# Modification of nano alginate-chitosan matrix for oral delivery of insulin

Mehrdad Mahkam

Chemistry Department, Azarbaijan University of Tarbiat Moallem, Tabriz, Iran
mmahkam@yahoo.com; mahkam@azaruniv.edu
Tel: +98 412 432 7541

**Abstract:** The objective of this study is modification of nano chitosan blended alginate matrix (CBAM) by grafting with poly methacrylic acid (PMAA) to utilize for oral delivery of insulin. firstly, chitosan blended alginate matrix converted to nano by freeze drying method and then, free radical graft copolymerizations were carried out at 70 °C, bis-acrylamide as a cross-linking agent and persulfate as an initiator. The cross-linked three-dimensional polymers were characterized by scanning electron microscopy and FT-IR. In the matrices with increase in the content of chitosan had shown increased bioadhesivity. 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). 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. 2009;7(8):1-7]. (ISSN 1545-0740).

**Keywords**: Blending, Chitosan, Alginate, Grafting, Oral delivery, Insulin

## Introduction

Oral delivery of drugs, especially therapeutic proteins, is the preferred route of administration because it offers advantages over injection, which is the presently accepted route of therapeutic protein administration. The oral delivery route is more natural and less invasive. However, there exist several problems for the development of oral protein delivery systems. 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]. Among the various methods that have been developed to assist to these problems [2-5], use of environmentally sensitive hydrogels, especially methacrylic acid (MAA)-based complexation and pH-sensitive hydrogels, is the most promising method.

Natural polymers have potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biodegradability. Alginates are natural polymers isolated from several species of brown algae. Alginates are composed of two monomers, α-L guluronic acid and β-D-mannuronic acid. The pH sensitive nature and its ability to control gel permeability means; that these materials have significant potential for drug delivery applications. However the bioadhesive potential of alginate is not sufficient to make suitable for prolonged contact with the intestinal mucosal surface in case of oral drug delivery of poorly absorbable agents. Chitosan, another natural polymer is partially or fully deacetylated derivatives of chitin. The polymer is linear consisting of D-glucosamine residues with a variable number of randomly located N-acetyl glucosamine groups. Chitosan is well known for its bioadhesive nature. Bioadhesion is defined as the ability of a material to adhere to a biological tissue for an extended period of time. In the cationic form, the Dglucosamine residue of chitosan could interact with the sialic acid residues of mucin by electrostatic interaction. The bioadhesive property of chitosan may allow a prolonged interaction of the delivered drug with the membrane epithelia facilitating more efficient absorption. Increased absorption of drugs at mucosal sites by chitosan could be due to prolonged interaction with the membrane epithelia or opening of the tight junctions between cells to facilitate transport [6, 7]. A number of reports suggest the utilization of alginate and chitosan for drug delivery [8, 9]; however, it may need to be further modified for some special applications. Among diverse

approaches that are possible for modifying polysaccharides, grafting of synthetic polymer is a convenient method for adding new properties to a polysaccharide with minimum loss of its initial properties [10]. Graft copolymerization of vinyl monomers onto polysaccharides using free radical initiation, has attracted the interest of many scientists.

In this study our aim was to utilize the pH sensitivity of alginate, (Alginate is stable in acidic pH of stomach, but it swells and starts dissolving slowly in the intestinal alkaline pH) which can be used for protecting insulin in stomach and the bioadhesivity of chitosan to make prolonged contact with the intestinal mucosae, so as to increase the absorption of insulin. The graft copolymerization poly methacrylic acid onto chitosan blended alginate matrix (CBAM) was carried out under free radical polymerization, bis-acrylamide as a cross-linking agent and persulfate as an initiator. Insulin was entrapped in these gels and the in vitro release profiles and stability of insulin in contact with these hydrogels during the release were studied.

## Experimental

### Materials

Chitosan blended alginate matrix (CBAM) was prepared by the methods described in the literatures [11-13] and then dried by freeze drying method. The insulin used was recombinant human insulin (AK2U Nobel France; lot # 821156, Batch L-00023822). Sodium alginate of medium viscosity (3500cps for a 2% solution at 25 °C) was obtained from Sigma chemicals Co. Chitosan was obtained from Aldrich (85% deacetylation). Methacrylic acid (MAA) and bis-acrylamide were purchased from Merck Co. All the other chemicals used were of analytical reagent grade. Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the literature [14].

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.

### Copolymerization: General Procedure

CBAM with different molar ratios of MAA were polymerized at 60-70 °C in a thermostatic water bath, bis-acrylamide as a cross-linking agent (CA), using persulfate as an initiator ([I] = 0.01 M) and water as the solvent (50 mL). After the desired time (48 h) the precipitated network polymers 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. The values are given in Table1. IR (KBr): 3380-2500 (broadened, -COOH group), 1720, 1520, 1240, 1225 $cm^{-1}$.

Table 1. Composition of copolymers

| Polymers | Molar composition in the feed | | | |
|---|---|---|---|---|
| | CBAM 1:10 | CBAM 1:12 | MAA | CA (%) |
| P-1 | 1 | --- | 3 | 5 |
| P-2 | 1 | --- | 3 | 10 |
| P-3 | 1 | --- | 5 | 5 |
| P-4 | 1 | --- | 5 | 10 |
| P-5 | --- | 1 | 3 | 5 |
| P-6 | --- | 1 | 3 | 10 |
| P-7 | --- | 1 | 5 | 5 |
| P-8 | --- | 1 | 5 | 10 |

### Measurement of swelling ratio

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:

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

Where, Ws and Wd represent the weight of swollen and dry samples, respectively. The study of swelling shows that swelling of hydrogels increases with time, first rapidly and then slowly, reaching maximum constant swelling (mass equilibrium swelling, MES). In all cases, the swelling weight reached its equilibrium after 5 hours. Time-dependent swelling behaviour of cross-linked polymers in pH 1 and pH 7.4 at 37°C are given in Table 2.

**Table 2. Percent of swelling and drug loading numbers**

| polymers | maximum constant swelling (%) pH 1 | maximum constant swelling (%) pH 7.4 | Percent of insulin-loading |
|---|---|---|---|
| P-1 | 400 | 1300 | 80 |
| P-2 | 340 | 1150 | 72 |
| P-3 | 310 | 1550 | 95 |
| P-4 | 280 | 1450 | 89 |
| P-5 | 390 | 1500 | 89 |
| P-6 | 340 | 1450 | 82 |
| P-7 | 300 | 1800 | 99 |
| P-8 | 260 | 1700 | 93 |

**Insulin Loading in Hydrogels**

Insulin can only be dissolved in acidic aqueous solutions of around pH 3.0; insulin was first dissolved at pH 3.0 and then the pH was increased to pH 7.2 using 0.1M KOH. Subsequently, 10 mg of each hydrogel was placed in 3 mL of insulin solution (1.0 mg/mL) to absorb the total amount of the insulin solution. After approximately 60 min, the completely swollen hydrogels loaded with insulin were placed in desiccators and dried under vacuum at room temperature.

**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 2.

**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.

**In situ Bioadhesivity Studies**

Bioadhesivity testing was done by a novel in situ method as described by Ranga Rao and Buri [15]. A freshly cut 5-6 cm long piece of small intestine of rat was obtained and cleaned by washing with isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of hydrogels were added evenly on the mucosal surface. The intestinal piece was maintained at 80% relative humidity for 30 mts in a desiccator. The piece was taken out and phosphate buffer pH 6 was allowed to flow over the intestinal piece for about 2 mts at a rate of 20 ml/min. The perfusate was collected and dried to get the particles not adhered. The percent of bioadhesion was estimated by the ratio of amount applied to adhere hydrogels. The values are given in Table 3.

**Table 3. Particles adhered onto rat intestine (%)**

| polymers | Percentage adherence |
|---|---|
| **P-1** | **76** |
| **P-2** | **75** |
| **P-3** | **73** |
| **P-4** | **71** |
| **P-5** | **73** |
| **P-6** | **71** |
| **P-7** | **69** |
| **P-8** | **69** |

**Results and discussions:**

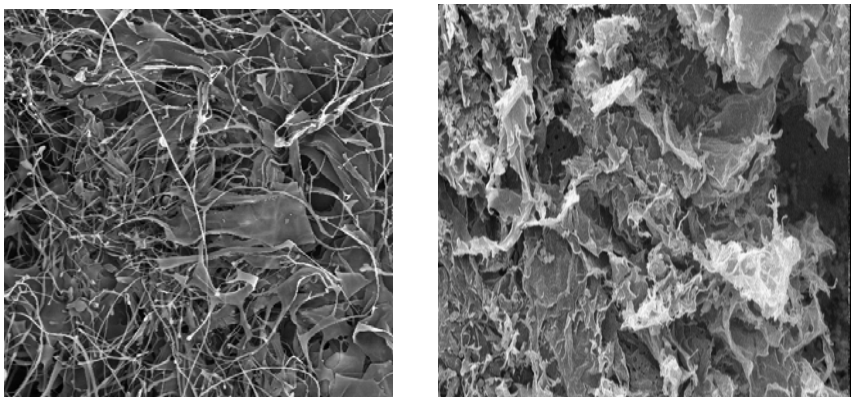
Fourier transform infrared (FT-IR) spectroscopy of matrix was carried out in order to detect any peak shift that could be attributed to interactions between the two polymers, such as hydrogen bonding or complexation. In general, the FTIR spectrum of blank chitosan-alginate particles showed a broad band around 3500-3100 cm-1, indicating enhanced hydrogen bonding compared to that of chitosan or sodium alginate alone [16]. Moreover, the N-H bending vibration of nonacrylated nonacrylated 2-aminoglucose primary amines of chitosan (1570 $cm^{-1}$) and asymmetric and symmetric –C-O stretching at 1407 $cm^{-1}$ of sodium alginate disappeared, indicating that the ($-NH_3^+$) of chitosan reacted with the ($–COO^-$) of alginate [17]. Absence of these bonds in the FTIR spectra of chitosan/sodium alginate matrix indicated the formation of a strong electrostatic bond between them.

The structure of polymers can be analyzed via using scanning electron microscope. As a sample, Figure 1 showed SEM image of chitosan blended alginate matrix of P-1.

The SEM studies show that due to the strong interaction from the intermolecular hydrogen bonds and electrostatic interactions between carboxyl groups on alginic acid and amino groups on chitosan, not only creates good miscibility between chitosan and alginate but also the prepared polymer adapts fibrous structure. In the grafting, by development of intermolecular hydrogen bonding between its amino groups and the carboxyl groups of PMAA, interaction of alginate/chitosan reduced and ruined fiber structures.

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. The composition of the polymer defines its nature as a neutral or ionic network and furthermore, its hydrophilic/hydrophobic characteristics. Ionic hydrogels, which could be cationic, containing basic functional groups or anionic, containing acidic functional groups, have been reported to be very sensitive to changes in the environmental pH. The swelling properties of the ionic hydrogels are unique due to the ionization of their pendent functional groups.

Hydrogels containing basic functional groups is found increased swelling activity in acidic conditions and reduced in basic conditions but on the other hand pH sensitive anionic hydrogels shows low swelling activity in acidic medium but very high activity in basic medium.



A B

Figure 1. SEM images of chitosan /alginate matrix before (A) and after (B) the grafting

The equilibrium swelling ratio of the hydrogels was a function of the network structure, crosslinking ratio, hydrophilicity and degree of ionization of the functional groups. With increased cross-linking and an increase in the reticulated degree of the polymer, diffusion of the water in the network's polymer is reduced and the swelling is slower. The 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 swelling 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.

The loading numbers in Table 2 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. Hydrogen bonding is a key contributor to the specificity of intermolecular interactions in polymer bonded drugs systems. The hydrogen-bonding and electrostatic interactions increased with MAA content in the copolymer networks. The hydrogen-bonding and electrostatic interactions increased with MAA content in the copolymer networks.

The preparation of CBAM was difficult to achieve with higher ratio of chitosan to alginate. Since due to ionic interaction immediately after mixing of chitosan with alginate rigid gel formation will occur, this makes the particle formation difficult. In our study we tried 1:10, 1:12 ratio of chitosan to alginate for preparation of matrices. The chitosan blending was done to provide additional bioadhesivity to alginate matrices. Among diverse approaches that are possible for modifying natural polymers, grafting of synthetic polymer is a convenient method for adding new properties to a natural polymer with minimum loss of its initial properties. All the matrices with the presence of chitosan had shown increased bioadhesivity (Table 3). The binding of chitosan residues with sialic acid residues make prolonged contact of the drug with the epithelium, also it was assumed that opening of the intercellular junctions by chitosan could lead to the enhancement of peptide absorption across the mucosa.

**Insulin Release**

To develop potential applications of polymer-bonded drugs (PBDs) containing insulin as the pharmaceutically active compound, we studied the hydrolysis behavior of hydrogel polymers under physiological conditions. Although the polymers were not soluble in water, they were dispersed in a buffer solution, and the hydrolysis was evaluated as a heterogeneous system. The degree of hydrolysis of the hydrogels containing insulin as a function of time is shown in figures 2 and 3.

The order of hydrolysis in this series was significantly affected by polymer composition. The increase of CBAM 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. In the other hand, with increases of percentage of chitosan in matrix, the pH sensitivity of alginate is altered or reduced. But, as the content of MAA in the feed monomers increased, hydrolysis rate decreased at pH 1 but increased at pH 7.4. This was because a higher MAA content in the polymer networks led to higher carboxylate anion concentration at high pH. In other words, the 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.

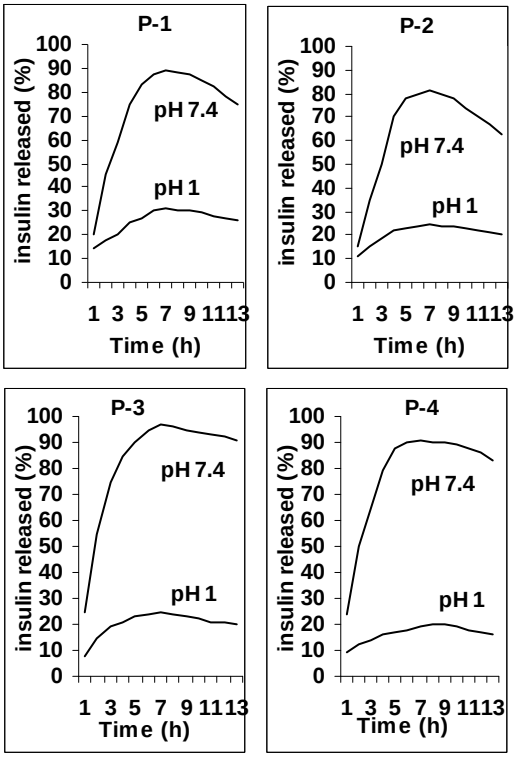



**Figure 2. Release of insulin from polymeric carriers containing (CBAM 1:10) as a function of time at 37 °C.**

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 [18]. In the other hand, At an incorporation pH 7.4, the carboxylic acid groups in hydrogels as well as the insulin, which has pI of 5, were negatively charged resulting in repelling each other. Because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH. This effect response to decrease or increase insulin release in pHs 1 and 7.4, respectively.

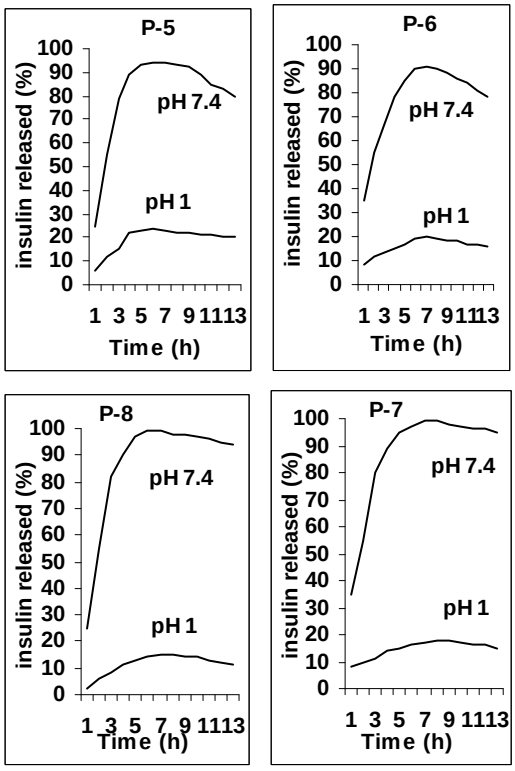



**Figure 3. Release of insulin from polymeric carriers containing (CBAM 1:12) as a function of time at 37 °C.**

## Conclusions

For colon-selective drug delivery, a polymer is needed that is able to withstand lower pH values, but disintegrates at the slightly alkaline pH values of the ileocecal junction and the large intestine. The use of carriers made of natural

polysaccharides has arisen as a promising alternative for drug delivery. The chemical modification of natural polymers by grafting has received considerable attention in recent years because of the wide variety of monomers available. Novel pH-responsive hydrogels containing chitosan and alginate were synthesized by the graft copolymerization poly methacrylic acid onto chitosan blended alginate matrix (CBAM). In our study all the hydrogels containing chitosan has shown greater bioadhesiveness, in the in-situ-study. By regulating the crosslinking percentage of the grafted polymers, pH-sensitive hydrogels with improved optimal hydrolysis rates were obtained. The hydrolysis of the drug-polymer conjugates were performed at pH 1 and 7.4 at 37 °C. The different systems available with varied release kinetics and bioadhesivity may help in tailoring the system suitable for the oral delivery of insulin for the management of diabetes.

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**Correspondence to:**
Mehrdad Mahkam
35 Km Tabriz-Marageh road
Azarbaijan University of Tarbiat Moallem,
Tabriz, Iran
Tel: +98 412 432 7541
mmahkam@yahoo.com; mahkam@azaruniv.edu

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