Novel lactose based pH-sensitive carriers for oral-insulin delivery

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Abstract: Novel types of hydrogels based on hydrophilic lactose acrylate (LA), poly (ethyleneglycol monomethyl ether methacrylate) and methacrylic acid were designed and synthesized. Hydrogel synthesis was carried out by free-radical polymerization of the monomers using persulfate as initiator and N, N′-methylenebisacrylamide as crosslinker. Equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids (SGF and SIF, respectively). Insulin was entrapped in these gels and the in vitro release profiles were established separately in both (SGF, pH 1) and (SIF, pH 7.4). All of the hydrogels were able to incorporate insulin and protected it from release in acidic media. Drug release studies showed that the increasing content of MAA in the copolymer enhances hydrolysis in SIF. In these cases, the biological activity of insulin was retained. These results were used to design and improve insulin release behavior from these carriers. [Nature and Science. 2010;8(1):81-86]. (ISSN: 1545-0740).

Key words: lactose acrylate, poly(ethylene glycol), pH-sensitive hydrogel, oral drug delivery, MAA

Introduction

Oral drug delivery is the most popular method for drug delivery. The goal of oral delivery systems is to protect the sensitive drug from proteolytic enzyme degradation in the stomach and upper portion of the small intestine. However, two problems exist in developing oral delivery systems for insulin. One major problem is the degradation of proteins by proteolytic enzymes and the acidic environment of the stomach. Another problem is the low penetration of proteins across the lining of the intestine into the blood stream [1-5]. This can be overcome by designing carriers that can protect the insulin from the harsh environments of the stomach before releasing the drug into more favorable regions of the GI tract, specifically the colon [6]. Additionally, researchers have attempted to incorporate protease inhibitors into oral insulin formulations, which serve to prevent insulin degradation by the proteolytic enzymes [7]. The large intestine may be optimal for drugs delivery because of high residence time and low digestive enzymatic activity [8-9]. One strategy for targeting orally administered drugs to the colon includes coating drugs with pH-sensitive hydrogels [10-13]. In general, MAA-based hydrogels can form polymer complexes in response to the environmental pH. In the acidic environment of the stomach, these hydrogels are in a collapsed state due to hydrogen bonding, which can protect proteins by not allowing them to diffuse out from the hydrogel. In the intestine, as the environmental pH increases, the complexes dissociate and the pore size of the hydrogels increases leading to protein [14-19]. Additionally, the ionized carboxylic acid groups of PMAA have the ability to bind calcium ions in the extracellular medium. Therefore, they can help to minimize the proteolytic activity of calcium-dependent enzymes like trypsin [20, 21] and increase the paracellular permeability of epithelial cell monolayers by opening of tight junctions between two epithelial cells [22, 23].

Natural polymers have potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biodegradability. Synthetic polymers containing side-chain carbohydrates are considered as high value polymeric materials because of their potential as biocompatible materials with medical applications. These applications are generally based on the fact that cell-cell interactions between oligosaccharides and lipids play an important role in various life processes [24].

Poly(ethylene glycol) (PEG) is widely used in the drug delivery system (DDS) for many reasons, with one being their low toxicity to cells [25]. Another reason is that PEGs bind relatively little with proteins [26], thereby enabling the long chain of the PEGs to protect the proteins and peptides in the DDS that are used to target the cells from reacting with other sites in the body [27]. PEGs may also increase the chances of the DDS carriers to reach the desired cells, as the large molecular weight of the PEGs have been reported to increase the circulation time of the DDS carriers in the bloodstream [28].

In this study, the feasibility of MAA-based hydrogels containing side-chain lactose and Poly(ethylene glycol) groups as oral delivery carriers for proteins was evaluated by investigating the pH-responsive release behavior of insulin in the physiological pH range and protective ability of

hydrogels for insulin in simulated gastric solutions. The release pattern and stability of insulin in contact with these hydrogels during the release were studied.

Experimental
Materials

The insulin used was recombinant human insulin (AK2U Nobel France; lot # 821156, Batch L-00023822). Lactose acrylate (LA) and poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) were prepared by the methods described in the literatures, respectively [29, 30]. Poly (ethylene glycol) monomethyl ether (PEGME) was purchased from Aldrich (Mn = 1000, 2000). 4-dimethylaminopyridine (DMAP) and reagents were obtained from Fluka Co. Methacrylic acid (MAA), acryloyl chloride, dicyclohexylcarbodiimide (DCC) and bis-acrylamide were purchased from Merck Co. The solvents and reagents were obtained from Fluka. The IR spectra were recorded on a Shimadzu FT IR-408 spectrophotometer. The amount of released drug was analyzed using a high-performance liquid chromatography- ultraviolet (HPLC-UV) Waters bus SAT/IN Module at 210 nm. Isocratic elution was performed using 30% acetonitrile and 70% buffer containing 0.1M KH_2PO_4 and 1% triethylamine adjusted to pH 3.0 with phosphoric acid. The column used was Nulcleosil-C185-m PHASE SEPARATIONS 4.6-250 mm Analytical Cartridge (part no. psl841020) equipped with a precolumn. Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the US Pharmacopeia [31].

Copolymerization: General Procedure

terpolymerization Lactose acrylate (LA), poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) with different molar ratios of methacrylic acid and bis-acrylamide as a cross-linking agent (CA) were polymerized by using persulfate as an initiator ([I] = 0.02 M) and water as the solvent (50 mL). The variable feed ratio as shown in Table 1. All experiments were carried out in Pyrex glass ampoules sealed off under vacuum. After the desired time (48 h) the precipitated network polymer bonded drug was collected, washed with deionized water for 1 week, and the water was changed every 12 hours in order to remove any unreacted monomers. After washing, the samples were dried in air and stored in desiccators until use (Scheme 1). The values are given in Table1. IR (KBr): 3450-2500 (broadened, -COOH group), 1720, 1660, 1590, 1410, 1230, 1210 cm^{-1}.

Swelling ratio

The resulting network polymers swell and become soft in solvents such as H_2O and most organic solvents without dissolving. To measure the swelling, preweighed dry drug-free hydrogels were immersed in various buffer solutions (pH 7.4 and pH 1) at 37° C. After excess water on the surface was removed with the filter paper, the weight of the swollen samples was measured at various time intervals. The procedure was repeated until there was no further weight increase. The degree of swelling was calculated according the relation:

$$SW\ (\%) = [(Ws\text{-}Wd)/\ Wd] \times 100$$

Where, Ws and Wd represent the weight of swollen and dry samples, respectively. Time-dependent swelling behavior of cross-linked polymers in pH 1 and pH 7.4 at 37° C are

plotted in figure 1. approximately 60 min, the completely swollen hydrogels loaded with insulin were placed in desiccators and dried under vacuum at room temperature. The values are given in Table 1.
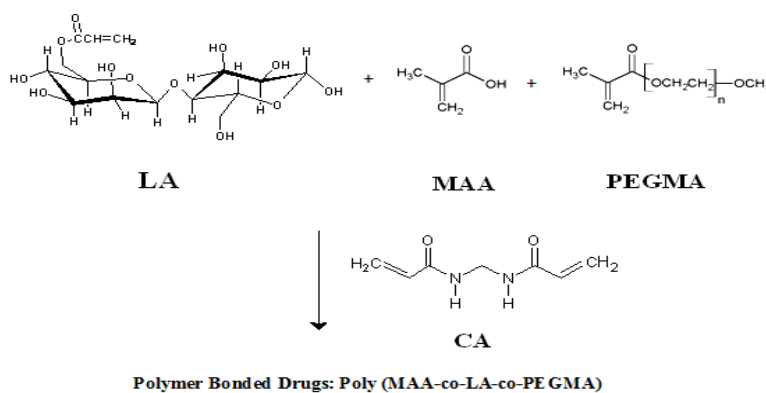
Scheme 1.   Synthesis of pH-sensitive hydrogels.

Insulin stability during release studies from hydrogels

In order to study the stability of insulin in contact with hydrogels, two different conditions were chosen: 37° C and darkness, 37° C and light. Insulin was loaded in hydrogels as described and then the peptide stability was investigated during release under the above mentioned conditions at two different pH values of 1 and 7.4. Samples were analyzed under each condition after 24 and 48 h. In this condition insulin remained fairly stable at both pH values during the course of experiments, indicating that adsorption of the peptide to the hydrogels and their release afterwards did not substantially influence the stability of this peptide drug.

To investigate the protective ability of the hydrogel for insulin in the harsh environment of the stomach, insulin and insulin-incorporated were treated with a simulated gastric solution that contained endopesidase pepsin. After the treatment in gastric solution, the biological activity of insulin was determined with HPLC. These results indicated that all insulin was degraded immediately after insulin was in contact with gastric fluid and the main cause of degradation was the proteolytic enzyme, pepsin. After being treated with gastric fluid, all of hydrogels demonstrated a protective effect on insulin and the biological activity remained after the treatment with gastric fluid of hydrogels. Studies of hydrogel showed that when the MAA content increased, degradation of insulin decreased.

Insulin release from hydrogels

Insulin release from the delivery systems was tested in the pyrex glasses. The powdered hydrogel (10mg) was poured in 5ml of aqueous buffer solution (pH=7.4 & pH=1) at 37° C. The rotation speed was adjusted with stirrer. Samples were measured using HPLC-UV at 210 nm. The flow-rate and injection volume were 1 ml/min and 60 μL, respectively. Insulin was detected at a retention time of 5.5 min and the detection limit was 0.3 μg/mL. Triplicate samples were used. The amounts of insulin released from hydrogels was collected by taking 60-μL samples at predetermined time intervals and analyzed by HPLC.

Quantitative analysis of insulin

Three milligrams of polymer-drug adduct was dispersed in 3 mL of mobile phase solution. The reaction mixture was maintained at 37° C. After 4 h the hydrolysis solution filtered and analyzed by HPLC for the determination of total insulin in hydrogels. The results obtained are presented in Table 1.

Table 1.   Composition of copolymers

Polymers	Molar composition of monomers in the feed			Percent of insulin-loading (%)
	LA	MAA	CA	
P-1	1	1	2	93
P-2	1	1	4	89
P-3	1	2	2	99
P-4	1	2	4	93
P-5	2	1	2	80
P-6	2	1	4	75

Results and Discussion

To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium. Lactose acrylate (LA) monomer was prepared by the method described in the literature [26]. The lactose monoester was isolated from the unreacted lactose and disubstituted derivatives by extracting the mixture with methyl ketone and butan-2-ol, i.e., by selective dissolution of monounsaturated lactose. In FT-IR of LA, the peak at 1640 cm^{-1} is due to the vinyl unsaturation. The peak due to ester carbonyl peak at 1730 cm^{-1} merged with the 1640 cm^{-1} vinyl peak. The ^{1}H-NMR spectrum of LA shows the characteristic vinylic proton signals at 6.2 and 5.8 p pm. All other proton signals are due to the lactose moiety.

As shown in Figure 1, an increase in the content of MAA in the feed monomer mixtures resulted in less swelling in SGF but greater swelling in SIF. The loading numbers in Table 1 shows existence of polar functionally groups as carboxylic acid need not only for loading insulin on the polymer but also for pH-sensitive properties of polymer. Insulin molecules have a tendency to attach to polar groups due to hydrogen-bonding. Hydrogen bonding is a key contributor to the specificity of intramolecular and intermolecular interactions in biological systems. Because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH. The increase of lactose content resulted in less collapsed networks at low pH. This led to a relatively large pore size of the networks. Thus, insulin could diffuse readily from the gel at low pH. For these hydrogels, the released insulin in the acid media increased with the molecular weight of the grafted PEG in the network. At the incorporating pH of 7.0, the carboxylic acid groups in the networks, as well as the insulin, (pI of 5), were negatively charged resulting in repulsion. Thus, the negatively charged insulin was mainly distributed in the

neutral PEG chain domains. A researcher shows that insulin appeared to partition into the PEG phase in hydrogels containing PEG and a negatively charged component [32]. When insulin-incorporated polymer bonded drugs were placed in acidic media, particles with longer PEG chains, where more insulin was distributed, had more chance to contact the outer aqueous environment, and as a result insulin was released by a concentration gradient at low pH. However, there was no significant difference of insulin release at high pHs from systems with different PEG molecular weights.

The degree of hydrolysis of the hydrogels containing insulin as a function of time is shown in figure 2. It appears that the degree of hydrolysis network polymers depends on their degree of swelling and reticulated degree. With increased cross-linking and an increase in the reticulated degree of the polymer, diffusion of the hydrolyzing agents in the networks polymer is reduced and the hydrolysis rate is slower. The increase of glucose content resulted in less collapsed networks at low pH. This led to a relatively large pore size of the networks due to the bulky sugar groups. Thus,

insulin could diffuse readily from the gel at low pH. On the other hand, a high different hydrolysis rate for polymers at pH 1 and pH 7.4 can be related to the number of carboxylic acid groups units along the polymer chain.

Existence of hydrogen-bonding interactions between –COOH groups in the polymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum hydrolyzing of the gel in a medium of pH 1. However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of –COOH groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged –COO$^-$ groups. These two factors ultimately result in a greater increase in the water uptake. In pH 7.4 with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing agents on polymer is increased and the hydrolysis rate increased [33].
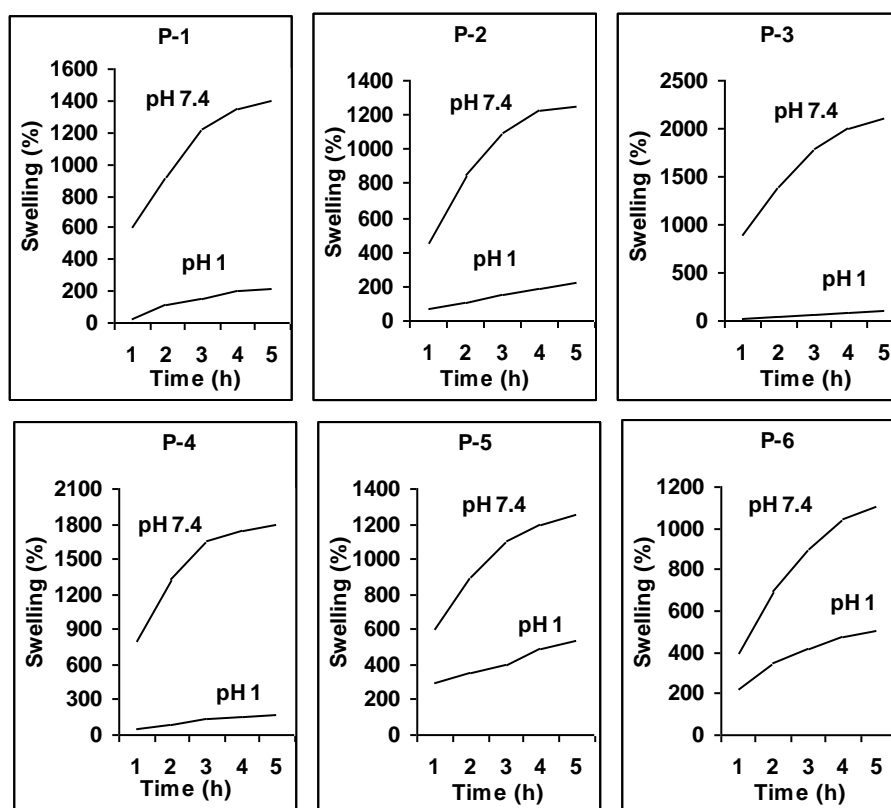

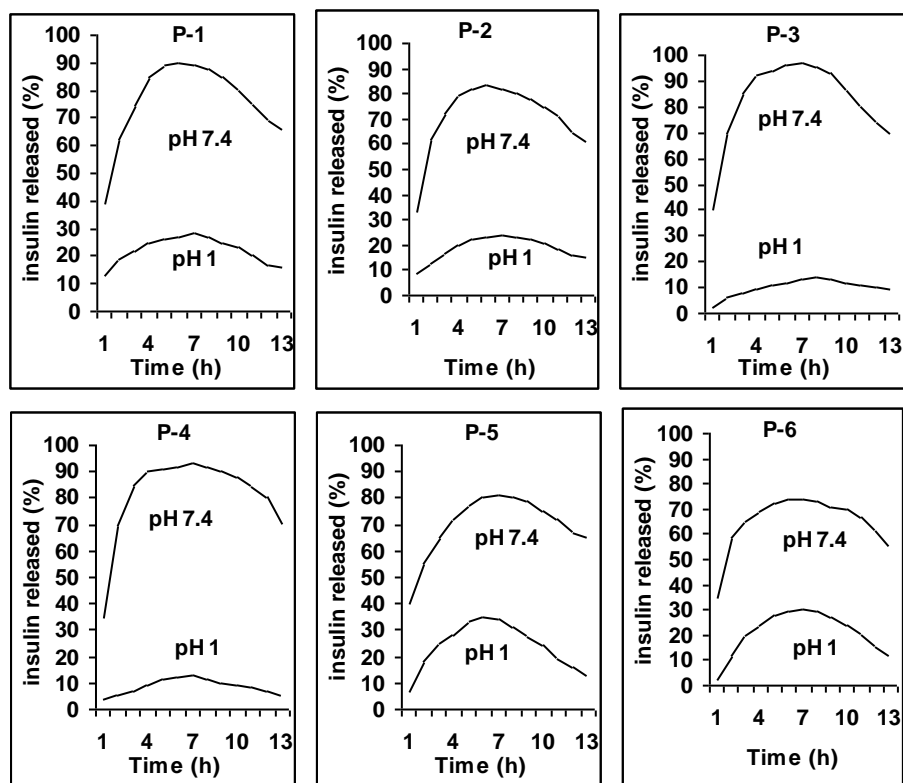**Figure 1.   Time-dependent swelling behavior of hydrogels in pH 1 and pH 7.4.**

Figure 2.   Release of insulin from polymeric carriers as a function of time at 37°C.

Conclusion

We incorporated a large amount of insulin from the insulin stock solution in all the formulations of hydrogels that contained MAA. The order of swelling and hydrolysis in this series was significantly affected by polymer composition. Incorporation of MAA made the hydrogels pH-dependent and the transition between the swollen and the collapsed states occurred at high and low pH. The swelling ratios of the hydrogels increased at pH 7.4, but decreased at pH 1 with increasing incorporation of MAA. Based on the great difference in hydrolysis rate at pH 1 and 7.4, these glycopolymers appear to be good candidates for colon-specific protein delivery.

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