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International Journal of Pharmaceutics 277 (2004) 11–17
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

# Devices based on intelligent biopolymers for oral protein delivery

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Received 30 September 2002; received in revised form 6 January 2003; accepted 16 March 2003

Available online 13 April 2004

**Abstract**

The primary goal of bioadhesive controlled drug delivery is to localize a delivery device within the body to enhance the drug absorption process in a site-specific manner. An important contributor to good adhesion is the presence of molecular adhesion promoters such as polymer-tethered structure (e.g., poly(ethylene glycol) chains grafted to crosslinked networks) or even linear chains which are free to diffuse across the gel/gel interface. Recently, we have developed a very promising class of carriers for drug and especially protein delivery. Copolymer networks of poly(methacrylic acid) grafted with poly(ethylene glycol) exhibit reversible, pH-dependent swelling behavior due to the formation of interpolymer complexes between protonated pendant acid groups and the etheric groups on the graft chains. Gels containing equimolar amounts of MAA/EG exhibited the lowest degree of swelling at low pH increased complexation. The average network mesh size or correlation length was dramatically affected by the pH of the swelling solution. The in vitro release of insulin from P(MAA-g-EG) gels containing PEG grafts of molecular weight 1000 indicates a significant release of insulin as the gel decomplexes and insulin is freed through the structure. The results of additional in vitro studies have shown that insulin release rates can be controlled by appropriate adjustment of the structure of the gels.


*Keywords:* Hydrogels; Protein; Insulin; Mucoadhesion; Poly(ethylene glycol)

## 1. Introduction

### 1.1. Tethered carriers and protein delivery

The utilization of hydrogels as carriers for protein delivery has been discussed in a number of recent reviews (Lowman and Peppas, 1999a; Byrne et al., 2002; Peppas, 1995). Particularly, the work of Lowman and coworkers (Lowman and Peppas, 1999a,b,c, 2000; Lowman et al., 1998a,b) has shown that diffusion controlled delivery of proteins from hydrogels can be possible and controlled by the three-dimensional structure. Recently, promising new studies have shown that especially a number of mucoadhesive carriers can be used for protein delivery. For example, Mathiowitz et al. (1997) showed that certain biologically adhesive engineered polymer microspheres prepared from biologically erodible polymers which displayed strong interactions with the mucosa of the gastrointestinal tract could be "developed as delivery systems to transfer biologically active molecules" (among which insulin and plasmid DNA are mentioned) to the circulation. Also, over the past 3 years, Lehr (1994, 1996) and his coworkers have reported that various forms of adhesive hydrogels based on poly(acrylic acid) exhibited an unusual property of inhibition of the degradation of various peptides and proteins

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0378-5173/$ – see front matter 
doi:10.1016/j.ijpharm.2003.03.001

(including insulin and hemoglobin) by the enzymes of homogenized intestinal rate mucosal cells. Thus, the use of mucoadhesive carriers for protein delivery is a subject of significant current research interest.

The impetus for controlled drug release is to maintain a steady flux of an active agent over an extended period of time. The primary goal of bioadhesive controlled drug delivery is to localize a delivery device within the body to enhance the drug absorption process in a site-specific manner. While substantial research has been done to characterize the macroscopic adhesive characteristics of a variety of bioadhesive polymers in contact with mucin or cells, the importance of specific adhesion mechanisms on the overall adhesion process has not been fully examined.

Understanding of gel carrier/mucosal adhesion is of utmost importance in bio- and mucoadhesion. An important contributor to good adhesion is the presence of molecular adhesion promoters such as polymer-tethered structures (e.g., poly(ethylene glycol) chains grafted to crosslinked networks) or even linear chains which are free to diffuse across the gel/gel interface. The idea of the use of adhesion promoters to achieve improved bioadhesion is relatively new and was first proposed in our laboratory (DeAscentiis et al., 1995a,b; Sahlin and Peppas, 1997; Huang et al., 2000, 2002). This method has been examined for possible use in novel buccal, transmucosal and vaginal drug delivery systems. While the role of chain diffusion in uncrosslinked polymer systems has been studied, less is known about linear polymer diffusion in swollen polymer networks (Peppas and Buri, 1985).

Although chain interpenetration is clearly a mechanism contributing to adhesion, surprisingly enough most of the data supporting diffusion in bioadhesion also agree with what would be anticipated for a wetting controlled process, due to the low value of the interfacial tension. We have previously analyzed the molecular aspects of this phenomenon and proposed several scaling laws for the description of bioadhesive systems (Huang et al., 2002; Mikos and Peppas, 1990). Two distinct mechanisms of adhesive failure were examined: chain pullout and chain scission. The equilibrium fracture energy, i.e. the fracture energy at long times, is expected to scale to the degree of polymerization when chain pullout is prevalent. For a rupture mechanism, the equilibrium fracture energy should be independent of molecular weight. Furthermore, the equilibrium volume fraction is expected to play a role in determining the equilibrium fracture energy. Although chain mobility is enhanced for low values of this parameter due to the increased swelling of the system, the fracture energy decreases since the number of free chains crossing the interface is reduced. As a result, it was predicted that highly crosslinked materials should be better bioadhesives for glycoproteinic networks. Furthermore, high degrees of swelling should reduce the fracture energy since the swelling ratio is inversely proportional to the polymer volume fraction.

### *1.2. Analysis of muco- and bioadhesives*

Our research group has been in the forefront of the development and investigation of the molecular and pharmacological behavior of new bioadhesion carrier materials for drug delivery applications. Potential bioadhesives have been evaluated for their adhesive properties, durability, and biological inertness (Duchêne et al., 1988). A variety of mechanical tests has been used to compare the adhesive strength of bioadhesive formulations. These methods have been used in both in vivo and in vitro experiments.

We examined the adhesive properties of poly(acrylic acid)/hydroxypropyl methylcellulose matrices as a function of PAA composition (Ponchel et al., 1987a,b; Peppas et al., 1987). The tablets were preswollen with a controlled amount of water while they were in contact with excised bovine sublingual mucosa. The work of adhesion increased as the PAA content of the specimens increased.

Similar studies were performed in our laboratories by DeAscentiis et al. (1995a,b) and Achar and Peppas (1994) who investigated a number of PAA and PHEMA microspheres of varying degrees of crosslinking and found little dependence of mucoadhesion on the crosslinking. However, in the work of DeAscentiis et al. (1995a) we found that if we incorporated free poly(ethylene glycol) (PEG) chains in the particles, mucoadhesion was improved significantly because of the penetration of the free PEG chains across the mucosa/polymer interface. This idea was first proposed by Sahlin and Peppas (1996, 1997) who studied the adhesion of two similar gels incorporating free PEG chains. We believe that these are the first references of use of adhesion promoters (more

specifically PEG) for mucoadhesive drug delivery systems (Huang et al., 2002, 2000).

Muco- and bioadhesion of hydrogels is the result of a combination of surface and diffusional phenomena that contribute to the formation of adequately strong interchain bridges between the polymer and the biological medium. Yet, clearly the idea of mucoadhesion promoters incorporated in the (polymer) carrier structure used for drug delivery is new and has not been studied to any extent. PEG is a preferred promoter that can be added to a carrier in contact with the mucosa providing a strong adhesive behavior. The main unanswered questions as to the use of such promoters in mucoadhesion are:

(i) development of PEG-promoted hydrogel carriers (PEG loaded in crosslinked network) with associated molecular analysis of PEG chain penetration into the mucosa;
(ii) development of PEG-tethered hydrogel carriers (PEG grafted on networks) and study of graft penetration into the mucosa;
(iii) use of ATR-FTIR to study PEG/mucin interactions;
(iv) comparison of tensiometric/fracture data with spectroscopic studies in vitro; and
(v) development of actual drug delivery devices using these principles.

Recently, we have developed a very promising class of carriers for drug and especially protein delivery. Copolymer networks of poly(methacrylic acid) grafted with poly(ethylene glycol) (henceforth designated as P(MAA-g-EG)) exhibit reversible, pH-dependent swelling behavior due to the formation of interpolymer complexes between protonated pendant acid groups and the etheric groups on the graft chains. In acidic media, the complexes form due to protonation of the pendant groups; thus, the gel is unable to imbibe much fluid. However, in neutral or basic media, the complexes dissociate due to ionization of the pendant groups, and the gels swell to a high degree. These gels have been found to respond rapidly to changes in the external pH and ionic strength. Due to complexation, these networks collapse at rates up to eight times faster than the corresponding rates of swelling. Additionally, solute permeation is severely hindered in complexed networks. Fig. 1 shows the general behavior of these gels, and indicate that the mesh size, $\xi$, expands and contracts due to hydrogen bonding complexation.

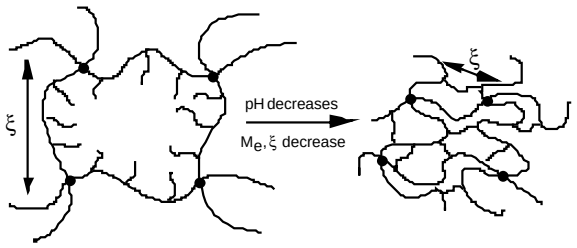


Fig. 1. The effect of complexation on the mesh size, $\xi$, and the effective molecular weight between crosslinks, $M_e$, in graft copolymer networks with permanent, chemical crosslinks (●).

Here we intend to show that in such systems the free PEG chains act as mucoadhesive anchors and that these carriers are promising systems for protein delivery.

## 2. Experimental

### 2.1. Synthesis

P(MAA-g-EG) hydrogels were prepared by free radical solution polymerization of methacrylic acid (MAA, Aldrich Chemical Co., Milwaukee, WI) and methoxy-terminated poly(ethylene glycol) monomethacrylate (PEGMA, Polysciences Inc., Warrington, PA) with PEG of molecular weight 200, 400, and 1000. The MAA was vacuum distilled at 54 °C/25 mmHg to remove the inhibitor, methoxyethyl hydroquinone. PEGMA was used as received. The monomers were mixed in ratios ranging from 1:1 to 4:1 MAA/EG repeating units. The solutions were diluted to 50 % by weight of the total monomers with a 1:1 by weight mixture of ethanol and water.

Tetraethylene glycol dimethacrylate (TEGDMA, Polysciences Inc., Warrington, PA) was added as the crosslinking agent in the amount of 0.75% moles of total monomers. Nitrogen was bubbled through the well mixed solution for 30 min to remove dissolved oxygen. The redox initiator pair, sodium metabisulfite and ammonium persulfate (Mallinckrodt Chemical Inc., Paris, KY), was added in the amount of 2% total monomer in a nitrogen atmosphere. The mixture was poured between flat plates to form films of 0.9 mm thickness. The monomer films were sealed under

nitrogen and allowed to react for 24 h at 37 °C. The polymers were then cut into the desired shapes and rinsed in deionized water for 7 days to remove unreacted monomer and the sol fraction. For the swelling experiments, polymer disks were cut so that the aspect ratio, the ratio of the gel radius to gel half-thickness, was greater than 10.

### 2.2. Characteristics

The gels were characterized by equilibrium and dynamic swelling studies in buffers of pH from 3.2 to 7.6 at constant ionic strength 0.1 M at 37 °C. The mesh size, $\xi$, was calculated by well-known techniques.

### 2.3. Mucoadhesive behavior

The gels tested were placed in a tensile tester at 25 °C and 90% RH. The samples were adhered to the upper holder of the tester, whereas a sample of gelled bovine submaxillary mucin (Sigma, St. Louis, MO) was placed on the lower jaws. The two jaws were brought together for 15 min and then separated at 1 mm/min. The detachment force was measured as a function of displacement. The work of fracture, equivalent to the work of bioadhesion, was calculated as the area under the curve.

### 2.4. Protein delivery

Protein incorporation was performed in a phosphate buffer solution of pH 7.4 by imbibition. Protein delivery studies were performed using the USP method in vitro at 37 °C. Protein concentration was determined by HPLC.

## 3. Results and discussion

Complexing hydrogels have the ability to respond to changes in their external environment. Macroscopic changes in the network structure can be attributed to changes in the polymer correlation length, $\xi$, or in the end-to-end distance of the polymer chains between junction points. The distinction is made here between junction points and classical covalently crosslinked structures, because in complexing hydrogels the hydrogen bonding forms regions of quasi-permanent nature which act as additional physical crosslinks or junctions. In our complexing P(MAA-g-EG) hydrogels, complexation resulted in the formation of temporary physical crosslinks due to hydrogen bonding between the PEG grafts and the PMAA pendant groups. These physical crosslinks were reversible in nature and dependent on the pH of the environment. Thus, the degree of crosslinking and the effective molecular weight between crosslinks of these systems, $M_e$, decreased due to interpolymer complexation in these gels. Additionally, the correlation length or mesh size, $\xi$, was reduced dramatically as more temporary, physical crosslinks were introduced into the system as a result of complex formation.

Equilibrium swelling studies elucidated the structure of the gels prepared. Materials which are highly complexed are unable to imbibe as much water as uncomplexed materials. The macroscopic swelling behavior of the copolymer networks was analyzed to investigate the critical parameters for complexation. The effects of parameters such as copolymer composition, PEG graft chain molecular weight and solvent pH on the conformational states of the networks were investigated (Lowman et al., 1999).

The effect of copolymer composition on the swelling of P(MAA-g-EG) hydrogels is shown in Fig. 2. In regions of low pH, lower than pH 4.6, all of the P(MAA-g-EG) hydrogels contained some degree

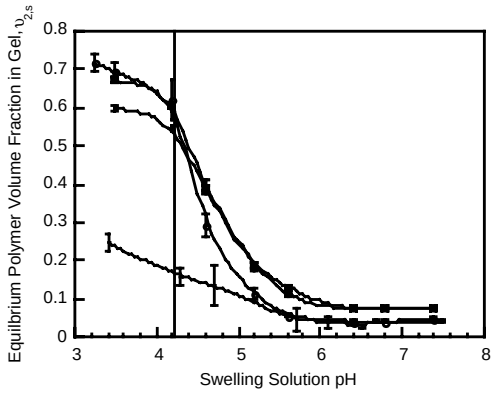


Fig. 2. Equilibrium polymer volume fraction in P(MAA-g-EG) hydrogels swollen in buffer solutions (at constant ionic strength, $I = 0.1$ M) at 37 °C plotted as a function of the swelling solution pH for gels with EG/MAA ratio of 1 containing PEG grafts chains of varying molecular weight (1000 (○), 400 (□), and 200 (△)). The value for pure PMAA is denoted by (▽).

of interpolymer complexes stabilized by hydrogen between protonated pendant acid groups and the ether groups of the PEG graft chains. Gels containing equimolar amounts of MAA/EG exhibited the lowest degree of swelling at low pH increased complexation. As the amount of MAA in the gels was increased, the gels swelled to a higher degree due to the presence of fewer interpolymer complexes. For solutions of pH greater than 4.6, all of the gels swelled to a higher degree due to dissociation of the complexes as the pendant groups ionized.

The equilibrium swelling of P(MAA-g-EG) was dependent also on the PEG graft chain molecular weight. At low pH (less than pH 4.6), all of the PEG-containing gels were in the complexed state. However, the degree of swelling was lowest in gels containing the longer PEG grafts (molecular weight 1000). Because of the longer graft chains, complexation was enhanced resulting in a lower degree of swelling in these gels. In solutions of pH greater than 4.6, the swelling of all of the gels increased significantly due to complex dissociation. Gels containing the longer PEG grafts (molecular weight 1000) swelled to the highest degree in the uncomplexed state. For these materials, the gel contained over 95% water in the highly swollen state.

The average network mesh size or correlation length was dramatically affected by the pH of the swelling solution (Fig. 3). In low pH solutions in which complexation will occur, the network mesh sizes for P(MAA-g-EG) hydrogels were low as 70 Å. However, as the pH was increased the physical crosslinks dissociated and the polymer chains elongated resulting in a significant increase in the network mesh size to almost 210 Å.

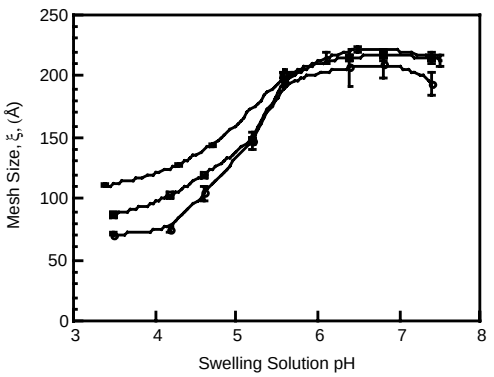


Fig. 3. Network mesh size in P(MAA-g-EG) hydrogels swollen in buffer solutions (at constant ionic strength, $I = 0.1$ M) at 37 °C plotted as a function of the swelling solution pH for gels with EG/MAA ratio of 1 containing PEG grafts chains of varying molecular weight (1000 (○) and 200 (□)). The data for PMAA hydrogels are represented by (△).

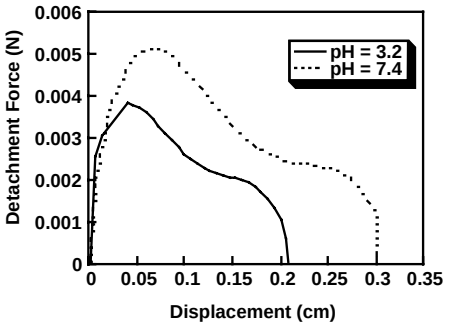


Fig. 4. Adhesive behavior of P(MAA-g-EG) gels at pH values of 3.2 and 7.4 in contact with bovine submaxillary gland mucin.

Fig. 4 shows the mucoadhesive behavior of these systems. The detachment force was plotted versus displacement for mucin adhesion of pH values of 3.2 and 7.4. The work of adhesion was significantly higher at the pH value of 7.4 indicating that the free PEG chains (Fig. 1) act as anchors for the mucoadhesion observed at those conditions.

Various delivery studies have been performed with a wide range of drugs and proteins. Fig. 5 shows the in vitro release of insulin from P(MAA-g-EG) gels containing PEG grafts of molecular weight 1000. Clearly

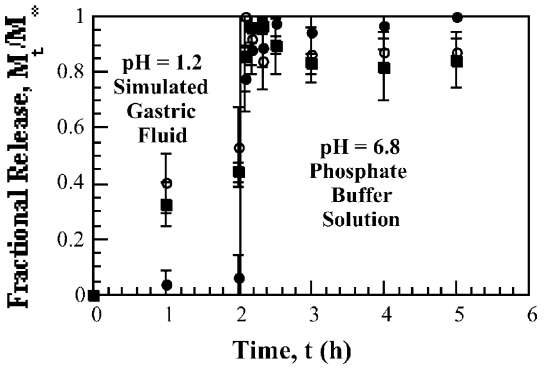


Fig. 5. Pulsatile release of insulin in vitro from P(MAA-g-EG) gels containing PEG grafts of MW 1000 and a MAA/EG molar ratio of (●) 1:1 and (■) 4:1 at 37 °C. The data for pure PMAA are represented by (○).

these systems indicate a significant release of insulin as the gel decomplexes and insulin is freed through the structure. The results of additional in vitro studies have shown that insulin release rates can be controlled by appropriate adjustment of the structure of the gels. In vivo results have been presented recently (Lowman et al., 1999; Morishita et al., 2002) showing that insulin-loaded gels induced sustained hypoglycemic effects, and their effects were dose related.

## Acknowledgements

This work was supported in part by a grant from the National Institutes of Health (no. EB-00246-11).

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