Published in final edited form as:

AICHE J. 2013 October 1; 59(10): 3578–3585. doi:10.1002/aic.14108.

Insulin Release Dynamics from Poly(diethylaminoethyl methacrylate) Hydrogel Systems

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Abstract

Novel glucose-sensitive systems for the release of insulin from poly(diethylaminoethyl methacrylate) (PDEAEM) micro-particles and nanoparticles decorated with glucose oxidase and catalase enzymes have been developed. The effect of polymer composition and loading conditions on the insulin loading efficiency and release was studied. The optimal conditions for loading insulin into PDEAEM microparticles were found to be at a loading pH of 5.6, particle to insulin mass ratio of 7:1, a concentration of 1.0 mg/mL insulin, and a collapsing pH of approximately 9.5. Microparticles exhibited a responsive (pH) or intelligent (glucose) release of insulin from a stimulus. Microparticles that had a nominal crosslinking ratio of 10% released a third of the insulin payload after a single stimulus, compared to nearly 70% for microparticles with a 3% crosslinking ratio. PDEAEM micro particles of 150 μ m diameter showed promise as components of a system of automated, intelligent delivery method for insulin to type I diabetics.

Keywords

poly(diethylaminoethyl methacrylate); insulin; glucose oxidase; microparticles; hydrogels

Introduction

There is a growing need in the medical field for devices that can provide patients with custom doses of a therapeutic agent¹ in response to high concentrations of selected biomarkers. More importantly, these “intelligent” drug delivery systems need to only release the drug when the patient is in need of its therapeutic effects.^{2–4} To provide this automatic and customized dosing, these devices must be able to first sense a specific biomarker associated with the diseased state and then release the drug at a predetermined biomarker level. Once the biomarker concentration drops and signals the end of the dosing period, the device must then limit its release of the therapeutic agent. Thus, a closed-loop system involving sensors, transducers, and actuators must be synthesized to provide this desired response.^{3,5–9}

An intelligent system of interest is based on cationic hydrogels for the intelligent delivery of therapeutics. A limited number of previous studies have been reported on hydrogel systems of various structures and on the release of small molecular weight solutes. For example,

Firestone and Siegel¹⁰ and Siegel et al.¹¹ studied the release of caffeine from dimethylaminoethyl methacrylate as a model drug. Oscillatory swelling and deswelling results were obtained by varying the pH. These gels exhibited a moving front mechanism of water sorption, which allowed the caffeine release from within these hydrogels. Caffeine release was dependent on the pH of the media.

Ishihara et al.^{12,13} investigated the release of imbibed insulin out of membranes made of 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA). This research showed that the release rate of insulin increased as the HEMA concentration increased, likely due to the increased hydrophilicity of the hydrogel at higher HEMA concentrations. Zero-order release out of these membranes was achieved because of the relatively constant concentration of insulin inside the membrane device. However, diabetes patients require fluctuating insulin release depending on their body's current condition; thus, zero-order release is not ideal for an insulin delivery device.

Schwarte and Peppas¹⁴ investigated several systems of intelligent, controlled release. They studied the permeation of proxiphylline, vitamin B₁₂, and several different dextran molecules from poly(dimethylaminoethyl methacrylate-grafted-poly(ethylene glycol)) hydrogel discs, henceforth designated as P(DEAEM-g-EG) gels. All solutes of interest were neutral, thus having no attraction or repulsion to the cationic hydrogels. Proxiphylline and vitamin B₁₂ had much lower permeation values than expected, due to their molecular weights and the mesh sizes of the hydrogels investigated.

Hariharan and Peppas¹⁵ investigated solute release from several hydrogel matrices, including P(DEAEM-co-HEMA) and poly(diethylaminoethyl acrylate-co-HEMA). Oxprenolol HCl, insulin, albumin, and myoglobin were loaded and released from these hydrogel discs. It was determined that the release rate of certain drugs (i.e., oxprenolol) was due primarily to polymer chain relaxation, whereas the others were Fickian controlled. More recently, other structures of cationic hydrogels have been studied in our laboratory.^{16–20} Such systems can be used for a number of new formulations for treatment of various diseases, especially diabetes.

To appreciate the limitations of design of glucose-dependent, hydrogel based, insulin delivery systems, we must address the physiological conditions of insulin release. Insulin is a peptide hormone of 51 amino acids with a monomeric molecular weight of ~5.8 kDa. However, in the presence of zinc, it tends to form a hexameric structure of approximately 35 kDa. At physiological relevant conditions (pH 7.4), X-ray scattering has shown that the average radius of gyration of insulin is 1.98 nm, which corresponds to the hexameric structure. The active form of insulin is the monomeric structure, and hexameric insulin will not bind to cellular receptors. Thus, hexameric insulin remains in equilibrium with its monomeric form, and it slowly breaks down into monomeric insulin.^{21–24}

A type I diabetic patient typically has to have a very specific insulin dosage regimen. A general guideline is that a diabetic patient needs to inject subcutaneously about 0.2 IU/kg/day of basal insulin, and between 0.05 and 0.1 IU/kg before consuming a meal. One IU, or international unit, of insulin is precisely defined as 1/22 mg (ca. 45.5 µg) of pure crystalline insulin. Thus, for a patient with a body mass of 75 kg (165 lb), these values correspond to a total daily insulin intake of about 30 mg. The basal insulin dose corresponds to roughly half of the daily dose, about 15 mg, whereas between 3.7 and 7.5 mg is necessary before the consumption of each meal.^{21,22}

The inactivity of hexameric insulin has been exploited in the treatment of diabetes. Pharmaceutical companies synthesize several different analogs of insulin, each having differing abilities to form hexamers. Thus, “long acting” insulin analogs are less soluble at

physiological pH and take a longer time to dissolve into the monomeric insulin form. These types of insulin are ideal for a long-term basal insulin delivery, such as a daily basal injection. Lispro insulin, on the other hand, was modified to prevent the formation of dimers and hexamers. This analog is entirely in its monomeric form, which makes it a “fast-acting” insulin ideal for postprandial subcutaneous delivery.²¹ Hexamers can also be prevented *in vitro* by using ethylenediaminetetraacetic acid (EDTA) to chelate the zinc ion.

In the present research study, insulin loading and release studies were performed to determine the feasibility of using polycationic-based hydrogel systems for treatment of type 1 diabetes. The physical properties of microparticles of PDEAEM-containing hydrogels containing poly(ethylene glycol) (PEG) as a stealth agent for the reticuloendothelial system (also known as P(DEAEM-g-EG) particles) were previously reported.²⁰ The mesh size was estimated for polymer samples with a crosslinking ratio of 3, 10, and 15%, which indicated that a collapsed mesh size of roughly 1 nm would potentially trap insulin (having a monomer hydrodynamic radius of 1.3 nm²⁵). Thus, experiments were conducted to determine if these hydrogel particles could indeed entrap insulin and release insulin only under certain conditions, specifically acidic pH or increased solution glucose concentrations.

In this work, physiologically relevant conditions *in vitro* were used to simulate how these polymers might respond *in vivo*. Insulin was loaded into the hydrogels via both equilibrium partitioning and ionic interactions. The release of insulin from these cationic hydrogels was measured as a function of time. Insulin release was triggered by either pH or glucose concentrations.

Materials and Method

Materials

N,N-diethylaminoethyl methacrylate (DEAEM), tetraethylene glycol dimethacrylate (TEGDMA), bovine insulin, glucose oxidase (GOX), catalase (CAT), SigmaCote®, Brij 30, Triton X-100, sodium metabisulfite (NaMBS), and EDTA were purchased from Sigma-Aldrich (St Louis, MO). Phosphate buffered saline (PBS), cyclohexane, ethyl ether, N,N,N',N'-tetraethylmethylenediamine (TEMED), trifluoroacetic acid (TFA), ammonium persulfate (APS), 1N sodium hydroxide (NaOH) and 1N hydrochloric acid (HCl) were obtained from Fisher Scientific (Fair Lawn, NJ). High pressure liquid chromatography (HPLC) grade water and acetonitrile were also acquired from Fisher Scientific. Polyethylene glycol 2000 monomethyl ether monomethacrylate (PEG2000MMA) was purchased from Polysciences (Warrington, PA). Acryloyl chloride was obtained from Alfa Aesar (Ward Hill, MA). DEAEM was passed through a basic alumina column (Fisher Scientific) to remove inhibitor and water was deionized and filtered (ddH₂O) through a Milli-Q Plus system (Millipore, Bedford, MA); all other chemicals were used as received.

Polymer synthesis

The hydrogels were synthesized by techniques reported before.²⁰ Briefly, microparticles were obtained by first forming polymer films, crushed under controlled conditions, and then wet sieved through a 150 μ m mesh. The polymers were synthesized by dissolving DEAEM, TEGDMA, and PEG2000MMA in PBS with acrylate-functionalized GOX and CAT (acryloyl chloride added dropwise to an ice-chilled enzyme solution). Typical ratios were 3 or 10% crosslinking, 10:1 PEG grafts (10 moles of DEAEM to 1 mole of PEG2000MMA), 6.6×10^{-4} mg/g GOX to polymer, and 4×10^{-3} mg/g CAT to polymer. The initiator APS and accelerant NaMBS (1 wt% APS, 4:1 in ddH₂O) were added to the solution after nitrogen purge at a final concentration of 0.5%. Polymer solutions were cast between two glass slides which had been SigmaCoted and separated by a Teflon spacer (740 μ m thick)

and allowed to polymerize overnight in a nitrogen-rich environment at 4°C. The films were then removed and crushed through a sieve, collected, and freeze dried.

Nanoparticles were synthesized by taking the above polymer solution and dispersing it in 85 mL cyclohexane with 4.7 g Brij 30 and 8.7 g Triton X-100. The suspension was homogenized (Ultra-Turrax T25, IKA, Wilmington, NC) at 24,000 rpm or sonicated in an ultrasonic bath for 5 min. After degassing with nitrogen, the accelerant TEMED was added and the reaction progressed very rapidly. The solvent was evaporated, the polymer was precipitated with ethyl ether, washed several times, and the collapsed nanoparticles were obtained via centrifugation.

The polymers were previously characterized via numerous methods.²⁰ Microparticles were characterized via Fourier transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and gravimetric swelling studies. Nanoparticles were characterized via FT-IR, proton nuclear magnetic resonance (¹H NMR) spectroscopy, DSC, dynamic light scattering (DLS), ζ-potential, and SEM. The swelling studies for both microparticles and nanoparticles were used to estimate collapsed and expanded mesh sizes.

Insulin loading

A stock solution of bovine insulin (30 USP U/mg) was prepared in PBS with EDTA at a concentration of 1.0 mg/mL. Insulin will only fully dissolve at acidic pH or after the addition of EDTA; thus, 0.04 mg EDTA per mg insulin was added to the PBS solution to facilitate insulin dissolution and loading. EDTA chelates the zinc ion which lies at the center of a hexameric insulin unit; thus, this procedure forced insulin into its monomeric form. After the insulin was dissolved, typically 7 mg/mL polymer sample (150 μm crushed microparticles) was added to the solution and the pH was lowered to about 5.6–5.8 using 1N HCl.

After allowing a suitable amount of time for loading, typically 4 h, the gels were collapsed by raising the pH to 9 with 1 M NaOH. This collapse physically trapped the insulin inside the microparticles. Excess insulin was washed off the surface of the particles with dilute (10 μM) NaOH and finally ddH₂O, and the measurement of insulin in the filtrate after each wash step was performed. The particles were then lyophilized and stored in a freezer until use.

To perform the washings on nanoparticles, special centrifuge tubes with dialysis membranes of a molecular weight cut-off of 20 kDa were used (iCON Concentrators #89886, Pierce, Rockford, IL). These membranes allowed the surface insulin to be washed away, while retaining the collapsed nanoparticles inside the dialysis membrane.

Insulin release

Insulin-loaded particles were suspended in appropriate media (i.e., PBS, saline) in SigmaCoted® glassware. Typically, a particle concentration of 1 mg/mL was used. To maintain physiological relevance, the temperature was kept at 37°C using a recirculating water bath. A dissolution apparatus (Distek Dissolution System 2100B, North Brunswick, NJ) was operated at 100 rpm using flat anchor impellers. Insulin concentrations were determined via HPLC using a Waters 2695 separations module with a Waters 2487 Dual λ Absorbance detector and a Symmetry300™ C4 column (particle size 5 μm; dimensions 3.0 mm ID × 150 mm length, Waters Corp., Milford, MA). A mobile phase of 70% water (0.1% TFA) and 30% acetonitrile (0.08% TFA) was used.

Results and Discussion

Polymer synthesis

Crushed polymer microparticles and nanoparticles were successfully synthesized and characterized, as previously reported.²⁰ As these hydrogels are cationic in nature, they exhibited a swelling transition at about pH 7.4, transitioning from a collapsed state in basic medium to a swollen state in acidic medium. An increase in crosslinking ratio resulted in a decrease in swelling. The wet-sieved microparticles were irregularly shaped and less than 150 μm in at least one direction in their relaxed state.

Previous work²⁰ indicated that these microparticles had mass swelling ratios (weight swollen divided by weight dry) of 2 in the collapsed state and 11 or 6.6 in the swollen state for the particles having 3 or 10% crosslinking ratio, respectively. The nanoparticles swelled from about 100 nm in their collapsed state to roughly 800 nm in their swollen state, as measured by DLS. The estimated mesh sizes for these microparticles were 10 $\text{\AA}$ in the collapsed state and 40 $\text{\AA}$ for 10% crosslinked gels or 68 $\text{\AA}$ for 3% crosslinked gels. Based on these mesh size estimates, the loading and controlled release of insulin ($r \approx 1.3 \text{ nm}$)²⁵ out of these gels was pursued.²⁶

Insulin loading

Insulin Loading Efficiency—The loading of insulin into PDEAEM microparticles was performed at a pH between 5.6 and 5.8. This pH range allowed insulin to retain a net negative charge, while still swelling the hydrogels with a net positive charge. Ionic interactions between the insulin and the polymer increased total loading of insulin into the hydrogels. Previous experiments^{3,27–33} relied solely on equilibrium partitioning of insulin into the polymer; however, these techniques yielded lower overall insulin loading.

The loading efficiency of insulin into the particles is defined in Eq. 1

$$\text{Loading Efficiency} = \frac{m_0 - m_i}{m_0} \times 100\% \quad (1)$$

where m_0 was the initial mass of insulin in solution and m_i was the mass of insulin in solution at condition i . Samples of the suspension medium were analyzed for insulin content using HPLC at the following conditions of interest: precollapse, postcollapse, and postrinse. Precollapse was the time immediately before the addition of NaOH (to raise the pH above the pK_a), which was after particles had been suspended in the insulin solution and time was allowed for the particles to imbibe insulin. Postcollapse refers to the time after the particles were collapsed to entrap insulin using NaOH, but prior to any surface rinsing. Postrinse was after rinsing surface-bound insulin from the particles using a filtration device, and consisted of the total mass of insulin recovered from the supernatant of the loading step plus all insulin collected after each wash step.

Figure 1 shows the relationship between the concentrations of particles in the insulin loading solution to the loading efficiency of the particles. The concentration of insulin remained constant at 1 mg/mL during these studies. As the concentration of 150 μm crushed microparticles was increased, the total amount of insulin imbibed into the particles increased substantially, which indicates that as the concentration of polymer particles was increased, more polymer was able to interact with the available insulin in solution, thus creating more ionic insulin-polymer interactions. Therefore, more insulin was removed from solution and imbibed into the hydrogel particles as the concentration of particles increased.

Even though these results indicated that increasing the particle concentration during insulin loading resulted in an increased amount of insulin being removed from solution, there was a practical limit to what polymer concentration was desired. This optimization is discussed below.

As shown in Figure 2, the loading efficiency for crushed microparticles was a function of crosslinking ratio. Particles with a crosslinking ratio of 10% yielded the highest loading efficiency, compared with the particles with a crosslinking ratio of 3% provided or 15%. It is interesting that as the particles with a 3% nominal crosslinking ratio were collapsed and rinsed, the loading efficiency dropped substantially. These results are due to the more loosely defined polymer network, which allowed the slight diffusion (and ultimately loss) of insulin out of the particles even in the collapsed state.

The particles with higher nominal crosslinking ratios, 10% and 15%, also exhibited some interesting characteristics. The amount of insulin entrapped in the polymer particles increased slightly between the precollapse and postcollapse steps. This result, which may be contrary to what was expected, could be explained by the extent of ionic interactions. As the particles were collapsed, the negative charge density of the insulin increased. Therefore, more insulin was imbibed into the hydrogel matrices due to increased ionic interactions. Since the highly crosslinked hydrogels had smaller overall mesh sizes, the insulin was not “squeezed out” after collapse. Finally, there was almost no difference between the postcollapse and the postrinse steps, indicating that nearly all insulin that was entrapped in the particles occurred on the inside of these particles and not washed away from the surface of the polymer.^{31–33} This result also indicates that the collapsed particles were bound tightly enough to prevent the premature diffusion of insulin out of the particles, which is important for controlled drug delivery applications. The nanoparticles, on the other hand, had an insulin loading efficiency of only about 32% postcollapse and 14% postrinse (data not shown).

Insulin Weight Fraction—A second important means of characterizing the amount of insulin loaded into the particles was the insulin weight fraction, defined in Eq. 2

$$\text{Insulin Weight Fraction} = \frac{m_0 - m_i}{m_0 - m_i + m_p} \times 100\% \quad (2)$$

where m_p was the mass of particles added to the insulin solution for loading. This relationship indicates what mass percent of a given loaded particle is insulin.

Figure 3 identifies the relationship between the concentrations of particles in the insulin loading solution to the insulin weight fraction of the particles. The concentration of insulin remained constant at 1 mg/mL during these studies. As the concentration of 150 μm crushed microparticles was increased, the relative amount of insulin per mass of particles imbibed into the particles decreased. These results indicate that as the concentration of polymer particles was increased, less total insulin was available per hydrogel microparticle. Therefore, even though more insulin was removed from the solution at higher particle concentrations, the total amount of insulin in each particle decreased.

Based on these results, a higher concentration of particles during the insulin loading was not necessary ideal. For a drug formulation, the amount of drug loaded into each particle is an important parameter. If the insulin weight fraction is very low for a given set of microparticles, then it would take more particles to deliver the same amount of insulin compared to a set of microparticles with a high insulin weight fraction. For example, 5 g of particles with an insulin weight fraction of 8% has 400 mg of insulin. However, 5 g of

particles with an insulin weight fraction of 13% has 650 mg of insulin, more than 60% more insulin per mass of particles. Thus, it would take 60% more particles of the lower insulin weight fraction to be able to deliver the same total amount of insulin as the particles with the higher insulin weight fraction.

The ratio of 7 mg particles per 1 mg insulin was used for all further studies due to the high loading efficiency and relatively high insulin weight fraction of microparticles loaded at these conditions.

As shown in Figure 4, the insulin weight fraction for crushed microparticles was also a function of crosslinking ratio. Particles with a crosslinking ratio of 10% yielded the highest mass of insulin loaded per total mass compared to the 3 and 15% crosslinked polymers. Similar to the loading efficiency, after the particles with a 3% crosslinking ratio were collapsed and rinsed, the insulin weight fraction dropped. Again, this phenomenon is most likely due to the more loosely defined polymer network which allowed some of the entrapped insulin to diffuse out during the hydrogel collapse.

As determined by the mesh size estimates, the crushed microparticles were indeed able to entrap insulin in basic media. However, the ability to load insulin into the nanoparticles was more problematic. The insulin weight fraction for the nanoparticles was roughly 7% postcollapse and 3% post-rinse (data not shown). Studies were inconclusive as to the effectiveness of trapping insulin inside these nanoparticles at elevated pH values. In acidic media, negatively charged insulin was attracted to the positively charged nanogels. However, upon addition of NaOH to collapse the nanogels and trap the imbibed insulin, most insulin appeared to simply diffuse out. This result is likely due to a few factors. First, the nanoparticles had higher swelling ratios than their respective microparticle counterparts, due to the differences in polymerization methods. Second, the substantially increased surface-area-to-volume ratio of the nanoparticles, compared with the microparticles, substantially increased the diffusional surface per unit mass by orders of magnitude. These results were further investigated with release studies performed with the nanogels, and discussed below.

Insulin release

pH-Sensitivity for Insulin Release—Initial studies were first performed on crushed microparticles that were exposed to different pH media. This proof-of-concept was used to ensure that insulin could be trapped in the hydrogel particles at elevated pH values (physiological pH of 7.4) and then be released at acidic conditions. Crushed microparticles were used in the study represented by Figure 5, where the pH of the media was 7.4 until time 120 min, at which time an injection of HCl was used to reduce the pH to 4.0. As desired, the insulin remained entrapped inside the hydrogel microparticles at the physiological pH. After the addition of acid, the tertiary amines of DEAEM became protonated, and the insulin took on a net negative charge ($pI = 5.3$). This charge repulsion caused particle swelling and the expulsion of imbibed insulin from the microparticles.

Rapid insulin release occurred after the addition of hydrochloric acid, releasing most releasable insulin after 30 min and nearly 100% after about 50 min. This experiment confirmed the ability to retain the insulin inside the hydrogel microparticles at elevated pH values and only release the insulin once the pH became acidic. Attempts were made to release insulin in a controlled manner from nanoparticles, Figure 6. However, large errors were encountered in acidic media and the pH of the media did not affect the release in a desirable manner, even in basic media. Thus, nanoparticle release was not exhaustively analyzed.

Glucose-Sensitivity for Insulin Release—The proof-of-concept results discussed above were promising and indicated that further studies were necessary to determine the release profile for hydrogels exposed to glucose solutions. Since the mechanism of insulin delivery with the proposed system is during elevated glucose levels, it was necessary to determine the effect glucose had on the release of insulin.

The first comparison was between two sets of crushed hydrogel microparticles which had differing crosslinking ratios. Figure 7 shows the insulin release profile of microparticles with a crosslinking ratio of 3 and 10%. As a guide to the eye, this figure uses the late-time

approximations of $\frac{M_t}{M_\infty}$

$$\frac{M_t}{M_\infty} = \left(1 - \frac{6}{\pi^2}\right) e^{-\frac{\pi^2 t D}{r^2}} \quad (3)$$

where M_t is the mass released at time t , M_∞ is the mass released at very long times, D is the diffusion coefficient, and r is the radius of the sphere. The term $\frac{D}{r^2}$ was solved for using least squares regression, yielding values of $1.6 \times 10^{-4} \text{ s}^{-1}$ and $5.3 \times 10^{-5} \text{ s}^{-1}$ for the 3 and 10% crosslinked particles, respectively. Since the microparticles with only 3% crosslinking swelled substantially more than those with 10% crosslinking, more insulin was able to easily diffuse out during the insulin release study. Thus, 70% of the total amount of insulin that was releasable in the microparticles was released for the particles having 3% crosslinking. This polymeric system would not be ideal for multiple, pulsed release due to over two-thirds of the total drug payload being released on the first pulse.

The higher crosslinked particles exhibited more ideal characteristics for an insulin delivery device. These microparticles released closer to one-third of the available insulin from within the matrix instead of 70%. Thus, multiple dosages of insulin would be possible with such a hydrogel, potentially up to three for the particles investigated. The actual amount of insulin released per mass of particles was $24 \text{ } \mu\text{g}/\text{mg}$ for the 3% crosslinked microparticles and $6 \text{ } \mu\text{g}/\text{mg}$ for the 10% crosslinked microparticles. Therefore, for a typical patient of about 75 kg that would require a postprandial insulin dose of between 3 and 10 IU, roughly 20–60 mg of dry microparticles (10% crosslinking ratio) would be required per day.

Figure 8 shows how the total amount of insulin able to be released from the particles was determined. This value may differ from M_∞ due to the method used. The term M_∞ typically refers to the amount of drug released at infinite time under the current conditions. However, we wanted to quantify the total amount of insulin not permanently bound to the polymeric carrier. After the insulin release due to the glucose stimulus, hydrochloric acid was added to the solution to lower the pH to below the pI of insulin. At a pH of 4, both the insulin and the hydrogel microparticles had net positive charges. Thus, ionic repulsion helped force out any excess insulin still entrapped in the hydrogel after the glucose stimulus. This amount of insulin was quantified, and used as the total amount of insulin that was able to be released from the microparticles.

Figure 9 indicated the effect of a buffering system had on the release of insulin from the hydrogel microparticles. The ionic strength of each solution was kept constant at 150 mM, and the only difference was whether there was a phosphate buffer (5 mM) present. The buffered media reduced the total amount of insulin released due to the Donnan equilibrium effect, which has an effect on the overall swelling of the particles.³⁴ This effect indicates that a semi-permeable membrane, such as the hydrogel itself, may balance net charges by partitioning certain ions into either the membrane or the solvent phase.³⁵ Previous modeling

studies indicated that the buffered medium substantially hindered the pH drop inside these particles.^{16,19} A pH profile along the radius of each particle was also modeled, thus preventing total swelling throughout each particle. With a decrease in the particle swelling, each insulin molecule was presented with a more tortuous diffusional path and was thus less likely to be able to leave the microparticle.^{34–39}

Conclusions

Microparticles with a crosslinking ratio of 10% were determined to have the optimal characteristics for insulin loading and release, both as a function of pH and as a function of glucose concentrations. The release of insulin was controlled by either pH or glucose concentration, and insulin release was minimal at physiological pH values. It was possible to obtain physiologically relevant amounts of insulin release from these microparticle systems. A patient of 75 kg would only need between 30 and 60 mg of microparticles per day for postprandial insulin delivery. The large surface area to volume ratio of the nanoparticles, as well as their larger swelling ratios, did not allow for controlled release of the insulin.

Acknowledgments

This work was supported in part by grant EB 000246 from the National Institutes of Health.

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Figure 1. Loading efficiency of 150 μ m crushed microparticles with 10% crosslinking ratio, varying particle loading concentration, insulin concentration of 1 mg/mL ($n = 3 \pm$ SD)

Figure 2. Loading efficiency of 150 µm crushed microparticles with varying crosslinking ratios, particle loading concentration of 7 mg/mL, insulin concentration of 1 mg/mL ($n = 3 \pm SD$)

Figure 3. Insulin weight fraction of loaded 150 μm crushed microparticles with 10% crosslinking ratio, varying particle loading concentration, insulin concentration of 1 mg/mL ($n = 3 \pm \text{SD}$)

Figure 4. Insulin weight fraction of loaded 150 μm crushed microparticles with varying crosslinking ratios, particle loading concentration of 7 mg/mL, insulin concentration of 1 mg/mL ($n = 3 \pm \text{SD}$)

Figure 5. Insulin release profile of loaded 150 μm crushed microparticles in response to a pH step change

At 120 min, 1N HCl was added to the solution to decrease the pH, and increase the amount of hydrogel swelling ($n = 3 \pm \text{SD}$).

Figure 6. Insulin release profile of loaded nanogels in PBS, pH 7.4 ($n = 3 \pm \text{SD}$)

Figure 7. Insulin release profile of loaded 150 μm crushed microparticles in response to glucose stimulus

At time 0, a glucose bolus was delivered to the particle suspension and the amount of insulin released was observed ($n = 3 \pm \text{SD}$).

Figure 8. Determination of total releasable insulin from loaded 150 μm crushed microparticles

To determine the total amount of insulin that can be released in Figure 7, 1 N HCl was added after the insulin release caused by a glucose bolus. This change in pH caused the pH to drop below the pI of insulin, forming ionic repulsion between the positively charged insulin and the positively charged hydrogel ($n = 3 \pm \text{SD}$).

Figure 9. Insulin release from loaded 150 μm crushed microparticles in response to glucose, in varying media: unbuffered saline (ionic strength $I = 0.15\text{ M}$) and buffered PBS ($I = 0.15\text{ M}$)
At a time of 125 min, 300 mg/dL glucose was added to each sample ($n = 3 \pm \text{SD}$).