, sophisticated, therapeutic agents that can target specific cellular functions, it is vital that these drugs reach the desired diseased cells. Environmentally responsive DDS can accomplish this [1–3]. Synthetic polymer-based hydrogels improve the solubility, bioavailability, and pharmacokinetics of a drug delivered orally. These materials are attractive for transmucosal and intracellular drug delivery because of their straightforward synthesis, their inherent biocompatibility, tunable physicochemical properties, and ability to be physiologically responsive [4].

The oral administration of small molecular weight agents has numerous obstacles that must be circumvented to create an effective delivery system. Compared to intravenous delivery, there is low bioavailability in transportation of the hydrophobic agents from the gastrointestinal (GI) tract to the bloodstream. Design of polymeric carriers must ensure the drug is carried from the GI tract to the bloodstream without it being inactivated. The drug must be protected from the low pH of the stomach, but concurrently the GI tract must be protected by the potential toxicity of these therapeutic agents.

In our laboratory we have developed a class of hydrogels containing an acid backbone composed of acrylic acid (AA) and/or methacrylic acid (MAA) for the development of pH responsive hybrid biomaterials, for oral delivery within the upper small intestine. Previously we've demonstrated that the physicochemical behavior of these hydrogels can be controlled by the three-dimensional architecture of the polymer networks as well as by their environmental conditions for biomacromolecular delivery [4–19]. Unfortunately, due to

their hydrophilic nature, traditional hydrogel-based carrier systems may not be the optimal systems for delivery of small molecular weight, hydrophobic therapeutic agents. Hybrid hydrogel systems that contain hydrophobic components have the potential to overcome this limitation.

Several novel hybrid hydrogel materials have recently been developed to overcome this shortcoming [4,16,17]. In their work, Schoener et al. [16] developed two types of hybrid hydrogels that had hydrophobic domains within the hydrophilic polymer network. The first hybrid hydrogels were amphiphilic interpenetrating networks (IPNs) that had a hydrophobic network composed of poly(*n*-butyl acrylate) (PBA) interwoven within a hydrophilic hydrogel of methacrylic acid grafted with poly(ethylene glycol) tethers P(MAA-g-EG) [16]. The presence of the PBA rendered the overall network more hydrophobic but it also decreased the swelling of the hydrogel network that could limit the loading efficiency and release of therapeutic agents.

To overcome this limitation, Schoener and colleagues developed a second hydrogel architecture composed of P (MAA-g-EG) hydrogels containing hydrophobic poly(methyl methacrylate) (PMMA) nanoparticles [17]. The presence of PMMA nanoparticles in the hydrogel network altered the swelling behavior, loading efficiency, and release profiles of model hydrophobic drugs. The swelling ratio of nanoparticle-containing hydrogels decreased with increasing nanoparticle content. Also, the presence of the PMMA nanoparticles influenced the physical properties of the hydrogel system. It appeared that the nanoparticles reduced ionic repulsion between the deprotonated carboxyl groups in the P (MAA-g-EG) hydrogel, reduced the free volume for polymer chain movement of the pH responsive network, and reduced water intake.

In the present work, microparticles composed of co-polymer blends of methyl methacrylate/acrylic acid (MMA/AA) were investigated for the development of an oral drug delivery system for small molecular weight drugs. The carriers' ability to load and release diltiazem hydrochloride, a hydrophilic low molecular weight drug and fluorescein, a model hydrophobic low molecular weight drug, was studied. P (AA-co-MMA) copolymers start swelling at pH values near the pKa of acrylic acid (~pH 5). Thus, these hydrogels are expected to remain collapsed in the acidic conditions of the stomach, but swell in the more basic conditions of the small intestine. In addition, the mixture of hydrophobic and hydrophilic monomers will give the polymer unique characteristics that may prove to be beneficial for loading a release of small molecular weight drugs and hydrophobic therapeutics.

It is well known that acrylic acid is hydrophilic whereas methyl methacrylate is more hydrophobic. Therefore, we believe that the hydrophobicity will improve the polymer's ability to load and retain a hydrophobic drug but the hydrophilicity makes aqueous swelling possible and improves the drug's solubility. In acidic conditions, the hydrogel will be collapsed and capable of retaining a small molecular weight drug, but in neutral conditions the hydrogel will swell allowing for release of the drug as the polymer network relaxes and imbibes water.

In vitro characterization of particles' biocompatibility and ability to effectively transport agents across the intestinal lining was investigated using a co-culture model developed by the Peppas Lab [19]. The co-culture model uses HT-29 MTX cells, a mucus-secreting subclone of human carcinoma cells, and Caco-2 cells to model the intestinal epithelial layer. The Caco-2/HT29-MTX co-culture model is a robust human gastrointestinal (GI) tract model. When grown out to confluence for 20 days the cells produces enzymes and mucus, possess tight junctions, and develop microvilli. These features create a GI tract model whose transport of molecules correlates well with *in vivo* absorption [19].

2. Methods

2.1. Materials

All solvents (ethanol, acetone) were from Fisher Sciences. The two agents for delivery were (+)-cis diltiazem hydrochloride and fluorescein sodium were purchased from Sigma Aldrich. Tert-butyl methacrylate (*t*-BMA), tetraethylene glycol dimethacrylate (TEGDMA), acrylic acid (AA), and methyl methacrylate (MMA) were from Sigma Aldrich. The ultraviolet (UV) photoinitiator, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1 propanone (Irgacure 2959) was purchased from Sigma Aldrich. Inhibitor was removed via a basic alumina column from TEGDMA, *t*-BMA, and MMA before being use. All polymer films were made in a Labconco controlled chamber glove box with a Dymax light curing system.

Caco-2 cells were obtained for the American Type Culture Collection (ATCC) and HT29-MTX cells were generously donated by 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) [20,21]. All cell types were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose supplemented with 10% heat-inactivated fetal bovine serum, with 1% streptomycin from Sigma Life Sciences. Dulbecco's Phosphate-Buffered Saline (DPBS) and was also obtained from Sigma Life Sciences. The dimethyl sulfoxide (DMSO) and 10 x Phosphate-Buffered Saline (PBS) was from Fisher BioReagents. The Hank's Buffered Saline Solution (HBSS) used was purchased from HyClone. The fluorescence and Costar UV 96-well plates were acquired from Corning. Cells were maintained in T-75 flask from Corning and Costar Transwell® plates with a polycarbonate membrane were used from transport studies from Fisher Scientific.

2.2. Hydrogel film synthesis

Polymer films of MMA/AA copolymers, henceforth designated as P (AA-co-MMA), were synthesized using UV-initiated free radical polymerization. Copolymers with 10, 20, and 30 mol % of MMA in the total monomer feed were produced. Tetraethylene glycol dimethacrylate (TEGDMA) was used as the crosslinking agent. Irgacure 2959 was used as the photoinitiator. A 1:1 mixture of ethanol and water were used as the solvent during polymerization to ensure complete solubility of the monomer components, cross-linking agent, and photoinitiator.

All components were added to an amber bottle, covered, and sonicated for 15 min. The amber bottle was then placed in a sealed glove box and purged with nitrogen gas for 20 min. The UV light source was set to an intensity of 16–18 mW/cm². The solution was transferred

between two glass slides separated by a Teflon spacer and placed under the UV light source for 30 min. After polymerization, the hydrogels were removed from the glass slides. Synthesized films were washed for 10 days in deionized water (DIH₂O), with daily water changes and then vacuum dried for 3–5 days. After drying, the film was crushed using a mortar and pestle. The particles were sieved to be between 10 and 40 μm . In this way, we could achieve a more uniform size of microparticles to be used in all characterization studies.

2.3. Characterization of hydrogel samples

2.3.1. FT-IR spectroscopy—Fourier transform infrared (FT-IR) spectroscopy was used to ensure that the synthesized polymer contained the components as predicted. The spectra were obtained using an FT-IR spectrometer equipped with a deuterated triglycine sulfate detector and potassium bromide (KBr) beam splitter. Readings were taken at wavenumbers from 400 to 4000 cm^{-1} and an average of 64 scans was used. The FT-IR pellets were prepared with 2 mg of crushed polymer sample and 200 mg of dried KBr pressed using 15,000 psi of compression force.

2.3.2. Particle sizing—After drying in a vacuum oven for 3–5 days, the films were crushed using a mortar and pestle. The particles were sieved to be between 10 and 40 μm to achieve a more uniform size of micro-particles to be used in all characterization studies. The size was verified using scanning electron microscopy (SEM).

2.3.3. Dynamic swelling studies of hydrogel samples—Bulk swelling studies were conducted to track the swelling behavior of the synthesized hydrogels in solutions of increasing pH. Discs with a diameter of 14 mm were cut from the polymer film in the relaxed state, before being exposed to water. The discs were washed in DIH₂O for 10 days to remove any unreacted monomer and were then allowed to dry in a vacuum oven for 3–5 days. After drying, the discs were weighed. The discs were then put in 1 x PBS solutions from pH 4–8 for 5 min each before being weighed again. The weight-swelling ratio was calculated from the results.

2.4. Drug loading and release studies

Loading and release studies were conducted to evaluate the synthesized microparticles' capability to encapsulate and release two different small molecular weight molecules of different levels of hydrophobicity. The hydrophobic fluorescent molecule, fluorescein, was compared to the hydrophilic drug diltiazem hydrochloride to observe the particle loading and release characteristics.

2.4.1. Fluorescein loading studies—To evaluate the carriers' utility as a drug delivery system, loading studies were conducted to quantify the amount of fluorescein loaded into the microparticles. P (AA-co-MMA) microparticles were incubated in PBS at pH 8 for 24 h to ensure that the particles were completely swollen prior to performing the loading procedure. The particles were allowed to settle to the bottom of their container and then the supernatant was removed. 10 mL of a 0.5 mg/mL fluorescein stock solution was added to each vial and after a quick shake, a 300 μL sample was taken. After 5 h and 24 h, additional samples were

taken. The amount of fluorescein remaining in the supernatant was measured using the BIO-TEK Synergy HT plate reader with an excitation of 485 nm and an emission of 528 nm to determine the amount of fluorescein loaded.

After 24 h, the hydrogels were collapsed by decreasing the solution pH to 4. The particles were rinsed three times with PBS at pH 4. Each wash cycle consists of centrifuging the particles, removing the supernatant, adding 10 mL of the wash solution, and placing on the rotating shaker for 5 min. Supernatant samples were collected after the hydrogel microparticles' network were collapsed and after each rinse. The amount of fluorescein remaining in the supernatant was measured and was used to determine the amount of fluorescein remaining encapsulated within the microparticles. Once the particles were collapsed and washed, they were freeze-dried for 3 days. Replicates of five were performed to increase the statistical significance of measurements.

2.4.2. Diltiazem hydrochloride loading studies—After testing the carriers' ability to encapsulate fluorescein, studies to determine the amount of diltiazem hydrochloride loaded were conducted in a similar manner. Particles were incubated in PBS at pH 8 for 24 h to ensure that the particles were completely swollen prior to performing the loading procedure. The particles were allowed to settle to the bottom of their container and then the supernatant was removed. A 0.5 mg/mL stock solution of diltiazem hydrochloride and 1 x PBS was prepared. The particles were removed from the rotator and allowed to settle and the supernatant was sampled. 10 mL of the diltiazem hydrochloride stock solution was added to each centrifuge tube and vortexed. The pH of the solution was recorded and a sample was taken. The centrifuge tube was placed back on the rotator for 1-h incubations for the first 5 h. A sample was taken after each incubation and then again after 24 h. Samples were analyzed using the BIO-TEK Cytation 5 plate reader absorbing at 220 nm. The particles were collapsed by decreasing the pH of the particle suspension to 4 and rinsed in pH 4 PBS. Supernatant samples were collected after the hydrogel microparticles' networks were collapsed and after each rinse. The amount of diltiazem hydrochloride remaining in the supernatant was measured and was used to determine the amount of diltiazem hydrochloride remaining encapsulated within the microparticles. The particles were frozen in liquid nitrogen and then lyophilized for 3–5 days before release studies were performed. Replicates of five were performed to increase the statistical significance of measurements.

2.4.3. Drug release studies—For the analysis of fluorescein and diltiazem hydrochloride release from the P (AA-co-MMA) microparticles as a function of pH, release studies were performed. PBS solution at a pH of 5.5, 6.5 and 7 were prepared. Particles were put into the PBS solution at a pH of 5.5 at a concentration of 1 mg/mL. The particles were allowed to stir for 10 min before a sample was taken and the pH was titrated to 6.5. This procedure continued, increasing by a 1 increment until a pH of 7 was reached. The supernatant continued to be sampled over time at pH 7 to determine the release of fluorescein and diltiazem hydrochloride. Samples were analyzed using the BIO-TEK Synergy HT (for fluorescein) and the Cytation 5 (for diltiazem hydrochloride) plate readers.

2.5. In vitro cellular studies

In vitro characterization of particles' biocompatibility and ability to effectively transport agents across the intestinal lining was investigated using a co-culture model developed by the Peppas Lab [19]. The co-culture consists of human epithelial colorectal adenocarcinoma cells (Caco-2) and HT-29 MTX cells, which are mucous-secreting cells. Cultures were maintained in T-75 flasks at 37°C and a humidified environment of 5% CO₂ in air. The medium was changed every other day. Cells were consistently passaged at 80% confluence, which occurred between 6 and 7 days after seeding. A passage operation consisted of two washes with Dulbecco's phosphate buffered saline (DPBS) without Ca²⁺ and Mg²⁺, then 1 quick rinse with 1 ml 0.5% trypsin/0.2% EDTA solution, followed by 5–8 min incubation with trypsin/EDTA solution after which cells were detached from the flasks and could then be counted and reseeded. Caco-2 cells were seeded at a density of 3.0×10^3 cells cm⁻² and used between passages 60 and 80. HT29–MTX cells were seeded at a density of 2.0×10^4 cells cm⁻² and used between passages 8 and 20.

2.5.1. Biocompatibility studies—Cytotoxicity experiments were performed to confirm our hypothesis that the co-polymers are biocompatible. In the cytotoxicity studies, the cell growth rate of control cells was compared to the cell growth rate of cells to the polymer microparticles. Caco 2 cells were used to quantify what effect the polymer particles have on cell viability. Caco 2 cells were plated in a 96-well plate at a cell concentration of 10^4 cells/cm² and allowed to adhere and grow for 72 h. Replicates of 6 for each data point were used to increase the statistical significance and reduce the error in results. Five different conditions were evaluated: with 1 mg/mL of 10 mol %, 20 mol %, 30 mol % PMMA in P (AA-co-MMA), 30 mol % PMMA in P(AA-coMMA) loaded with diltiazem hydrochloride, and control cells with no particles. The CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega) was used to measure and compare cell viability. After these preliminary cytotoxicity studies, the same five conditions were tested with the higher particle concentrations of 2.5 mg/mL and 5.0 mg/mL.

2.5.2. Drug transport studies—All transport and transepithelial electrical resistance (TEER) experiments were conducted using a Costar Transwell® plates with a polycarbonate membrane (0.4 µm pore size) and a cell growth area 1 cm² (12-well). Caco-2 and HT29–MTX cells were maintained separately in T-75 flasks as previously described [19]. After subculturing, the cells were counted using a Millipore Sceptor precision cell counter, and mixed together in a 1:1 ratio before seeding onto the Transwell® plate at a density of 1×10^5 cells cm⁻² (12-well) after a passaging procedure and cultured for 21–24 days. Culture medium was changed every other day in the co-culture and TEER values were measured with an EVOM volt-ohm meter and a chopstick electrode (World Precision Instruments) to monitor the development of tight junctions. Transport studies were performed to determine the *in vitro* transport conditions of the drug. Previous research demonstrated that a 1:1 seeding ratio produced TEER values closest to those reported *in vivo* for human intestinal epithelia [22]. It has also been well studied that Caco-2 cells form an absorptive polarized monolayer, develop an apical brush border, and secrete enzymes indicative of human intestinal epithelia after 21 days in culture [23,24].

Experiments were performed to quantify the amount of diltiazem hydrochloride transported across a cellular monolayer in both the presence and absence of microparticles. After 22 days in culture, the medium in the both the apical and basolateral chambers of the Transwell plates was removed and cells were rinsed 3 times with pre-warmed HBSS. The monolayers were then incubated in HBSS to equilibrate for 1 h and TEER measurements were taken at 0.5, 1, 2 and 3-h time points. Once the 1-h incubation was completed, 200 μ L samples were taken from both the apical and basolateral chambers of each well. These samples were immediately refrigerated. The co-cultures were then exposed to either free diltiazem hydrochloride or microparticles and diltiazem hydrochloride. The final concentration of drug was kept constant at 4.5 mg/mL and the concentration of particles introduced to the monolayer was 5 mg/mL. Media from the apical chambers was removed and replaced with 0.5 mL of its respective sample. 100 μ L samples were taken from the basolateral chamber at 0.5, 1, 2, and 3 h, replacing with 100 μ L of HBSS each time. At the 3-h time point, a sample from the apical chamber was also taken. Fresh, pre-warmed DMEM was added to both the apical and basolateral chambers and the TEER values were monitored over the next 24 h. The amount of diltiazem hydrochloride transported across the monolayer was determined using absorbance measurements using the BIO-TEK Synergy HT plate reader.

3. Results and discussion

Microparticles composed of co-polymer blends of methyl methacrylate/acrylic acid (MMA/AA) were investigated for the development of an oral drug delivery system for small molecular weight drugs. A series of studies were conducted to evaluate the synthesized carriers *in vitro* performance for drug delivery applications.

3.1. Carriers characterization

FT-IR was used to confirm the chemical composition of the synthesized particles. Fig. 1 depicts the FT-IR spectrum for the three different polymer particles synthesized. The absorbance is just relative to the rest of the data for that polymer; only the shapes not the absolute values of the absorbance curves are valuable for comparison. The location of various peaks indicates the presence of certain functional groups.

The broad peak between 3300 and 2500 cm^{-1} wavenumbers indicates the presence of a carboxyl group –in this case, the carboxyl group on acrylic acid. The sharp peak sticking out of this broad peak at 2900 cm^{-1} and the peak at 1450 cm^{-1} represents the presence of a –O–CH₃ functional group. The two different peaks are indicative of symmetric stretching vibrations and deformation vibrations. The –C=O and –C–O–C – groups found in the methyl methacrylate are observed in the strong peaks at 1600–1700 cm^{-1} and 1200 cm^{-1} , respectively. The peaks are not as prominent for 10 mol-% PMMA polymer particles as for the 30 mol-% PMMA since there are less of these types of functional groups in the polymer.

SEM was used to evaluate the size and shape of microparticles formed. The SEM images (Fig. 2) confirm that the size of the microparticles is in the desired range of 10–40 μm . The SEM images also show the surface properties of the microparticles after being crushed and sieved.

Bulk swelling studies were conducted to track the swelling behavior of polymer film in solutions of increasing pH. The swelling behaviors of various MMA-AAc blends were observed with swelling ratios of up to 5.8 at pH 8 (Fig. 3). Copolymers with lower feed ratios of methyl methacrylate swelled more than copolymers with higher methyl methacrylate feed ratios. This behavior is expected because methyl methacrylate is hydrophobic and polymers with higher percentages of hydrophobic monomers swell less. The highest weight-swelling ratio was the 10 mol% formulation (Fig. 3), which also demonstrated an increase in swelling ratio after pH 6. Swelling studies were also repeated with the 30 mol% MMA, with *t*-BMA incorporated at 1.5% mass of MMA. The weight-swelling ratio of the P (AA-co-MMA) with 30 mol % MMA after *t*-BMA was incorporated increased by a factor of 1.5 and the swelling ratio constantly increased (Fig. 3). The incorporation of an additional hydrophobic monomer solved the problematic behavior with the 30 mol% MMA weight ratio (Fig. 3) decreasing. The additional hydrophobic monomer appears to help stabilize the swelling behavior.

Each polymer formulation was tested for its loading capabilities of small molecular weight drugs: fluorescein, a hydrophobic drug analog, and diltiazem hydrochloride, a hydrophilic drug. As the hydrophobic character of the polymer formulation increased, so did the percent of drug loaded. The 30 mol % formulation showed over 88% loading of fluorescein and had a very small standard deviation, which indicated very replicable results. Measurements were taken with a sample set of 5 and are shown in Table 1 with standard deviations reported. As the amount of hydrophobic components increased the amount of hydrophobic drug encapsulated increased. Interestingly, the same trend occurred with the hydrophilic drug just at a significantly lower loading efficiency. The loading efficiency of the hydrophilic drug diltiazem hydrochloride was not as good as that of fluorescein. The results obtained from the diltiazem hydrochloride suggest that increased hydrophobicity may not be the only factor affecting loading of low molecular weight drug. As the amount of MMA increased in the hydrogels, the swelling ratio decreases (Fig. 3). Results from the loading studies suggest that decreasing the swelling ratio, and therefore the hydrogel network mesh size, will increase the loading efficiency of low molecular weight drugs.

In release studies presented here, we wanted to mimic the pH changes the microparticles would experience *in vivo* when passing through the stomach and into the small intestine all in one experiment. Fig. 4 shows the release profile for all 3 formulations of P(AA-co-MMA) microparticles for both hydrophobic drug model (Fig. 4a) and hydrophilic drug model (Fig. 4b). As the pH was increased, release from P (AA-co-MMA) hydrogels were observed for both drugs. At low pHs all formulations limited release of diltiazem hydrochloride. P (AA-co-MMA) 30 mol% formulation was the only composition that limited release of fluorescein at low pHs. Limited release of less than 10% of loaded fluorescein was observed from the 20 mol % hydrogels at pH 5.5. The 10 mol % P(AA-co-MMA) reached maximum amount of fluorescein release within 8 min at pH 5.5 solution. This rapid release of 25–35% of the loaded fluorescein at a low pH demonstrates that the 10% MMA networks are swelling too quickly to maintain encapsulation of low molecular weight hydrophobic drugs. For both drugs, the 20 mol% formulation achieved the maximum percent drug release at pH 7. After 30 min in the pH 7 buffer, 60% release of loaded fluorescein was achieved from the 20 mol

% P (AA-co-MMA) microparticle. Release of diltiazem hydrochloride from all P (AA-co-MMA) formulations occurred later when the solution pH was 7. Maximum release of 25% of loaded diltiazem hydrochloride did not occur until after 1 h of incubation in pH 7 solution. Table 2 shows a side-by-side comparison of the loading and release capabilities of the 30 mol% P (AA-co-MMA) microparticles. This data indicates that the 20 and 30 mol% P (AA-coMMA) hydrogel compositions is more suitable for loading and release of small molecular weight, hydrophobic drugs.

3.2. In vitro characterization of carriers

3.2.1. Cytotoxicity studies—To evaluate the biocompatibility of the synthesized carriers, cytocompatibility studies were conducted using the MTS assay, which monitors the mitochondrial activity of the cells. The results from the cytotoxicity experiments (Fig. 5) show that the P (AA-coMMA) polymer microparticles with 10, 20, and 30 mol % MMA as well as the loaded 30 mol % MMA were not toxic to the cells at 1, 2.5, and 5 mg/mL concentrations. The concentrations of microparticles did not seem to affect the particle toxicity to the cells greatly, although there is a slight decrease in proliferation observed for all formulations at 5 mg/mL. Fig. 5 shows reported percent cytocompatibility that are above 100%. The results could be due to differences in cell seeding in individual wells on the plate.

3.2.2. Diltiazem hydrochloride transport studies—The carriers' ability to effectively transport agents across the intestinal lining was investigated using an HT-29 MTX and Caco-2 co-culture model. The TEER values for the co-culture (Caco-2 and HT-29 MTX) were monitored for the first 20 days in culture. Once the values plateaued to a value of $\sim 200 \Omega\text{cm}^2$ (Fig. 6a), indicating the cells had formed a monolayer, the transport studies began. The results of the transport studies show a slight decrease in TEER values (Fig. 6b) over the 3-h experiment. The P (AA-co-MMA) with 10 mol%, 20 mol%, and 30 mol% MMA all showed similar transport compared to the transport of the native drug alone. The presence of the microparticles does not significantly enhance permeability. The transport profile of the loaded P(AA-co-MMA) microparticles with 30 mol% MMA (Fig. 7) is much lower because drug first has to diffuse out of the microparticles before it is available for transport across the monolayer.

4. Conclusion

The focus of the studies presented here was to evaluate the effect hydrophobicity has on loading and release of small molecular weight therapeutics. We demonstrated the ability to synthesize pH-responsive hydrogel materials that can be used for oral delivery of small molecular weight therapeutic agents. Three P(AA-coMMA) hydrogel formulations were evaluated with the percentage of the hydrophobic MMA varied. As hypothesized as the amount of hydrophobic components increased the amount of hydrophobic drug encapsulated increased. Interestingly, the same trend occurred with the hydrophilic drug just at a significantly lower loading efficiency. The loading efficiency of the hydrophilic drug diltiazem hydrochloride was not as good as that of fluorescein. It is reasonable to assume that as the hydrophobic character of our polymer increasing the uptake of hydrophobic

agents, such as fluorescein. The results obtained from the diltiazem hydrochloride suggest that increased hydrophobicity may not be the only factor affecting loading of low molecular weight drug. As the amount of MMA increased in the hydrogels, the swelling ratio decreases (Fig. 3). Results from the loading studies suggest that decreasing the swelling ratio, and therefore the hydrogel network mesh size, will increase the loading efficiency of low molecular weight drugs. From these results we can confirm that small molecular weight, hydrophobic drugs can efficiently be loaded into P(AA-co-MMA) micro-particles and released into aqueous solutions.

In vitro cytotoxicity studies showed that our formulations are biocompatible with Caco-2 or HT29-MTX cells. The transport studies demonstrated that comparable bioavailability profiles could be achieved from drug-microparticle suspension and free drug samples. In contrast, lower concentrations of drug was transported across the cell monolayer when it was loaded and being release directly from the microparticles. The cumulative amount of drug transported from loaded particles could be increased by switching to P (AA-co-MMA) nanoparticles because the higher surface to volume ratio would increase the release rate and therefore the amount of drug available to be transported across the cell monolayer.

Acknowledgments

This work was supported in part a grant from the National Institutes of Health (grant EB-000246-21).

References

1. Caldorera-Moore M, Guimard N, Shi L, Roy K. Designer nanoparticles: incorporating size, shape and triggered release into nanoscale drug carriers. *Expert Opin Drug Deliv*. 2010; 7(4):479–495. [PubMed: 20331355]
2. Caldorera-Moore M, Kang MK, Moore Z, Singh V, Sreenivasan SV, Shi L, Huang R, Roy K. Swelling behavior of nanoscale, shape- and size-specific, hydrogel particles fabricated using imprint lithography. *Soft Matter*. 2011; 7(6):2879.
3. Caldorera-Moore M, Peppas NA. Micro- and nanotechnologies for intelligent and responsive biomaterial-based medical systems. *Adv Drug Deliv Rev*. 2009; 61(15):1391–1401. [PubMed: 19758574]
4. Liechty WB, Caldorera-Moore M, Phillips MA, Schoener C, Peppas NA. Advanced molecular design of biopolymers for transmucosal and intracellular delivery of chemotherapeutic agents and biological therapeutics. *J Control Release*. 2011; 155(2):119–127. [PubMed: 21699934]
5. Blanchette J, Kavimandan N, Peppas NA. Principles of transmucosal delivery of therapeutic agents. *Biomed Pharmacother*. 2004; 58(3):142–151. [PubMed: 15082336]
6. Carr DA, Peppas NA. Molecular structure of physiologically-responsive hydrogels controls diffusive behavior. *Macromol Biosci*. 2009; 9(5):497–505. [PubMed: 19016502]
7. Carr DA, Peppas NA. Assessment of poly (methacrylic acid-co-N-vinyl pyrrolidone) as a carrier for the oral delivery of therapeutic proteins using Caco-2 and HT29-MTX cell lines. *J Biomed Mater Res Part A*. 2010; 92(2):504–512.
8. Fisher OZ, Kim T, Dietz SR, Peppas NA. Enhanced core hydrophobicity, functionalization and cell penetration of polybasic nanomatrices. *Pharm Res*. 2009; 26(1):51–60. [PubMed: 18751960]
9. Fisher OZ, Peppas NA. Polybasic nanomatrices prepared by UV-initiated photopolymerization. *Macromolecules*. 2009; 42(9):3391–3398. [PubMed: 20526378]
10. Kavimandan NJ, Losi E, Peppas NA. Novel delivery system based on complexation hydrogels as delivery vehicles for insulin–transferrin conjugates. *Biomaterials*. 2006; 27(20):3846–3854. [PubMed: 16529810]

11. Peppas NA, Kavimandan NJ. Nanoscale analysis of protein and peptide absorption: insulin absorption using complexation and pH-sensitive hydrogels as delivery vehicles. *Eur J Pharm Sci.* 2006; 29(3):183–197. [PubMed: 16777391]
12. Marek SR, Conn CA, Peppas NA. Cationic nanogels based on diethylaminoethyl methacrylate. *Polymer (Guildf).* 2010; 51(6):1237–1243. [PubMed: 20436948]
13. Marek SR, Peppas NA. Insulin release dynamics from poly (diethylaminoethyl methacrylate) hydrogel systems. *AIChE J.* 2013; 59(10):3578–3585. [PubMed: 24634515]
14. Peppas NA, Carr DA. Impact of absorption and transport on intelligent therapeutics and nanoscale delivery of protein therapeutic agents. *Chem Eng Sci.* 2009; 64(22):4553–4565. [PubMed: 20161384]
15. Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. *Adv Drug Deliv Rev.* 2004; 56(11):1649–1659. [PubMed: 15350294]
16. Schoener CA, Hutson HN, Fletcher GK, Peppas NA. Amphiphilic interpenetrating networks for the delivery of hydrophobic, low molecular weight therapeutic agents. *Ind Eng Chem Res.* 2011; 50(22):12556–12561. [PubMed: 22247592]
17. Schoener CA, Hutson HN, Peppas NA. pH-responsive hydrogels with dispersed hydrophobic nanoparticles for the delivery of hydrophobic therapeutic agents. *Polym Int.* 2012; 61(6):874–879. [PubMed: 23087546]
18. Wood KM, Stone GM, Peppas NA. Wheat germ agglutinin functionalized complexation hydrogels for oral insulin delivery. *Biomacromolecules.* 2008; 9(4):1293–1298. [PubMed: 18330990]
19. Wood KM, Stone GM, Peppas NA. The effect of complexation hydrogels on insulin transport in intestinal epithelial cell models. *Acta Biomater.* 2010; 6(1):48–56. [PubMed: 19481619]
20. Lesuffleur T, Barbat A, Dussaulx E, Zweibaum A. Growth adaptation to methotrexate of HT-29 human colon carcinoma cells is associated with their ability to differentiate into columnar absorptive and mucus-secreting cells. *Cancer Res.* 1990; 50(19):6334–6343. [PubMed: 2205381]
21. Lesuffleur T, Porchet N, Aubert JP, Swallow D, Gum JR, Kim YS, Real FX, Zweibaum A. Differential expression of the human mucin genes MUC1 to MUC5 in relation to growth and differentiation of different mucus-secreting HT-29 cell subpopulations. *J Cell Sci.* 1993; 106(3): 771–783. [PubMed: 8308060]
22. Hilgendorf C, Spahn-Langguth H, Regårdh CG, Lipka E, Amidon GL, Langguth P. Caco-2 versus caco-2/HT29-MTX co-cultured cell lines: permeabilities via diffusion, inside-and outside-directed carrier-mediated transport. *J Pharm Sci.* 2000; 89(1):63–75. [PubMed: 10664539]
23. Behrens I, Kissel T. Do cell culture conditions influence the carrier-mediated transport of peptides in Caco-2 cell monolayers? *Eur J Pharm Sci.* 2003; 19(5):433–442. [PubMed: 12907294]
24. Delie F, Rubas W. A human colonic cell line sharing similarities with enterocytes as a model to examine oral absorption: advantages and limitations of the Caco-2 model. *Crit RevTM Ther Drug Carr Syst.* 1997; 14(3)

Fig. 1.

FT-IR spectrum for (a) 10 (b) 20 (c) 30 mol % PMMA in P(AA-co-MMA). The broad peak between 3300 and 2500 cm⁻¹ wavenumbers indicates the presence of a carboxyl group –in this case, the carboxyl group on acrylic acid. The sharp peak sticking out of this broad peak at 2900 cm⁻¹ and the peak at 1450 cm⁻¹ represents the presence of a -O-CH₃ functional group. The two different peaks are indicative of symmetric stretching vibrations and deformation vibrations. The -C=O and -C-O-C – groups found in the methyl methacrylate are observed in the strong peaks at 1600–1700 cm⁻¹ and 1200 cm⁻¹.

Fig. 2.
SEM images of the sieved P (AA-co-MMA) microparticles.

Fig. 3. Weight Swelling Study for 10, 20, and 30 mol % PMMA in P (AA-co-MMA) and 30 mol % MMA in feed solution of P (AA-co-MMA) with *t*-BMA ($n = 6$).

Fig. 4. (a) Cumulative release profile of fluorescein and (b) Release profile of diltiazem hydrochloride from P (MMA-co-AA) microparticles at varied % mol of PMMA (n = 5 for all samples).

Fig. 5.
Cytocompatibility of P (AA-co-MMA)microparticles at varied concentrations (n= 6).

Fig. 6. (a) Co-culture TEER values before transport studies (n = 24) and (b) TEER values during the transport of diltiazem hydrochloride (n = 4).

Fig. 7.
Cumulative diltiazem hydrochloride transported across co-culture cell monolayer (n = 4).

Table 1

Loading results of P (AA-co-MMA) microparticles (n = 5).

Polymer formulation	% Loaded fluorescein	% Loaded diltiazem
10 mol% MMA	50.46 ± 14.10	7.80 ± 3.4
20 mol% MMA	72.54 ± 13.05	5.59 ± 2.3
30 mol% MMA	88.71 ± 1.57	13.4 ± 3.9

Table 2

Loading and release of fluorescein and diltiazem hydrochloride with 30 mol% MMA in P (AA-co-MMA) (n = 5).

Agent for delivery	% Loaded	% Released
Fluorescein	88.7 $\pm$ 1.6	11.6 $\pm$ 1.5
Diltiazem	13.4 $\pm$ 3.9	6.4 $\pm$ 0.4