11 Polymeric Systems for Oral Protein and Peptide Delivery

Richard A. Gemeinhart, Ph.D.

CONTENTS

INTRODUCTION

In recent years many peptide and protein drugs have been identified for use in humans (Table 11.1). With rapid progress in human genome analysis and as the structures of more proteins are deciphered, better mimetic peptides will be designed that have higher activity, better stability, and improved physical properties when compared to the original protein. Many biomacromolecules and their synthetic analogs are very potent. Correspondingly, the therapeutic index for the protein or peptide may be quite narrow. Since most biomacromolecules have short *in vivo* half-lives, repeated injections would be necessary for the drug to be continually active.

TABLE 11.1
Protein and Peptide Drugs Currently in Use or Being Investigated that Could Be Delivered via the Oral Route

Protein or Peptide	Disease
Insulin	Diabetes
Calcitonin	Osteoporosis
Erythropoietin	Anemia
Interleukin-2	Renal carcinoma
Interferon	Multiple sclerosis
Hepatitis B subunit	Vaccine for hepatitis B
Human growth hormone	Growth disorders
Tissue plasminogen activator	Heart attack
β-Cerebrosidase	Enzyme disorder
Deoxyribonuclease	Cystic fibrosis

New biomacromolecules are not the only recent development. Production methods for biomacromolecules have become more reasonable. Modern cell culture methods allow the production of gram quantities of proteins from bacterial, yeast, or mammalian cells (Hauser and Wagner 1997). Efficient methods are now available to recover the proteins from their producing cells (Seetharam and Sharma 1991). The breakthroughs in protein production allow for more expensive methods to be investigated for the delivery of the protein; as the price of the protein drops, the price of the delivery device can go up while the cost of the final dose and profit for the company remains the same. The combination of new active proteins and the lower cost of the proteins has promoted increased research in the area of protein delivery.

Most biomacromolecules are delivered by injection, but many patients will not submit to the repeated injections that are needed to maintain sufficient levels of protein in the bloodstream. Because of this, alternative methods to deliver biomacromolecules are desired. Pulmonary delivery is promising for certain agents (see Chapter 10), however, many patients are not willing to use inhalation therapy and tend to reduce their dosage because they are not happy using this type of dosing. Transdermal delivery is also possible for delivery of some proteins, but usually not without the use of dermal abrasion or iontophoretic mechanisms. Since the oral route of delivery is the most accepted by patients, many investigators have focused on identifying possible methods for oral peptide delivery. New methods for the delivery of biomacromolecules must be economically feasible, as healthcare costs are currently such a concern. Many of the oral protein and peptide delivery systems to date use polymers.

Polymers are macromolecules composed of specific repeating units. The properties of the polymer, such as viscosity of a solution, elasticity, and solid strength, are determined by the number of repeating units, or monomers, and ultimately the radius of the polymer. The properties of a polymer can be predicted based upon

theoretical calculations (Flory 1953). The diversity and predictability of properties are the reason that polymers are so useful in controlled drug delivery. Careful choice of the polymer leads to dosage forms that can deliver an active agent with reproducibility and accuracy. In this chapter, polymers useful in delivery of biomacromolecules are described. The delivery mechanism of the dosage forms containing this type of polymer is explained and future usefulness of the polymer is discussed.

POLYMERS USED FOR CONTROLLED DRUG DELIVERY

The classification of polymers for oral drug delivery can be done by using various means. To make this discipline readily accessible to the novice reader, the hydrophobic-hydrophilic nature of the polymer was chosen to group polymers since the mechanism of biomacromolecule release from most hydrophobic polymeric devices is similar; the mechanism of release from most hydrophilic polymeric devices also have similar mechanisms. Hydrophobic polymers are described first, followed by hydrophilic polymers.

Hydrophobic Polymers

Hydrophobic polymers are often used to deliver biomacromolecules regardless of the route of administration. The rapid transit time is approximately 8 hours, which limits the time of a device in the gastrointestinal (GI) system, consequently the mechanisms possible for oral drug release are limited. The predominant method of release from hydrophobic polymers has been degradation, or biodegradation, of a polymeric matrix by hydrolysis (Figure 11.1). In fact, all of the hydrophobic polymers described in this chapter for use as oral protein or peptide delivery are hydrolytically unstable. The hydrophobic polymers are described in the following sections beginning with the most frequently described hydrophobic polymers used for oral

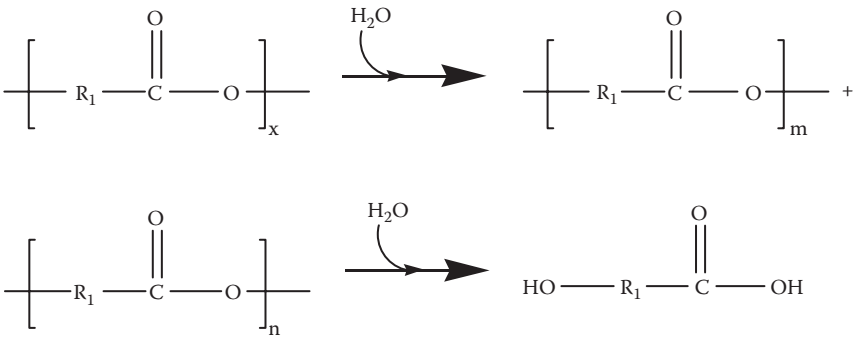


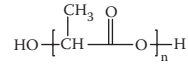
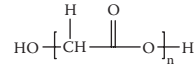
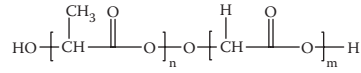
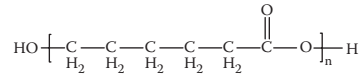
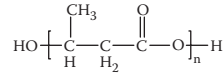
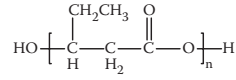
FIGURE 11.1 Chemical scheme for degradation of poly(esters). Water reacts with the hydrolytic unstable ester bonds and finally produces monomers or short oligomers of the monomer.

protein and peptide delivery. Some of these polymers have been used for oral peptide and protein delivery, while others have not. Those polymers that have not been used to date for protein or peptide delivery have the potential for future use in devices for oral peptide and protein delivery and should not be overlooked. Each has been used *in vitro* or in animal studies that suggest that the polymer could be used for oral protein or peptide delivery.

Poly(esters)

Poly(esters) (Table 11.2) are the first class of polymers discussed, as they are the most widely investigated of all of the polymer families for oral protein delivery. Poly(esters) used for oral drug delivery have primarily been biodegradable polymers (Figure 11.1). Biodegradation is the primary delivery mechanism for poly(ester) polymers used for protein and peptide delivery. The degradation properties of poly(esters) are dependent on the monomers used to produce the poly(ester). Several poly(esters) are discussed in detail in the following sections.

TABLE 11.2
Structures of Poly(esters) Used in Oral Protein and Peptide Delivery

Polymer	Structure
Poly(lactic acid)	
Poly(glycolic acid)	
Poly(lactic acid-*co*-glycolic acid)	
Poly(caprolactone)	
Poly(β-hydroxybutyric acid)	
Poly(β-hydroxyvaleric acid)	

Poly(lactic acid-co-glycolic acid)

The most investigated poly(esters) for oral peptide delivery have been poly(lactic acid-co-glycolic acid) (PLGA) copolymers (Spenlehauer et al. 1989; Wang et al. 1990; Odonnell and McGinity 1997). In actuality, PLGA itself is a group of copolymers with distinctly different properties depending on the composition. PLGA is a combination of D-lactic acid, L-lactic acid, and glycolic acid. Typically, PLGA polymers are referred to by the percent lactic acid to glycolic acid content that is present. A PLGA with 25% D-lactic acid, 25% L-lactic acid, and 50% glycolic acid would be described as 50:50 poly(D,L-lactic acid-*co*-glycolic acid) or 50:50 poly(D,L-lactide-*co*-glycolide). The physical and chemical properties of PLGA vary greatly with poly(glycolic acid) being poorly soluble in most solvents and poly(D,L-lactic acid) being soluble in many organic solvents.

The molecular weight and polydispersity of the polymer is an important factor in determining the properties of PLGAs. The starting molecular weight is important because as the PLGA is degraded, the delivery rate changes with a resultant decrease in molecular weight. When the molecular weight of the polymers is sufficiently low, the remaining chains become soluble in water. These small molecular weight oligomers and lactic and glycolic acid monomers have a placticizing effect on the higher molecular weight portions of the device (Grizzi et al. 1995). The increased water content of the polymeric device contributes to the degradation process that is observed with poly(esters) and may even account for the diffusion-like delivery of some hydrophilic molecules (Figure 11.2A). The bulk degradation properties of PLGA have frequently been cited as a disadvantage. In bulk degradation of the matrix, the drug molecule may diffuse from the interior and exterior of the matrix; however, in surface erosion, which will be discussed in detail later, the drug will only be released from the surface of the matrix as it dissolves or degrades (Figure 11.2B).

PLGAs are one of the most biocompatible polymers currently used. This is due to the fact that the degradation products, lactic acid and glycolic acid, are produced naturally in the body. PLGA devices have been shown since the late 1960s to be an acceptable material for implantation and have been utilized since that time as surgical sutures (Cutright et al. 1971; Frazza and Schmitt 1971). Inflammatory responses occur with these materials due to causes directly related to the degradation mechanism. As the polymer degrades, an acidic environment is produced surrounding and within the polymer matrix (Fu et al. 2000). As the backbone of the polymer is hydrolyzed, more carboxylic acid groups are produced in the matrix, decreasing the pH of the surrounding fluid. It has been proposed that basic salts can be used to control the local pH of the microparticles (Agrawal and Athanasiou 1997). Control over the internal pH of the microparticles may produce a better system for delivery of peptides as the decreased pH of the current microparticles may contribute to low activity of delivered peptide. Despite the decrease in pH, many biomacromolecules can be delivered using PLGAs in the delivery device.

Due to the short transit time of materials taken orally, large PLGA matrices cannot deliver their entire contents prior to being eliminated. For this reason, microparticles have been the dosage form of choice for not only PLGAs, but also the majority of the hydrophobic polymer based systems. Microparticles of PLGA deliver their contents over a period from hours to years, depending on their composition

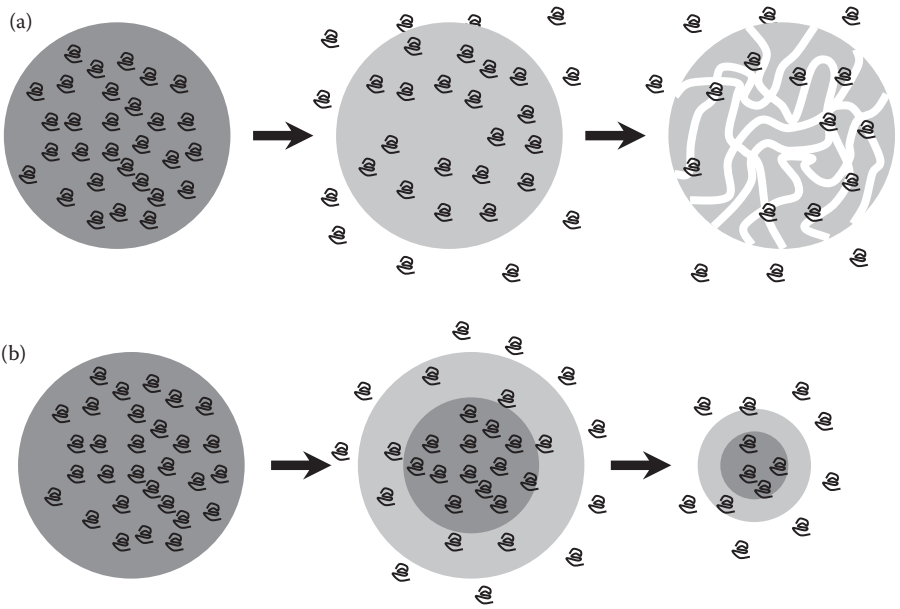


FIGURE 11.2 Degradation of polymer matrices by (A) bulk degradation or (B) surface erosion. In bulk degradation, the degradation occurs as fluid enters the entire matrix and the encorporated molecule can diffuse from the degrading matrix. During surface erosion, only the biomacromolecules in the degrading layer are released; therefore, there is drug being released as long as the matrix is present in the body.

and site of deposition, but there is a typically large burst of delivery that will occur in the time that the microparticles are retained in the gastrointestinal tract. The time course of delivery is not always a problem, however, as some microparticles are actually absorbed by the gastrointestinal border cells (Figure 11.3). Those particles that are absorbed deliver their contents while the particles degrade in the body. This is typically in the lymphatic tissue of the intestine. To increase the uptake of microparticles by the M-cells in the Peyer's patches of the intestine, nonspecific bioadhesives and cell-specific molecules are being placed on the surface of the PLGA microparticle (Gabor and Wirth 2003). Uptake of microparticles is quite low, so a large excess of microparticles is needed to achieve sufficient drug transport. Bioadhesive and cell-specific molecules increase the contact time between the cells and microparticles, thus increasing the cellular uptake of the particles.

The water-in-oil-in-water (w/o/w) emulsion method (Figure 11.4) is the predominant method used for encapsulation of biomacromolecules in these microparticles. Protein solution forms the internal water phase of the w/o/w emulsion. Loading efficiency of the microparticles has not been optimal using water or buffer as an internal phase, so water is sometimes substituted with polymeric liquids, such as low molecular weight polyethylene glycol. The primary emulsion is then added to a secondary liquid phase, forming the secondary emulsion. The solvent for the

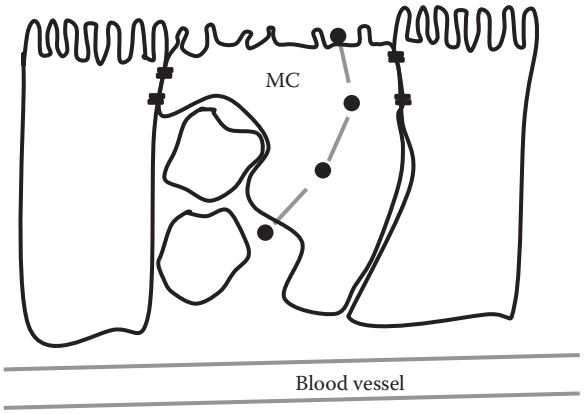


FIGURE 11.3 Structure of the intestinal wall including an M cell (MC). M cells are part of the immune system and have been shown to be phagocytotic, engulfing particles and imparting an immune response.

polymer, usually methylene chloride, chloroform, or other organic solvent evaporates leaving hardened microparticles filled with water and protein. The evaporation step is one of the most important steps for control of the size and morphology of the microparticles, as heat and agitation have profound effects (Jeyanthi et al. 1997; Capan et al. 1999). Despite numerous attempts, the loading efficiency of the poly(ester) microparticles is quite low. Sufficient loadings are possible for low dose drugs, but large amounts of the peptide are needed to get the high loadings necessary. Due to this, much research is still needed in the formulation of poly(ester) microparticles, and other methods may be shown to be better despite the acceptability of these systems (Kompella and Koushik 2001).

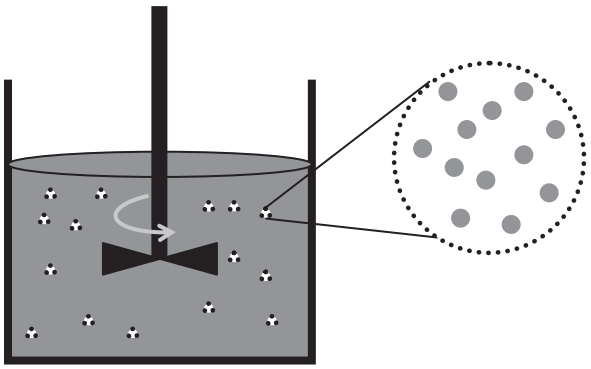


FIGURE 11.4 Schematic representation of a water-in-oil-in-water emulsion. The *gray* represents the water phase, where the protein or poly(nucleic acid) would be present. The *black dots* surrounding the white oil phase represents a surfactant, typically poly(vinyl alcohol) or serum albumin.

The final obstacle for delivery of biomacromolecules from PLGA microparticles is sterility. Oral delivery of microparticles allows for more bacterial contamination than parenteral or pulmonary dosage forms as the gastrointestinal system is well equipped to control bacterial outgrowth. However, if the particles traverse the intestinal lining and begin circulating, bacterial incorporation will be problematic. To combat bacterial incorporation in the microparticles, one must work under sterile conditions, but the PLGAs may bring bacteria that cannot be removed. Particles sterilized by γ-irradiation after production contain protein with decreased activity and polymers of lower molecular weight than in unirradiated particles. This has caused an increase in delivery rate based upon polymer degradation over the initial days following irradiation with a combined decrease in active protein (Shameem et al. 1999).

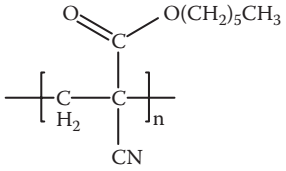
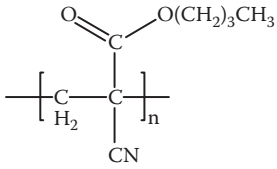
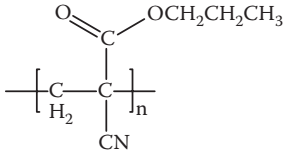
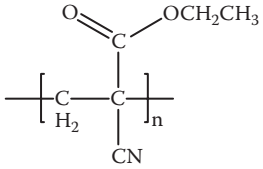
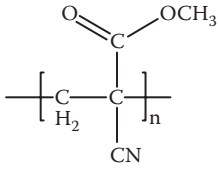
Other Poly(esters)

Poly(ε-caprolactone) (PCL) is another of the degradable poly(esters) that has been utilized to deliver biomacromolecules orally. The polymer is more hydrophobic than any of the PLGAs, and can be used by itself or as a copolymer with lactic acid, glycolic acid, or any hydroxy acid (Pitt 1990). Due to its higher hydrophobicity, the degradation rate of the polymer is somewhat slower than PLGA; PLGAs are typically the fastest degrading of the poly(esters) as hydrophobicity is directly related to degradation rate. The uptake of particles that are formed with PCLs was also proposed to be higher than that of PLGAs (Eldridge et al. 1990). Other poly(esters) (Table 11.2) have been used for peptide incorporation in the form of microparticles. The delivery from these materials is long term so they can be used to decrease dosing upon injection or implantation (Engelberg and Kohn 1991); however, few have been successfully used in oral delivery of biomacromolecules. All of the poly(esters) are excellent candidates for the delivery of biomacromolecules in the form of microparticles based on the ability of the polymeric particles to protect the proteins and peptide from degradation in the digestive tract. The uptake of the particles by the gut-associated lymphoid tissue can increase the amount of protein that reaches the bloodstream. Unfortunately, a sufficient amount of microparticles is not retained for substantial blood levels of most proteins for this method to be used on a regular basis. Regardless of the type of polyester, much research is still needed to improve microparticle preparation for protein incorporation.

Poly(cyanoacrylate)

An alternative hydrophobic microparticulate dosage form can be produced using poly(alkyl cyanoacrylates) also referred to as simply poly(cyanoacrylates) (PCAs) (Table 11.3). Poly(cyanoacrylates) are a class of addition polymers that undergo polymerization under mild conditions, and even upon the addition of water or ethanol. Poly(cyanoacrylates) have been widely investigated for delivery of biomacromolecules. Due to their properties, cyanoacrylates can easily be formed into two types of particles: spheres (Couvreur et al. 1982) or capsules (Al-Khouri Fallouh et al. 1986), both of which can be used to deliver biomacromolecules. The most used of the poly(cyanoacrylates) is poly(isobutyl cyanoacrylate) (PBCA). The reason

TABLE 11.3
Structures of Some Common Poly(cyanoacrylates)

Polymer	Structure
Poly(isohexyl cyanoacrylate)	
Poly(isobutyl cyanoacrylate)	
Poly(isopropylcyanoacrylate)	
Poly(ethyl cyanoacrylate)	
Poly(methyl cyanoacrylate)	

PBCA has been extensively investigated is that it can easily be formed into microparticles with the aid of ethanol as a solubilizer. Other PCAs tend to polymerize in the presence of ethanol, including poly(isohexyl cyanoacrylate) (PIHCA) (Chouinard et al. 1991).

Since the polymerization procedure is mild, there is less activity loss during the incorporation of peptides and proteins into PCA microparticles as compared to

poly(ester) microparticles. Also, there is a greater amount of incorporation in the PCA microparticles than the poly(esters) microparticles. One of the major disadvantages of the PCA over the poly(hydroxy acids) is the toxicity of the monomers (Muller et al. 1988). The degradation products of PCAs are alkyl cyanoacetate and formaldehyde, both of which are more toxic than the lactic acid, glycolic acid, or other hydroxy acids formed from the degradation of poly(esters). The relative toxicity may not be as much of a factor as anticipated since the PCA degradation rate can be controlled to limit the amount of degradation product that is present.

The rate of degradation of PCAs is much faster than PLGAs which do not necessitate the overloading of the body with excess polymer that is still present long after initial treatment (Lherm et al. 1992). The alkyl cyanoacetate that is formed and the rate of formation are dependent on the length of the alkyl chain of the monomer used to produce the microparticles. The degree of toxicity is also dependent on the length of the alkyl chain. Poly(cyanoacrylates) has been approved by the FDA in several systems, so it is one of the more acceptable of the polymeric systems.

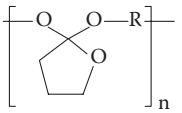
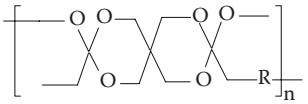
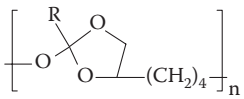
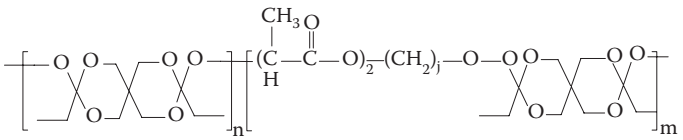
Poly(ortho esters)

Poly(ortho esters) (POEs) were one of the first polymers produced that exhibited delivery predominantly via surface degradation (Figure 11.2B). Four distinct types of POEs have been developed since the early 1970s (Heller et al. 2000) (Table 11.4). The design of each poly(ortho ester) particular family of POE is inherently different, and has specific properties. Type I poly(ortho esters) were developed at Alza Corporation (Mountain View, CA) and are described by a series of patents (Choi and Heller, 1976, 1978a,b, 1979a,b). Upon degradation, type I poly(ortho esters) form the appropriate alkane diol and γ-butyrolactone. The lactone easily hydrolyzes to form γ–hydroxybutyric acid. The acid accelerates the degradation of the polymer unless neutralized with a basic excipient. Polymers of this family are not well described in the literature, so little information can be given for the specific structures of the polymer. No oral delivery devices have been produced with this type of poly(ortho ester). Insulin-like growth factor has been delivered using this type of poly(ortho ester) indicating that loading and release from this class of polymer is possible (Busch et al. 1996).

Type II poly(ortho esters) are very stable due to their hydrophobic nature (Heller 1990). The degradation rate can be controlled using acidic and basic excipients; acidic excipients increase the degradation rates and facilitate a zero-order release rate over a 2-week period (Sparer et al. 1984). Basic additives increase the degradation time of the polymers and create a polymer that degrades specifically at the surface (Heller 1985). By careful choice of the excipient added, the degradation rate can be closely controlled. No experiments have shown the use of these polymers with proteins or peptides. This is not, however, indicative of the fact that these polymers are not compatible with proteins or peptides, but they are probably not the most appropriate polymeric carrier for oral delivery of biomacromolecules.

Type III poly(ortho esters) are very similar to the type I poly(ortho esters) in that they are based on a five member ortho ester ring (Heller et al 1990). The linkages are quite different with the degradation process not forming a lactone, but an acid

TABLE 11.4
Structures of Poly(ortho esters) Used for Drug Delivery

Polymer	Structure
Poly(ortho ester) type I	
Poly(ortho ester) type II	
Poly(ortho ester) type III	
Poly(ortho ester) type IV	

Adapted from Heller et al. 2000.

and a triol. Type III poly(ortho esters) developed to date are more hydrophilic than other poly(ortho esters), and for this reason, they erode quite quickly (Merkli et al. 1996). Some polymers of this type are semisolid at room temperature (Einmahl et al. 1999), which can facilitate their mixing with biomacromolecules. The semisolid nature has been exploited for specific applications; however, no significant effort has been identified for advancing these materials for oral biomacromolecule delivery.

Type IV poly(ortho esters) are very similar in structure to type II poly(ortho esters), but they do not need to have excipients in the formulation due to the incorporation of no acidic moieties in the polymer backbone (Ng et al. 1997). Rods of poly(ortho ester) loaded with recombinant human-growth hormone and bovine serum albumin have been created. The rods are the products of polymer-protein mixture extrusion at a temperature between 50° and 70°C. Particles have also been produced from these rods (Heller et al. 2000). The size of these particles, >106 μm, was much larger than would be expected to be absorbed by the gastrointestinal lining (Florence 1997). If the particle size can be reduced, this type of polymer system may be made to be acceptable for oral administration.

Despite the fact that these polymers show delivery patterns comparable to gastric transit, there have not been any substantial reports of oral delivery of peptides or proteins based on this type of polymer. Further investigations into this polymer may

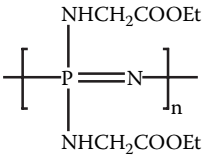
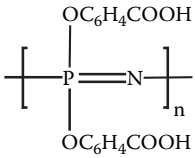
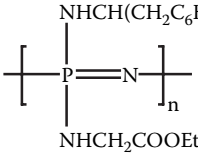
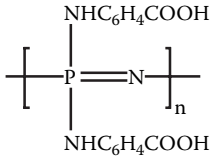
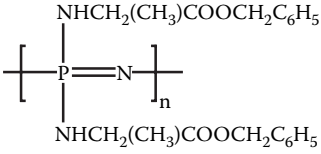
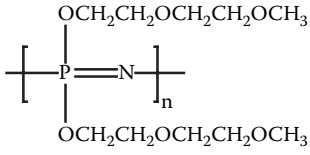
be desirable since the degradation properties of the polymers can be very tightly controlled.

Poly(phosphazenes)

Poly(phosphazenes) are another class of biodegradable polymers that have varying degradation rates (Scopelianos 1994). The rate of degradation of poly(phosphazenes), however, is not dependent on the changes to the backbone of the polymer, but rather to the changes of the pendant group properties. A wide variety of polymers can be formed by the molecular substitution on poly(dichloro phosphazene). In fact, poly(phosphazenes) can be produced as either hydrophobic or hydrophilic polymers depending on the exact nature of the pendant groups (Table 11.5). Almost unlimited possibilities exist concerning the structure and properties. The production of the various poly(phosphazenes) is very simple, an alcohol or 1° or 2° amino terminated molecule can be incorporated onto poly(dichlorophosphazene). The hydrophobic polymers will be examined in this section, and the hydrophilic polymers formed with poly(phosphazenes) will be discussed later.

The degradation of the poly(phosphazenes) is very well understood, with the production of ethanol, phosphate, ammonium salts, and the pendant groups (Andrianov and Payne 1998). When the pendant group is an amino acid, all of the degradation

TABLE 11.5
Structures of Some Example Poly(phosphazenes) Used in Drug Delivery

Hydrophobic	Hydrophilic
	
	
	

products are natural products normally found in the body. The exact properties of these types of polymers are controlled by the selection of the amino acid substituents added (Crommen et al. 1992). Other labile groups have been used to create degradable polymeric systems including ethylamino (Tanigami et al. 1995), imidazolyl, oligopeptide, amino acid esters, and depsipeptide groups. As with other degradable polymers, the more hydrophobic, or bulky, side-groups are more hydrolitically stable (Allcock et al. 1994). The degradation and release related directly to the relative mixture of the multiple components on the backbone. Combinations of the various poly(phosphazenes) also show a direct relation between the addition of faster degrading species and the degradation and delivery pattern of the polymer.

Poly(phosphazene) microparticles have shown the potential for oral protein or peptide delivery (Vandorpe et al. 1996; Veronese et al. 1998; Passi et al. 2000). The processing of these materials is very similar to poly(esters) and the biocompatibility of the polymers is exceptional. After the implantation of matrices of poly(phosphazene), no gross areas of inflammation were observed at explanation (Laurencin et al. 1987). The only negative aspect of these materials is that FDA approval of this polymer class has no precedent.

Hydrophilic Polymers

Hydrophilic polymers are currently undergoing investigation for improving the transport of biomacromolecules across the intestinal walls. Hydrophilic polymers have been shown to protect proteins and peptides from proteolysis. Multiple methods utilize the properties of polymers to protect biomacromolecules without removing them from the aqueous environment of the intestines.

Polymer-Protease Inhibitor Conjugates

Polymers may also inhibit proteolytic enzymes or be used to augment the activity of proteolytic inhibitors (Table 11.6). The administration of protease inhibitors in conjunction with the peptide, however, has been examined with some success (Fujii et al. 1985); however, the ability of these molecules to protect the protein has been hampered by the fact that they tend to cause systemic side effects (Yagi et al. 1980; McCaffrey and Jamieson 1993; Plumpton et al. 1994). Many protease inhibitors have been specific for the proteases in the stomach and intestine, but some of these factors are not specific and some control over absorption of the inhibitors was

TABLE 11.6
Polymers that Act as Protease Inhibitors

Polymer Backbone	Protease
Polyacrylate	Trypsin,chymotrypsin, carboxypepsidase A, elastase
Polymethacrylate	Trypsin,chymotrypsin, carboxypepsidase A, elastase
Chitosan	Trypsin, carboxypepsidase A, aminopepsidase N
Carboxymethylcellulose	Elastase, pepsin

desired. The activity of the inhibitors varied leading to more problems in determining methods for controlling the inhibitory effects.

To alleviate problems with administration of protease inhibitors, it was hypothesized that a polymer-based inhibitor could prevent peptide degradation. The fact that the inhibitor was attached to a polymer would localize the inhibitory affect, minimizing the effect in the intestine and allowing normal digestion (Melmed et al. 1976; Otsuki et al. 1987). This would also prevent the systemic effects of the inhibitors by decreasing the possibility for absorption if poorly absorbed polymers are used. The only problems with conjugating the inhibitors with proteins were possible degradation, loss of activity, and changes in activity. By carefully choosing the polymers for conjugation, it was thought that inhibition could actually be augmented with little loss or change in the activity of the inhibitors.

Various groups have proposed using this mechanism for protection of peptides with protease inhibitor-polymer conjugates within the digestive tract (Bernkop-Schnürch 1998). Some synthetic polymers have been proposed to be advantageous since they possess mucoadhesive properties (Lueßen et al 1996), but some natural polymers also have mucoadhesive properties and have also been used (Bernkop-Schnürch and Apprich 1997). Poly(acrylates) have been well described as mucoadhesive polymers because they have hydrogen bonding and chain entanglement with the mucin of the stomach and intestine (Park and Robinson 1987). The mucoadhesive properties of the polymer would be beneficial in that the dosage form would localize at the surface of the intestinal walls. This would have multiple benefits, including minimizing the diffusional distance that the protein or peptide prior to absorption and increase the residence time of the dosage form in the gastrointestinal tract.

The carboxylic acid moieties of the poly(acrylate) are also involved in the inhibition of proteolytic enzymes. Divalent cations are necessary for many proteases to act, specifically Ca^{2+} and Zn^{2+}. Whether these polymers can be effective *in vivo* at protecting peptides has been of some concern (Bernkop-Schnürch and Göckel 1997), but the poly(acrylates) do exert an inhibitory effect on many of the proteases present in the gastrointestinal tract (Lueßen et al. 1996; Madsen and Peppas 1999). Polycarbophil, Carbopol® (Noveon, Cleveland, OH), and synthetic poly(methacrylic acid-*g*-ethylene glycol) hydrogels have been shown to decrease the activity of trypsin by simply adding the polymer to a solution of the enzyme and a substrate (Figure 11.5). Poly(acrylates) have also been shown to increase the permeability of the intestine (Borchard et al. 1996). Since these polymers, e.g., carbophil, have received GRAS (generally regarded as safe) status, the use of these polymers as inhibitor conjugates should be examined closely.

Bowman-Birk inhibitor (BBI), a peptide analog from soybean, was conjugated to poly(acrylic acid) using carbodiimide chemistry as were chymostatin, bacitracin, and elastinal (Bernkop-Schnürch and Göckel 1997). Of these, bacitracin has been the only conjugate that did not possess any bioadhesive properties, while all of the conjugates showed decreased protease activity. The use of the polymer-inhibitor conjugates is not the only mechanism that has been used for oral biomacromolecule delivery. A selection of hydrophilic polymers that have potential for oral biomacromolecule delivery are described below while occasionally revisiting the idea of protease inhibition.

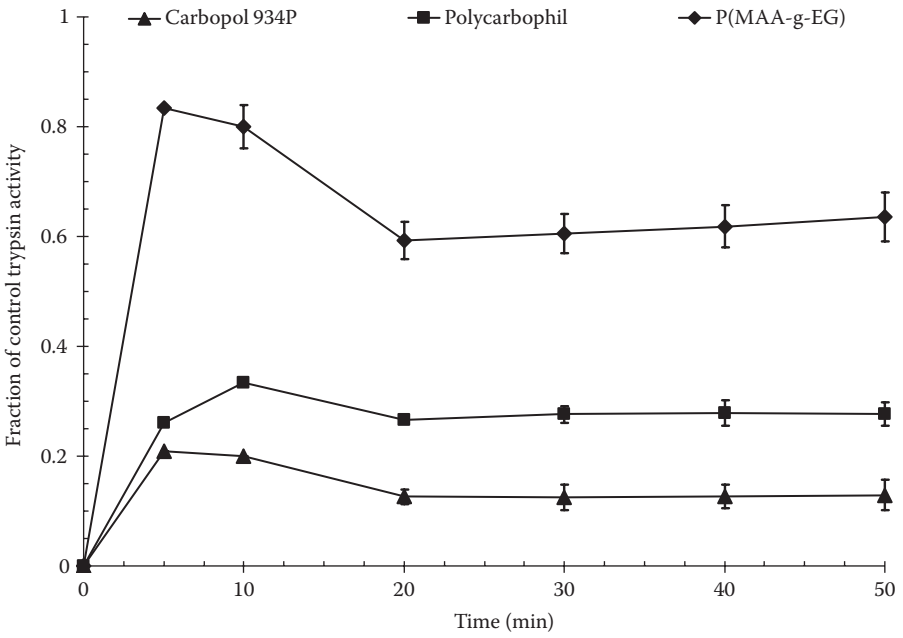


FIGURE 11.5 Degradation of N-a-benzoyl-l-arginine ethyl ester by trypsin. All three carboxyl containing polymers have anti-trypsin activity, but the activity of trypsin in the presence poly(ethylene glycol) modified poly(methacrylic acid) is not greatly reduced. (Adapted from Madsen and Peppas 1999.)

Poly(alkyl methacrylates)

Eudragit® (Röhm/Pharma Polymers, Darmstadt, Germany) is a class of polymer that will dissolve at specific pH values. Eudragit polymers are members of the poly(alkyl methacrylate) family of polymers. This family of polymers has a wide degree of properties due to the many modifications used in the formation of the polymers. The dissolution properties are based on the modification of the carboxylic moieties of methacrylic acid. The degree of modification and the specific modification determine at what pH and how quickly the polymers dissolve.

Microparticles prepared using Eudragit polymers were able to deliver protein in the intestine based upon the solubility properties of the Eudragit used (Morishita et al. 1991). Entrapment efficiency in the microparticles has been as high as 78% for the emulsion method used. These particles delivered their contents in the intestine due to the low solubility of both Eudragit polymers used in acidic conditions; Eudragit S100 and L100 are soluble at pH values above 7.0 and 6.0, respectively. The *in vivo* activity of insulin delivered by oral force feeding rats, showed decreased levels of glucose for several hours following the administration. These levels were reported as a percent decrease, but it is still clear that active insulin was being absorbed into the bloodstream of the animals.

The delivery of proteins from non-crosslinked microparticles has been hampered by the fact that the amount of protein that crosses the gastrointestinal lining is not augmented. Many of these systems only allow the material to be delivered in the vicinity of the intestinal wall. If particular proteins can easily traverse the GI lining, it will have increased *in vivo* activity.

Poly(methacrylates) and Poly(acrylates)

Chemical crosslinks have been added to materials similar in nature to the Eudragit polymers to further control the delivery of biomacromolecules. Most of these systems would fall under the category of hydrogel particles. Hydrogels are polymers that are physically or chemically crosslinked and are unable to dissolve in water. Hydrogels retain their shape upon swelling and usually swell in an isotropic manner (Peppas 1986). The rate of delivery of the protein or peptide has been shown to be dependent upon the swollen state of the hydrogel, the size of the diffusing peptide, and the mesh size of the hydrogel (Lustig and Peppas 1987). A general equation describing the diffusion coefficient of a solute as a factor of the swollen state of the hydrogel indicates that the more swollen the hydrogel, the faster the molecule will be delivered. The diffusion coefficient of the solute, D, can be related to the mesh size of the hydrogel, ξ, the molecular radius of the protein, r, a constant that typically can be assumed to be 1 for most systems, Y, and the swollen ratio of the hydrogel, Q. For insulin in a poly(diethylaminoethyl methacrylate-*g*-ethylene glycol) hydrogel microparticle, the swollen diffusion coefficient was found to be 21 times that of the collapsed diffusion coefficient. This would correlate with a significant increase in diffusional drug delivery for insulin in the swollen hydrogel particle (Podual et al. 2000).

$$D \cong \left(1 - \frac{r}{\xi}\right) e^{[-Y/(Q-1)]} \tag{1}$$

These hydrogels contain a large number of ionic pendant groups leading to increased electrostatic repulsion on the chains when they are in the ionized form. Since the pKa of poly(acrylic acid) is approximately 4.5, the hydrogels swell the most when they are placed in media that has a pH greater than 5.5. Poly(ethylene oxide) chains have been grafted to the hydrogel to increase the sensitivity of the hydrogels to their ionic environment. Whereas poly(diethylaminoethyl methacrylate) has a pKa of approximately 7, hydrogels of this polymer are highly swollen in media with a pH lower than 6.0. Since there is such variability between the pH of the stomach and intestine in humans, these polymers can be used as a delivery device dependent on the delivery site. Many other pH sensitive polymers have been examined to be used in drug delivery devices, but the majority that are currently used are in the form of either poly(acrylic acid) or poly(methacrylic acid).

The main reason that poly(acrylic acid) and poly(methacrylic acid) hydrogels and hydrogel particles have been utilized in oral drug delivery is the fact that they also possess bioadhesive properties (Park and Robinson 1987). Bioadhesive polymers

adhere to biologic matter, specifically the walls of the gastrointestinal tract. Bioadhesion may be specific or nonspecific in nature depending upon the mechanism of adhesion to the gastrointestinal tract (Ponchel and Irache 1998). Nonspecific interactions that may play a role in bioadhesion are van der Waals, hydrogen bonding, and ionic interactions. Mucin in the gastrointestinal tract is comprised of high molecular weight glycoproteins that vary in thickness throughout the gastrointestinal tract (Allen et al. 1982). Nonspecific bioadhesion has been shown to increase the gastrointestinal transit time; however, the increase is not great due to turnover of the mucin layer of the GI tract every 2 hours (Lehr et al. 1991).

Another type of hydrogel based on methacrylic acid that has been used in oral delivery of proteins is a hydrogel system that is degradable at a specific point in the intestinal tract. Since there are flora in the colon that are significantly different than that of the small intestine, polymers have been designed to be degraded by the enzymes only produced in this region of the intestine. One type of enzyme specific to the colon is azoreductase (Saffran et al. 1986). The azo-bonds of a specially designed crosslinker are degraded specifically in the colon (Brondsted and Kopecek 1992). Proteins can be delivered specifically to the colon using these polymers and this has been shown to be effective when penetration enhancers are added to hydrogel disks (Yeh et al. 1995). Penetration enhancers are needed because the absorption capacity of the colon is significantly lower than that of the small intestine. The fact that the protein and polymer would be delivered in the colon decreases the chances for proteolytic degradation. Colon specific delivery of biomacromolecules may be the preferred route due to this decreased degradation, but the decreased absorptive capacity of the colon may limit the delivery by this route.

Alginates

Physically crosslinked systems are somewhat similar to chemically crosslinked systems with the major difference being the type of crosslinking that actually takes place. The major polymer that is crosslinked using a physical crosslinking mechanism is alginate. Alginate is a naturally occurring polymer derived from seaweed. The benefit of the physical crosslinking systems as opposed to chemical crosslinking is the mild conditions needed to form a microparticle. Alginate is present as single polymer chains when in the presence of monovalent cations, such as Na^+ or K^+. However, when in the presence of divalent cations, i.e., Ca^{2+}, Zn^{2+}, and Mg^{2+}, the polymers associate into an ordered structure that is solid (Rees and Welsh 1977). Particles of alginate can be formed by simply adding a solution of alginate polymer containing the biomacromolecule to a solution of divalent cations. The exact form of the particles produced depends on the conditions used.

Large particles can be produced by simply injecting viscous solutions of sodium alginate from a large diameter needle (Badwan et al. 1985). It was found that anything above 5% (w/v) alginate was too viscous to prepare. Poly(L-lysine) (Dupuy et al. 1994) and chitosan (Takahashi et al. 1990) have been shown to increase the aggregation of alginates by forming a complex with the alginate, thereby strengthening the beads. Microparticles have been produced by several methods, but three

methods have predominated: atomization (Matsumoto et al. 1986), emulsification (Wan et al. 1992), and coacervation (Arneodo et al. 1987). Each of these methods for the production of alginate microparticles has its advantages and disadvantages according to the specific proteins or peptides being delivered. Since the emulsification method used harsher chemicals, it is thought to be the least useful for most biomacromolecules.

Alginates are well accepted and are generally accepted as safe by the Food and Drug Administration. Because they are safe and produce particles with high encapsulation efficiency, alginates have been well studied for drug delivery. Unfortunately, alginates do not possess the mucoadhesive properties of the acrylic polymers and hydrogels. It should be possible to modify the alginate polymers to impart an adhesive ability onto the chain so that the high encapsulation efficiency and mild encapsulation conditions can be combined with a mucoadhesive system.

Chitosan

Chitosan is a polymer produced from hydrolysis of natural chitin. Chitosan is not readily soluble in aqueous solutions, but can be solubilized and is thus considered with other water soluble polymers. In the hydrophobic form, chitosan has been treated in a similar manner to other hydrophobic polymers with microparticles produced by emulsion and phase separation techniques. Microparticles can be taken up by the gastrointestinal lining in a manner similar to that discussed for other hydrophobic microparticles.

The biggest difference between chitosan and other polymers is that it has both chelating and bioadhesive properties. Chitosan has been conjugated to antipain, chymostatin, elastatinal, ethylene diamine triacetate (EDTA), and combinations thereof (Bernkop-Schnürch and Scerbe-Saiko 1998). Cation binding inhibits proteins without the addition of proteolysis inhibitors in a similar manner to poly(acrylates), but the inhibitory effect of chitosan-EDTA toward Ca^{2+}-dependent proteases was not always found to be significant (Bernkop-Schnürch and Krajicek, 1998). These polymers can be incorporated into conventional tablets that will slowly dissolve and deliver the protein. When chitosan/EDTA/BBI conjugate was included at only 10% (w/w) of a formulation, more than half of the insulin activity can be maintained when administered orally compared to less than 10% insulin activity when no conjugate is added to a tablet. The inner portion of the tablet contained somewhat more active insulin, but even on the outer surface of the tablet, most of the activity still remained.

Polymers that are protease inhibitors and polymer-inhibitor conjugates are now widely investigated for their ability to protect proteins and peptides from proteolytic degradation. These molecules are effective in the immediate area surrounding the delivery device, so the effects on proteins that have diffused far from the delivery device are limited. Due to the fact that bioadhesives were used as the conjugating polymer, the delivery device may adhere to the intestinal lining. If this does happen, the diffusional distance of the protein from the device to the intestinal wall will be quite short. One barrier that the protease inhibitors do not affect is the cellular barrier. Biomacromolecules must still find a method to enter the cells or be taken up by phagocytosis.

Polyphosphazene Hydrogels

Polyphosphazene hydrogels, as indicated earlier, are hydrogels prepared using the polyphosphazene backbone but that contain hydrophilic moieties pendant from the backbone. A matrix and crosslinking can be produced by incorporating multifunctional groups into the mixture. Mild conditions are needed for production of the hydrogels, so biomacromolecules are not exposed to harsh conditions. Release from polyphosphazene hydrogels is by diffusion as has been described for other hydrogel microparticulate systems, but can be controlled by appropriate control of the polymer and secondary components. As with any of the microparticulate systems, a coating can be applied to the particles, thus preventing initial release of encapsulated molecules. By making the surface of the hydrogel semipermeable, the release of bovine serum albumin can be greatly reduced, but not completely stopped. Further investigations using semeipermeable or nonpermeable coatings should be investigated for various hydrophilic systems to decrease the release of biomacromolecules prior to reaching the lower intestines or the area of the intestine desired (Andrianov and Payne 1998).

Poly(ethylene glycol) or Poly(ethylene oxide)

The use of conjugates of polymers to proteins has been investigated extensively for parenteral administration of proteins (Veronese et al. 1985). A polymer conjugated to a protein prevents proteolytic enzymes from contacting the protein by steric hindrance. Since proteolysis is minimized, circulation of the peptide can be increased greatly. Some degradation is still possible either by alternate proteolytic mechanism, degradation of the conjugated polymer, or hydrolytic degradation. The protein or peptide would still be in contact with the acidic environment of the gastrointestinal tract and this may be the reason behind the limited investigation of this type of polymer for oral delivery.

Another reason that oral polymer-protein conjugates have not been investigated widely is that there may be some difficulty in the ability of the conjugate to traverse the intestinal wall. The hydrophilic protecting chain of the conjugate would decrease the solubility of the protein in the lipid bilayer of the cells of the intestine. The decrease in solubility could cause a decrease in the bioavailability of the protein. The fact that particles are well absorbed by the intestine could indicate that further research is necessary in this area. Conjugates of proteins and polymers are very effective when administered parenterally, and the increase in half-life of circulating protein conjugates could also indicate an increase in the half-life of absorbed proteins delivered orally. This area of oral drug delivery should be investigated more carefully in the future as it could greatly increase the absolute amount of active protein and peptide that can be delivered.

CONCLUSIONS

The use of polymers to deliver protein and peptides has had some success. Most of these systems have been based on encapsulating the protein or peptide within a polymer. The protection that the polymer gives the peptide from proteolysis was the

major benefit that was originally sought. Studies have shown, however, that polymers also play a role in uptake of biomacromolecules, as the polymeric carrier particles are themselves taken up by the intestinal cells. The majority of the particles do not reach the systemic circulation, and in fact, only a rare few make it to circulate in the blood. Blood levels of active biomacromolecules have been shown to increase to significant levels following oral administration, encouraging further investigation into methods that would increase the fraction of particles that are absorbed by the intestinal cells, or at least increasing the amount of time that the particles spend in the digestive tract prior to elimination from the body. Bioadhesive particles are currently thought to be a major breakthrough for oral peptide delivery and novel bioadhesive particles are being developed (Lehr 2000). The increase in absorption and residence time in the gastrointestinal tract could greatly increase the delivery of proteins.

Inhibitors of proteases have also been developed from polymers. These molecules are as simple as a polymer chain, or much more complex. The mechanisms for protease inhibition are variable depending upon the type of molecule used; specific protease inhibitors are available for conjugation to a polymer while other polymeric inhibitors inhibit all divalent cation dependent proteases. The development of these inhibitory polymers and polymer conjugates greatly increase the possibility to protect proteins from degradation in the gastrointestinal tract.

With all of the advances in polymer science and conjugation technology, many methods have been developed to increase the feasibility of oral peptide and protein delivery. There is still no single mechanism that can be used to protect a protein or peptide from degradation and increasing oral availability, but with the multitude of new methods for allowing a protein to negotiate natural barriers, oral delivery of any systemically active protein is a definite possibility at some point in the future.

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