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Review Article

The controlled parenteral delivery of polypeptides and proteins

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Summary

Strategies for the controlled parenteral delivery of polypeptides and proteins are reviewed. The different approaches are divided into five classes: (a) hydrogels, (b) self-diffusion systems, (c) microparticles, (d) biodegradable polymers, and (e) porous membranes; self-regulated delivery represents an additional subset. These methods are discussed in the light of difficulties associated with delivery of proteins, including their low permeability, rapid proteolysis, and denaturation within the delivery system.

Introduction

The advent of genetic engineering has resulted in a proliferation of new biopharmaceuticals that are orally inactive and must be administered by subcutaneous (sc) injection or intravenous (iv) infusion. A considerable research effort is now being invested in techniques, e.g. iontophoresis and permeability enhancement, that have the potential to overcome the barriers presented by natural biological membranes and avoid the trauma of sc or iv injections. Some successes with nasal, rectal, transdermal and gastrointestinal (GI) administration have been achieved with lower molecular weight peptides (Lee, 1986; Banga and Chien, 1988; Eppstein and Longenecker, 1988). However, for the immediate future, parenteral administration remains the only viable route as well as the only means by which sustained, controlled delivery can be achieved.

The extreme susceptibility of proteins to proteolysis and rapid clearance from the bloodstream argues for the use of controlled delivery systems. Conventional methods of controlled delivery based on Fickian diffusion through hydrophobic polymer membranes such as silicone rubber are not applicable to peptides and proteins. The high molecular weight and hydrophilicity of proteins cause the diffusion process to be impractically slow. This physical restriction is compounded by the instability of many proteins in hydrophobic matrices and in concentrated aqueous solution, and their tendency to bind strongly to hydrophobic surfaces. Furthermore, zero-order delivery may not be the optimum kinetic profile because of the potential down-regulation of biological receptors; in many cases pulsed or self-regulated delivery systems may be more efficient and economical.

Because of these restrictions, novel strategies for the delivery of proteins are necessary. The different approaches that have been explored are conveniently divided into five classes: (a) hydrogels, (b) self-diffusion systems, (c) microparticles,

(d) biodegradable polymers, and (e) porous membranes. Self-regulated delivery systems represent an additional subset.

Hydrogels

A log-log correlation between the Fickian diffusion coefficient (D) and the molar volume of solutes has been demonstrated (Michaels and Bixler, 1961). For larger solutes, the molecular weight (M_w) is conveniently substituted for molar volume (Baker and Lonsdale, 1974) (Eqn. 1, Fig. 1):

$$\log D = a \log M_w + b \quad (1)$$

It is evident from Fig. 1 that the value of D in synthetic polymers decreases rapidly as the molecular weight increases and is impractically small for solutes of M_w value greater than $M_w \sim 500$. In contrast, the value of D in water (and other low molecular weight, low viscosity solvents) decreases relatively slowly. It is therefore not surprising that many of the early studies of the controlled release of proteins focussed on hydrogels. Simplistically, the hydrogel provides a restricted aqueous environment for diffusional migration of the macromolecular drug. Experimental studies (Jhon and Andrade, 1973; Zentner et al., 1979) suggest that, for small molecules, a distinction be made between transport through a domain composed of bulk water (pore mechanism) and a domain composed of polymer segments, interfacial water, and bound water (partition mechanism); for hydrophilic macromolecules, the pore mechanism is dominant.

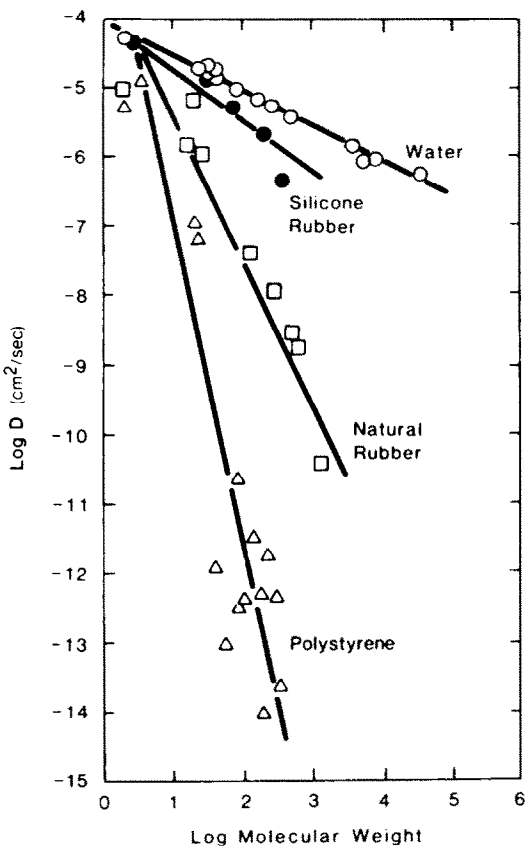

Fig. 1. Relationship between the logarithms of the diffusion coefficient and molecular weight of solutes in water, silicone rubber, natural rubber, and polystyrene (Baker, 1987).

The first application of hydrogels to controlled delivery of macromolecules is due to Davis, who used subcutaneously implanted polyacrylamide gels as a matrix for the administration of insulin to prolong the life of diabetic rats (Davis, 1972). In a second pioneering paper, Davis showed that polyacrylamide and polyvinylpyrrolidone gels crosslinked with 20% w/w N,N'-methylenebisacrylamide could be used for controlled delivery of IgG, luteinizing hormone (LH), bovine serum albumin (BSA), prostaglandin F_2 and sodium iodide (Davis, 1974). Implants were prepared as rods (2 cm × 1.5 mm o.d.) by photo-initiated polymerization of the vinyl monomers mixed with the ^{125}I-labeled drug in phosphate-buffered saline (PBS) in glass tubes. Diffusion coefficients were derived by fitting the rates of release of the various drugs from the hydrogel matrix in PBS (pH 7.2, 37°C) to the theoretical expression for radial diffusion in a cylinder. The diffusion coefficients exhibited an inverse logarithmic dependence on the fractional polymer content of the gel (C_p) and the drug molecular weight (M_w). Using the mean values of in vivo and in vitro measurements, this dependence was expressed mathematically as given in Eqn. 2, where D and D_w are the diffusion coefficients of the solute in the hydrogel and water, respectively.

$$\ln(D/D_w) = -(5 + 10^{-4} M_w) C_p \quad (2)$$

The duration of release of LH in hamster was increased from 1 to 36 days by increasing the polyacrylamide concentration in the gel from 5 to 40% (Fig. 2). The rates of release of LH were approximately proportional to the square root of time, consistent with the kinetics of diffusion from a monolithic cylindrical device. The release of LH showed a discontinuity after 4 days in vitro, which was tentatively attributed to partial denaturation of the protein. The in vivo release rates of LH and BSA were approx. 50% less than those observed under in vitro conditions. This result was too great to be explained by the difference in the viscosities of PBS and serum, and Davis suggested contributions from either tissue encapsulation of the implant or reduced hydration under in vivo conditions. This paper contains one of the first references to the use of an artificially derived concentration gradient within the gel to predetermine release kinetics.

Sefton and Nishimura (1980) measured the permeability of ^{125}I-labeled insulin in hydrogel membranes of polyhydroxyethyl methacrylate (37% water), polyhydroxyethyl acrylate (52% water), polymethacrylic acid (67.5% water), and cuprophane PT-150 (45% water). While the contribution of free ^{125}I to the data is uncertain (Albin et al., 1985), the measured permeabilities (P) of the series correlated directly with the degree of hydration (H) of the hydrogel (Eqn. 3).

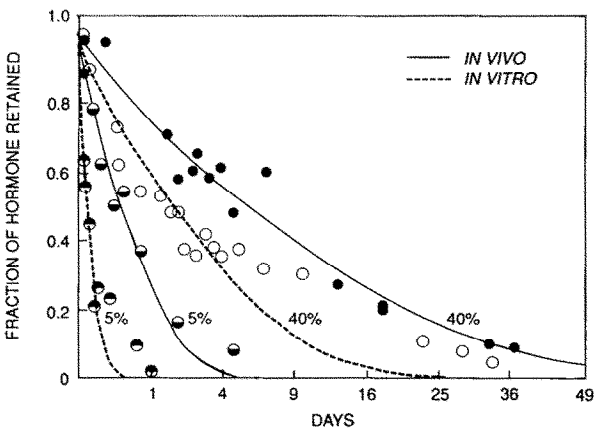

Fig. 2. Depletion of ^{125}I-labeled luteinizing hormone (LH) from 5 and 40% polyacrylamide gels (20% crosslinked) in 10 mM PBS (pH 7.2, at 37°C) and in mature female hamsters. The curves were calculated using the average apparent diffusion coefficients obtained from the amount of solute retained by the gels at the indicated times. Continuous lines denote implants, and broken lines refer to depletion in vitro (Davis, 1974).

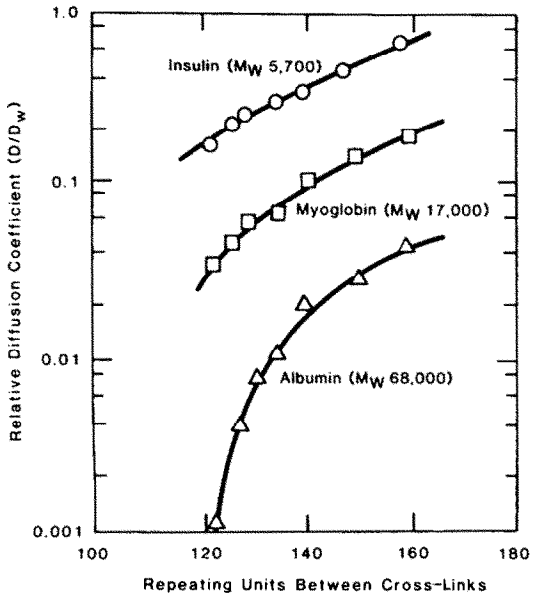

Fig. 3. Diffusion coefficients (D) of three proteins in crosslinked polyvinyl alcohol as a function of the number of repeating units between crosslinks. Diffusion coefficients are expressed as a fraction of the diffusion coefficient (D_w) in water (Sorensen and Peppas, 1979).

$$P = kHD_w \tag{3}$$

Sato and Kim (1984) described the use of hydrogels of hydroxyethyl methacrylate (HEMA), methoxyethyl methacrylate (MEMA), and methoxyethoxyethyl methacrylate (MEEMA) as membranes for the diffusion-controlled delivery of the following water-soluble solutes with a wide range of molecular weights: sodium acetate, glucose, maltose, insulin, cytochrome c and albumin. Both dense and porous membranes were studied. The diffusivity was dependent on the solute size and the membrane hydration, and was consistent with the model of permeation involving both 'pore' and 'partition' mechanisms.

The crosslink density of hydrogels has been used as a means of controlling both the degree of hydration and the permeability of hydrogels to

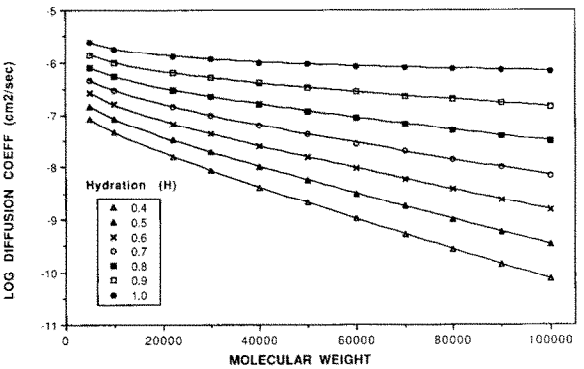

Fig. 4. Relationship between the diffusion coefficients and the molecular weights of proteins in hydrogels with different degrees of hydration, derived from Eqn. 4.

proteins. This is illustrated by the change in the permeability of crosslinked polyvinyl alcohol to serum albumin, insulin and myoglobin which is achieved by systematically varying the distance between crosslinks (Fig. 3) (Sorensen and Peppas, 1979). The greater the size of the protein, the greater is the sensitivity of the diffusion coefficient to changes in crosslink density.

An estimate of the diffusion coefficients of solutes in hydrogels with different degrees of hydration may be made by applying Eqn. 1 to estimate D_w, then combining with Eqn. 2. Using literature data for the diffusion coefficients of various solutes in water to determine the coefficients of Eqn. 1, one obtains Eqn. 4, and the family of curves in Fig. 4.

$$\log D = -4.0 - 0.43 \log(M_w) - (2.2 + 4.4 M_w 10^{-4}) C_p \quad (4)$$

The validity of Eqn. 4 has not been tested, but optimization of the coefficients for a wider range of hydrogels may make it a useful means of narrowing the choice of polymers to evaluate in the development of a diffusion-controlled delivery system.

Eqn. 5, derived from free volume theory by Yasuda et al. (1969), is a theoretically more sound correlation of the diffusion coefficient, the degree of hydration of the hydrogel, and the size of the solute:

$$\ln(D/D_w) = (kV_s/V_f)(1 - H)/H \quad (5)$$

Here k denotes a constant, V_s is proportional to the solute volume or cross-section, V_f is the free volume of water in the gel, and H is the degree of hydration. * A number of studies have established the validity of the relationship between D, V_s and H expressed by Eqn. 5 (Colton et al., 1971; Sato and Kim, 1984; Gilbert et al., 1988). For example, Gilbert et al. (1988) measured the permeabilities of dense and porous fibril collagen membranes crosslinked to different degrees with either glutaraldehyde or polyglycerol polyglycidyl ether. Using lysozyme, carbonic anhydrase, ovalbumin and serum albumin as a series of model proteins with increasing size, relationships between the diffusion coefficient and both the degree of hydration and the radius of the protein were demonstrated.

Insulin has been the most frequently studied protein. Albin et al. (1985) critically reviewed the literature on insulin permeabilities in hydrogel membranes, including pHEMA, polyhydroxyethyl acrylate, polymethacrylic acid, pHEMA–co-methyl methacrylate, polyhydroxypropyl methacrylate–co-diethylaminoethyl methacrylate, pHEMA–co-methoxyethyl methacrylate, and polyHEMA–co-methoxyethoxyethyl methacrylate (Shatayeva et al., 1979; Sefton and Nishimura, 1980; Ronel et al., 1983; Ishihara et al., 1984a,b; Sato and Kim, 1984; Sato et al., 1984a,b). It was suggested that insulin permeabilities reported to be greater than 10^{-9} cm^2 s^{-1} in pHEMA (40% water) may be the result of contamination of ^{125}I-labeled insulin with the more permeable $^{125}I^-$. The permeability of pHEMA can be successfully increased by polymerization of HEMA in > 50% water; for example, the permeability of insulin in pHEMA polymerized in 75% water is 2.8×10^{-7} cm^2 s^{-1} (Ronel et al., 1983).

A reservoir device with a rate-limiting non-degradable hydrogel membrane for the controlled delivery of peptides and proteins is disclosed in a patent (Sanders and Domb, 1987). A polyacryla-

* A reviewer has pointed out that, since V_s is approximately proportional to M_w, and in the limit of high hydration $(1 - H)/H$ approaches C_p, it follows that the term $M_w \cdot C_p$ is essentially common to both Eqns. 2 and 5.

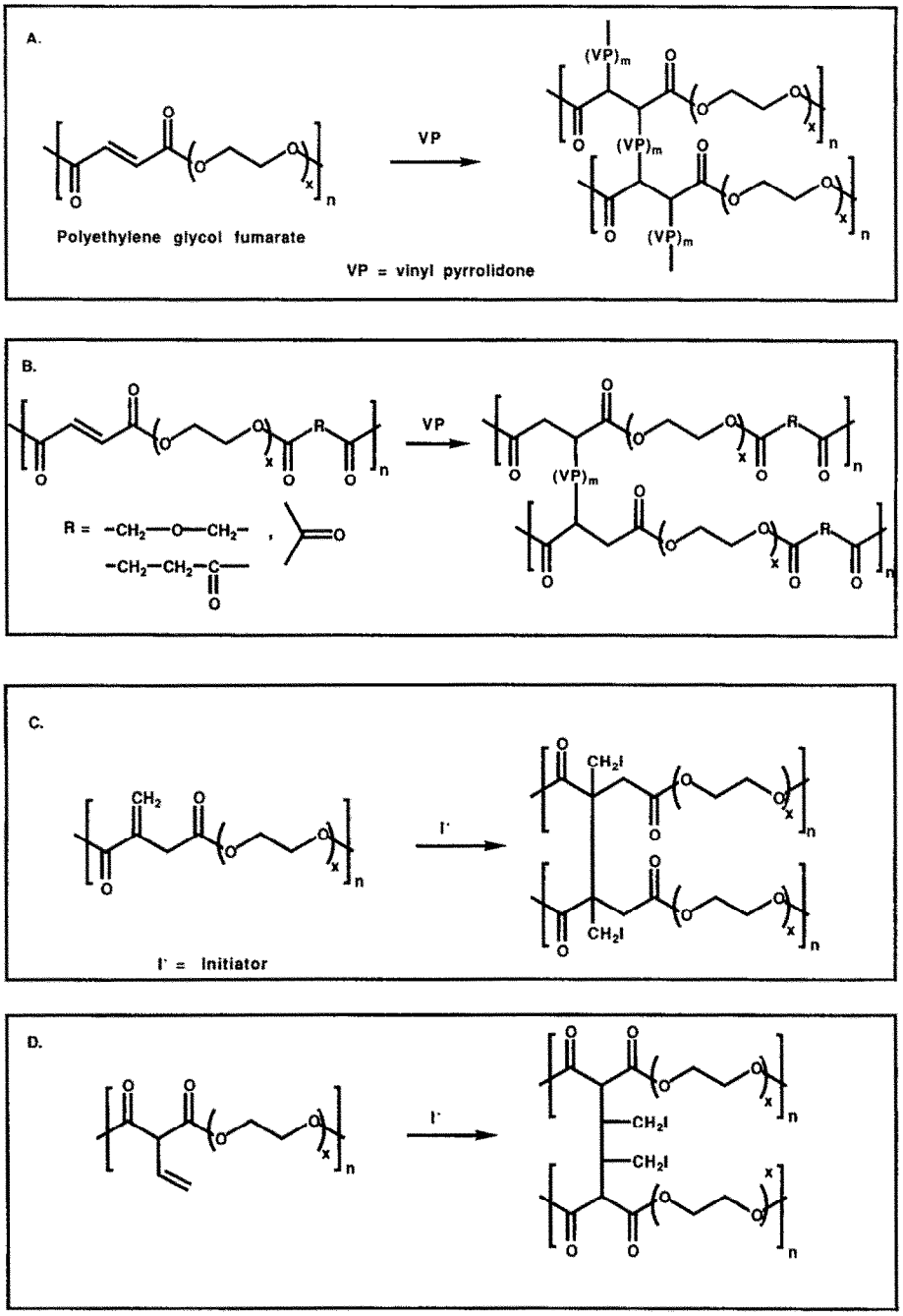

Fig. 5. Preparation of biodegradable hydrogels from: (A) esters of polyethylene glycol and fumaric acid, crosslinked with *N*-vinylpyrrolidone; (B) esters of polyethylene glycol, fumaric acid, and either ketomalonic, diglycolic or ketoglutaric acid, crosslinked with *N*-vinylpyrrolidone; (C) esters of polyethylene glycol and itaconic acid, radical crosslinked; and (D) esters of polyethylene glycol and allylmalonic acid, radical crosslinked (Heller et al., 1983).

mide-agar hydrogel for the delivery of proteins has also been claimed (Charmot and Daniel, 1987).

Biodegradable hydrogels

The use of biodegradable polymers for protein delivery serves the dual purpose of eliminating the need to remove polymer residues from the implant site after the drug is exhausted and providing an alternative mechanism of drug release from the polymer matrix. Torchilin et al. (1977) showed that polyvinylpyrrolidone (PVP) hydrogels crosslinked with *N,N′*-methylenebisacrylamide undergo biodegradation if the crosslink density is less than 1%. The methylene group of the crosslinker is subject to slow hydrolysis at pH 7. Cleavage reduces the small number of crosslinks below the critical value necessary to maintain the gel state and results in the controlled dissolution of the hydrogel. This delivery system was demonstrated by incorporation of chymotrypsin into the gel by mechanical entrapment during emulsion polymerization of *N*-vinylpyrrolidone. The amount of chymotrypsin released during a 50 h period at 25 °C from a gel with 0.3, 0.6, and 1.0% crosslinking agent was 100, 81, and 64%, respectively. The time required for dissolution of the same gels was listed as 2–3 days, > 10 days, and 'insoluble'.

The usefulness of this delivery system was limited by the fact that the crosslink density controlled the rates of both the hydrogel dissolution and the diffusional release of the chymotrypsin. Only gels with a very low crosslink density dissolved within a reasonable time period; such gels, by virtue of their highly porous structure, were unable to retain the entangled macromolecular solute, and rapid diffusional release occurred. Heller et al. (1983) sought to simplify this design problem by limiting the release mechanism to biodegradation. To this end, a more tightly crosslinked system was used to eliminate diffusional release, while at the same time the hydrolytic instability was increased by introduction of fumaric or malonic ester linkages. Water-soluble polyester prepolymers were first prepared from polyethylene glycol and fumaric acid, and then crosslinked by copolymerization with *N*-vinylpyrrolidone (Fig. 5A). Structurally similar but more rapidly hydrolyzed hydrogels were prepared from

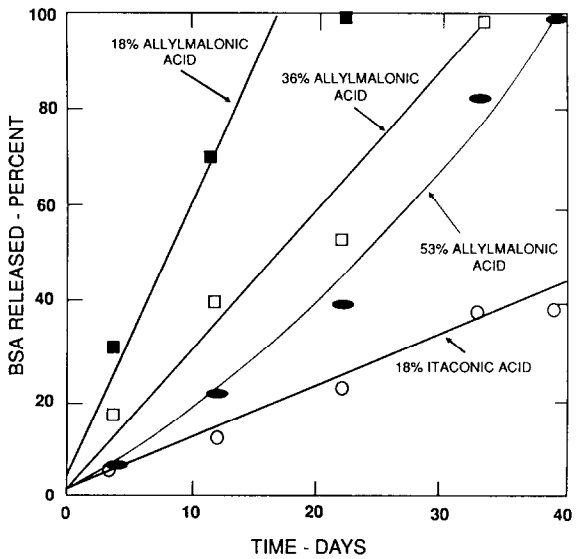

Fig. 6. Release of bovine serum albumin at pH 7.4 and 37 °C from microparticles prepared from various unsaturated polyesters of allylmalonic and itaconic acids, crosslinked by free radical initiation. (○) Itaconic acid, 18% concentration; (■) allylmalonic acid, 18% concentration; (□) allylmalonic acid, 36% concentration, (●) allylmalonic acid, 53% concentration (Heller et al., 1983).

combinations of fumaric acid with ketomalonic acid, 1 : 1-ketoglutaric acid or 1 : 1-diglycolic acid (Fig. 5B). These structures are more hydrolytically labile because of the presence of electron-withdrawing groups proximate to the ester function. BSA entrapped in microspheres of these gels was released as chain scission resulted in slow dissolution. The duration of release could be varied between 10 days and greater than 7 weeks by the choice of ester structure and the amount of vinylpyrrolidone crosslinks; zero-order kinetics were observed.

Erosion of these hydrogels produced the water-soluble but non-degradable polymer PVP as a by-product. A second group of hydrogels, totally degradable to low molecular weight fragments, was prepared by vinyl polymerization of the polyesters of itaconic and allymalonic acid (Fig. 5C,D) (Heller et al., 1983). The rate of release of BSA from these hydrogels was governed by the chemical structure (itaconic vs. allymalonic), and the concentration of the unsaturated ester in solution prior to gelation (Fig. 6).

Crosslinking of hydrogels can be achieved by physical as well as chemical means, e.g., by association of crystalline or hydrophobic polymer blocks. This approach is illustrated by a patent issued to Churchill and Hutchinson (1983), which discloses the preparation of block copolymers consisting of a hydrophilic component and a degradable hydrophobic component. Examples of the hydrophilic component are polyvinyl alcohol, polyethylene glycol, and polyvinylpyrrolidone, while the degradable block may be polyesters such as polylactic acid, polyglycolic acid, or polyamides. Release proceeds by diffusion through the hydrated domains, further promoted by the degradation process. Delivery of polypeptides such as LHRH, growth hormone releasing factor, EGF, ACTH, and somatostatin was claimed, although the kinetics were not described.

Dissolving hydrogels

A third subset of hydrogel systems is derived from polymers that hydrate rapidly but dissolve slowly on exposure to an aqueous environment. The mechanism of release can be drug diffusion through the hydrated gel or surface erosion, or both processes, depending on the relative rates of hydration and dissolution. Such systems have been studied extensively as a means of delivery of low molecular weight drugs. Since the rate of diffusion of the drug in the unhydrated polymer is generally insignificant, the rate of diffusional drug release from a monolithic device is determined by the rate of advancement of the water front. This process, termed Case II transport (Hopfenberg and Hsu, 1978), can be used to achieve zero-order drug delivery from monolithic slabs. The rate of water access may be controlled by partially or completely coating the hydrophilic polymer core with a semipermeable polymer (Colombo et al., 1987).

Recently, this strategy has been used to achieve controlled release of LH, LHRH and EGF (Oppenheim et al., 1988). Coating a compressed pellet of the polypeptide and a standard hydrophilic excipient with polymethyl methacrylate provided implants which released the polypeptide over periods varying from 1 week to several months, depending on the coating thickness. The kinetics were first order and the total amount of EGF decreased as the duration of delivery increased, suggesting decomposition of the protein in the pellet. Release of LHRH was used to induce ovulation and mating in anestrous ewes. The controlled release of Spectrum Orange, a dye-labeled dextran with a molecular weight of $14–16 \times 10^3$, from a hydrophilic poly(2-vinylpyridine 1-oxide) core coated with hydrophobic poly(2-vinylpyridine) was achieved using the same strategy (Hasirci and Kamel, 1988).

A number of patents claim controlled release of proteins from other materials of this class, including polyvinyl alcohol (Masao, 1982), polyethylene/propylene glycols (Morikawa, 1986; Blum et al., 1987), and cellulose ethers (Janski and Yang, 1987). Aqueous solutions of certain block copolymers of ethylene and propylene glycol are liquid at room temperature but gel at body temperature. This property facilitates administration by injection and results in a subcutaneous hydrogel depot. The kinetics of protein release from such systems have not been reported; however, the inability to control the permeability of the hydrogel by varying the water content whilst maintaining the transition temperature between 25 and 37 °C seriously restricts the utility of this concept. In a series of papers, Korsmeyer et al. (1983) discussed the mechanism of release of BSA and low molecular weight drugs from porous hydrophilic polymers, using poly(vinyl alcohol) discs as experimental models. The initial drug diffusion was attributed to dissolution into and diffusion through water-filled pores near the surface of the polymer matrix. Subsequent polymer swelling leads to structural changes in the polymer and its porosity. As the swelling progresses, diffusion of the drug occurs through the hydrated polymer matrix as well as through the water-filled pores. The rate and kinetic law, i.e., zero vs. first order, depend on the relative contributions of these processes.

An unusual example of a hydrogel delivery system is use of the protein itself as a hydrated matrix for controlled release. A patent (Azain et al., 1988) claims controlled delivery of bovine somatotropin from a compressed pellet of the protein which has been partially coated to control the initial dissolution rate. The protein aggregates under these conditions and forms a crosslinked

network which controls the degree of swelling of the hydrated matrix. A related patent is based on the controlled delivery of low molecular weight drugs from a crosslinked protein matrix (Friedman et al., 1987).

Self-diffusion (or Monolithic) Systems

While classical Fickian diffusion of macromolecules through the polymeric bulk of semipermeable membranes is impractically slow, Langer and his group have shown that many different macromolecules are released from hydrophobic polymers by a self-diffusion process (Langer and Folkman, 1976, 1978; Folkman and Langer, 1979, 1983; Langer et al., 1980, 1985a–c; Brown et al., 1983). The second of the two original patents (Folkman and Langer, 1983) describing this work claims a variety of hydrophobic polymers but most of the published results are derived from polyethylene–co-vinyl acetate (EVA) systems. Several studies have been devoted to the elucidation of the mechanism of release of macromolecules from EVA (Miller et al., 1983; Siegel and Langer, 1983, 1986; Bawa et al., 1985). The mechanism does not involve dissolution of the drug in EVA or swelling of the polymer bulk as in the case of a hydrogel. Rather, it involves diffusion through aqueous channels created by the protein itself. This is consistent with the finding that a minimum drug loading is necessary before release of the drug from the EVA matrix is observed. Microscopy identified pores and connecting channels occupied by the drug (Bawa et al., 1985). A morphological study demonstrated that there is considerable swelling of the pores due to water uptake by the protein, and collapse of some pores occurs after loss of the protein (Miller et al., 1983).

Initially, difficulties in obtaining reproducible release rates were experienced. However, procedures for fabricaton by mixing the dry, powdered macromolecule with the polymer solution at $-80\,^{\circ}C$, then solvent evaporation at incrementally higher temperatures, minimized drug settling and provided the necessary reproducibility (Rhine et al., 1980). A more convenient fabrication procedure involves mixing the micronized protein and

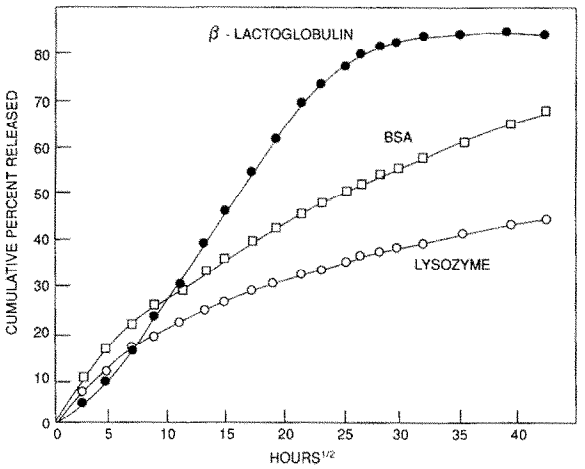

Fig. 7. Cumulative release of three proteins from an EVA slab, plotted vs. square root of time: (●) α-lactoglobulin; (□) BSA; and (○) lysozyme (Rhine et al., 1980).

polymer in a piston mold below the T_g of the polymer ($-36\,^{\circ}C$), then compression at $37\,^{\circ}C$; this method avoids the use of solvent (Siegel and Langer, 1983; Cohen et al., 1984). The list of drugs that have been combined with the monolithic EVA system includes BSA, α-lactoglobulin A, lysozyme, soybean trypsin inhibitor, alkaline phosphatase, catalase, insulin, heparin, and DNA. Three examples are shown in Fig. 7. The release rate, expressed as a percentage of the total drug load, increases with the drug load but diminishes with the particle size of the protein in the range 150–425 μm. The need to use a minimum load of a protein to achieve a finite release rate is not restrictive, for an inert protein (e.g., HSA) may be included with the active constituent.

The rate of diffusion in EVA is not directly related to the molecular weight of the protein, and is too slow to be explained by the aqueous diffusivity of the protein, or the tortuosity of the polymer pores. Rather, it has been suggested that the primary determinant is penetration of constrictions which connect the pores (Fig. 8) (Siegel and Langer, 1983).

A potential limitation of the use of a monolithic system arises from the fact that the kinetics of drug release are typically not zero order. For example, protein release from monolithic EVA slabs is proportional to the square root of time.

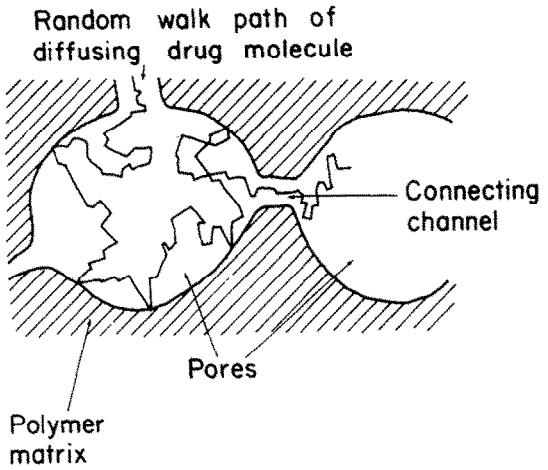

Fig. 8. Schematic of passage through which a self-diffusing protein molecule must pass in a hydrophobic matrix, showing the narrow rate-limiting channels connecting larger pores (Siegel and Langer, 1983).

This shortcoming has been addressed by changing the design of the monolith, for several different geometries which compensate for the monolithic characteristics and provide nearly zero-order release are known (Brooke and Washkuhn, 1977; Yalkowsky and Kuu, 1985). Hsieh et al. (1983) showed that the inward releasing hemisphere is one such geometry, illustrating its use with the in vitro release of BSA (Fig. 9).

A second, more serious limitation of the monolithic delivery system is the instability of many proteins in the concentrated aqueous solution which constitutes the pores of this type of device. For example, while 37% of fibroblast growth factor (FGF) was released from an EVA matrix over a 2 week period, 99% of its bioactivity was lost

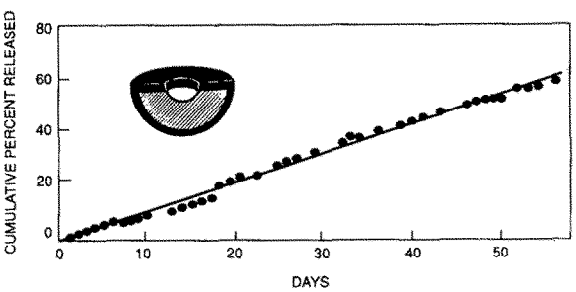

Fig. 9. Cumulative release of BSA from an inward releasing hemisphere of EVA, plotted vs. time (Hsieh et al., 1983).

(Edelman et al., 1989). When the protein was first stabilized by binding to heparin-Sepharose beads, bioactivity was retained in alginate microspheres but not in EVA.

The versatility of the monolithic EVA system was significantly enhanced by the discovery that the rate of release of the drug may be reversibly increased by ultrasound (Kost et al., 1985a, 1986). Release rates up to 20-times the baseline rate can be achieved, depending on the intensity of the source. Mechanistic experiments suggest that the enhancement of the release rate is due to cavitation; local temperature increases and improved mixing (i.e., removal of any boundary layer effects) are inconsequential. These findings provide a mechanism by which the recipient may control the rate of drug delivery, a considerable advantage in therapy such as insulin administration to diabetics, where delivery based on need is desirable. The application of a pulsed magnetic field to monolithic EVA-macromolecule delivery systems containing small magnetic beads has been used to achieve a similar effect (Edelman et al., 1983, 1987; Kost et al., 1985b; Langer et al., 1985a,c).

With the exceptions of EVA and PVA, relatively little work on self-diffusion systems has been done. Polydimethylsiloxane elastomer has been used in conjunction with polyethylene glycol (M_w 20 000) as a delivery system for heparin (Kim and Kim, 1986). It was suggested that the polyglycol serves the purpose of imbibing water and creating aqueous pores. The heparin release rate increased with the heparin and polyglycol loading. Glycerine has been used for the same purpose of creating aqueous pores in a silicone matrix (Hsieh et al., 1985). Similarly, sodium chloride has been mixed with BSA in a polydimethylsiloxane elastomer with the purpose of promoting sustained release of the protein (Carelli, 1989). Here, the excipient was thought to function by osmotically induced polymer cracking, the BSA being released by a combination of convective and diffusive fluxes through cracks. The effect of the particle size, the BSA : NaCl ratio and the loading of the NaCl on the in vitro release rate was studied. Zero-order kinetics were observed during the swelling of the monolithic pellets. A patent claims the use of silicone elastomer for the sus-

tained release of macromolecules (Fujioka et al., 1987).

Cholesterol pellets have long been used for the sustained subdermal delivery of contraceptive steroids (Shimkin and White, 1941). A paper (Wang, 1987) and a patent (Kent, 1984) describe the extension of the formulation to the delivery of insulin and other proteins and peptides. In none of these systems is the mechanism of release known with certainty.

Microparticulate Systems

While Fickian diffusion of low molecular weight drugs from microcapsules and nanoparticles of hydrophobic polymers is often extremely rapid, because of the short diffusion path, simple calculations show that this is not a viable mechanism for protein release. Instead, release of proteins must be achieved by polymer biodegradation, self-diffusion through pores (Langer mechanism), or by rupture of the polymer matrix as a result of sorption of water by the hydrophilic protein. In many cases, no distinction between these mechanisms has been made. The obvious advantage of microparticulate formulations is their ease of administration by injection of a suspension; by contrast, larger dosage forms may require a surgical incision. This convenience must be balanced with the potential need for retrieval, the chances of which increase with the duration of action of the delivery system.

The first example of the application of microcapsules to the delivery of macromolecules is due to Chang (1974), who in 1976 reported the release of insulin from polylactic acid microcapsules. It was stated that the release rate could be varied from 50% in 5 h to 2.5% in 24 h. The mechanism of release was not identified, but was most likely diffusion through defects or osmotic bursting. Asparaginase was incorporated in nylon-6,10 or polylactic acid microcapsules with retention of enzymatic activity (Chang and Chang, 1974; Chang, 1976); however, in these cases activity was achieved by diffusion of asparagine into the microcapsules and not release of the enzyme into the environment.

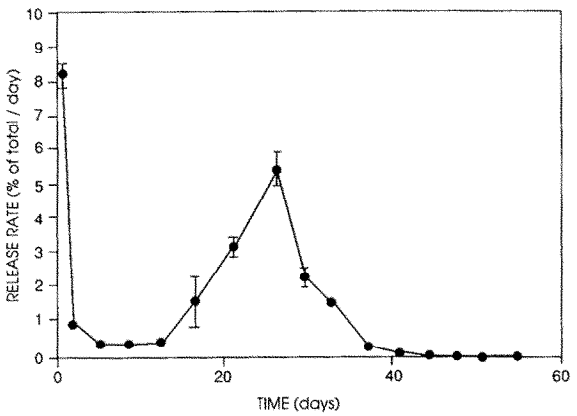

Fig. 10. Illustration of biphasic kinetics of release of Nefarelin, a peptide analog of LHRH, from polyglycolic acid–co-lactic acid microcapsules, in vitro at 37 °C, achieved by selection of the appropriate copolymer composition and molecular weight (Sanders et al., 1985).

A number of publications from the Syntex group have described the use of biodegradable polyglycolic acid–co-DL-lactic acid (PGLA) microcapsules for the delivery of the LHRH agonist Nafarelin (Anik et al., 1984; Sanders et al., 1984a,b, 1985, 1986; Kenley et al., 1987). Microcapsules were obtained by coascervation. The release of the peptide was shown to be polyphasic. The first phase was attibuted to diffusion and the loss of superficial drug. The second phase was characterized by a low or negligible release rate, and was followed by one or more phases of measurable release (Fig. 10). The latter did not occur until bulk hydrolysis of the polymer progressed to the point of erosion and break up of the polymer-drug matrix. As with other ester copolymers (Pitt et al., 1981; Ogawa et al., 1988b,c), the rates of polymer chain scission and the onset of the matrix erosion were determined by the initial polymer molecular weight and the glycolic acid : lactic acid ratio. By judicious choice of these polymer properties, it was possible to minimize sufficiently the second phase of low delivery to provide essentially continuous efficacy in rat for greater than 8 months, with partially effective levels of release continuing beyond 15 months. An essentially identical approach was subsequently reported by Ogawa et al. (1988a–c) for the delivery of leuprolide acetate, a second LHRH analogue. After

evaluating microcapsules of polylactic acid and PGLA, a formulation derived from PGLA, of M_w 14 000, GA : LA ratio 25 : 75, was reported to be ideal for a 1 month delivery system.

Ruiz et al. (1989) studied the microencapsulation of a LHRH analogue with PGLA using silicone oil to effect phase separation from methylene chloride. This coascervation method avoided peptide loss to an aqueous phase. Microencapsulation of hydrophilic peptides and proteins using water presents obvious problems because of loss of drug to the aqueous phase.

No release of interleukin-2 from PGLA microspheres (50–150 μm) was observed in vitro, apparently because of the insolubility of the protein (Hora et al., 1989). This difficulty was overcome by conversion of interleukin-2 to its polyethylene glycol conjugate.

Kwong et al. (1986) studied the delivery of insulin using polylactic acid microspheres and pellets, and proposed that release occurred via pores in the polymer matrix. The role of the emulsifier, polyvinyl alcohol, in promoting crystallization of insulin on the surface of microspheres was demonstrated. Such crystallization, which was not observed with gelatin, caused dumping of about 50% of the drug load within 1 h. Pellets showed a relatively small burst effect in vitro, and glucose blood levels in rats were reduced for 9–18 days depending on the dose of pellets administered.

Sefton et al. (1984) reported a method of preparation of EVA microspheres in which a suspension of the protein in a methylene chloride solution of EVA was extruded through a 16 gauge needle into ethanol at $-78°$ C. The ethanol served both to remove the solvent and to harden the microspheres. Release of BSA was initially proportional to the square root of time and increased with the protein loading.

Artursson et al. (1984) described the use of biodegradable microparticles prepared from crosslinked polysaccharides. The method of preparation involved chemical derivatization of the polysaccharide, either maltodextrin or hydroxyethylstarch, with acrylic acid glycidyl ester (Fig. 11). This derivative, the protein to be encapsulated, a surfactant and ammonium peroxodisulfate were dissolved in buffer, and homogenized in chloro-

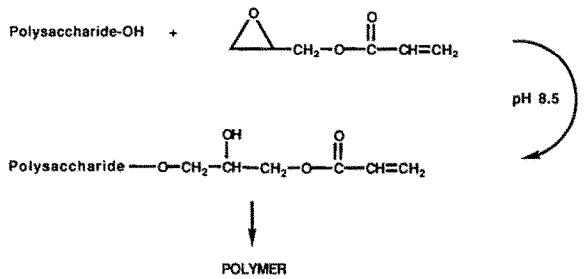

Fig. 11. Synthesis of the acrylic acid glycidyl esters of maltodextrin or hydroxyethylstarch, used for preparation of biodegradable microparticles by emulsion polymerization (Artursson et al., 1984).

form-toluene in the presence of a surfactant to produce a water-in-oil emulsion. Polymerization of the acrylate residues, initiated by addition of an amine, served to stabilize the suspended microspheres. The size range achieved was of the order of 1–10 μm and up to 40% (dry weight) of proteins such as human serum albumin, lysozyme, immunoglobulin, and carbonic anhydrase could be incorporated. Very rapid biodegradation of these microspheres in serum, and in the target organelle, the lysosome, was demonstrated. Release of the protein was sustained over a 12 week period but was not zero order. The mechanism of release was believed to be diffusion through pores in the microspheres. The inclusion of the crosslinking agent N,N'-methylenebisacrylamide in the formulation increased the release rate, consistent with previous findings that the microsphere pore size is increased by addition of a crosslinking agent. A recent European patent application (DeLuca and Rypacek, 1987) describes a very similar system differing by the use of a copolymer of acryloyl hydroxyethyl starch and acrylamide. An example claims the encapsulation of α_1-proteinase inhibitor. The entrapment of proteins in microparticles of crystalline carbohydrates is claimed as a means of providing both a stable environment and a means of controlled release (Schroder, 1984). The biodegradation of polysaccharides by rat liver lysosomal enzymes was studied using an in vitro model (Schacht et al., 1987), and the following order of conversion to glucose established: maltose > amylose > isomaltose $\gg$ dextran. De-

gradation of starch was substantially slowed by hydroxyethylation.

Gelatin microspheres (< 2 μm) of α-interferon were prepared in 50% yield by sonication of an aqueous solution of the protein and gelatin in toluene/chloroform containing Span 80, then treatment of the emulsion with glutaraldehyde to effect crosslinking (Tabata and Ikada, 1989). No information on the extent of glutaraldehyde reaction with α-interferon was presented. The microspheres were subject to degradation by collagenase in vitro at a rate determined by the gelatin crosslink density. They were phagocytosed by macrophages in vitro at a rate independent of crosslinking, resulting in the slow intracellular release of α-interferon.

Lee et al. (1981) described the development of serum albumin microbeads as an injectable, biodegradable system for the delivery of progesterone. This formulation was subsequently adapted to the delivery of insulin by Goosen et al. (1982). Insulin crystals were suspended in phosphate buffer containing BSA, and crosslinking was initiated by addition of glutaraldehyde (2.5 or 5%). The suspension was stirred rapidly in a mixture of corn oil and petroleum ether until the water in oil emulsion was fixed. The activity of the resulting microbeads, which ranged in size from 50 to 1000 μm, was measured in diabetic rats. Blood insulin levels (RIA) decreased from 67 to 10 μU over a 60 day period (Fig. 12), while resorption of the BSA microbeads required about 5 months. The decrease in the release rate after about day 12 was attributed to fibrous encapsulation of the beads, which had a greater effect when the beads were dispersed rather than implanted as a clump.

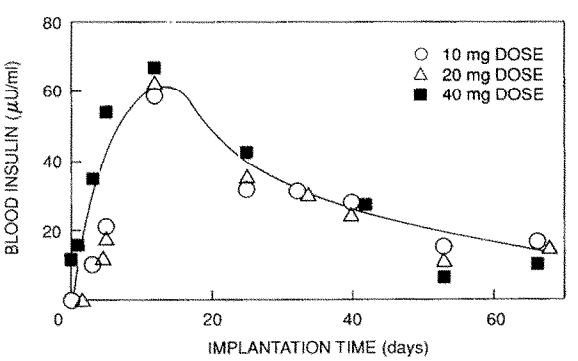

Fig. 12. Blood-insulin levels of diabetic rats after implantation of varying doses of insulin-albumin microbeads (200 mg insulin/g microbeads). Doses: (○) 10 mg, (△) 20 mg and (■) 40 mg (Goosen et al., 1982).

Couvreur and his co-workers (Damge et al., 1988) described the prolonged hyperglycemic effect of insulin-loaded nanocapsules (220 nm mean) prepared by interfacial emulsion polymerization of alkyl cyanoacrylates. The prolonged effect was achieved with both subcutaneously and orally administered preparations.

An early report (Patel and Ryman, 1977) of the oral activity of liposomal preparations of insulin could not be verified reproducibly, although papers on this method of insulin administration continue to appear. The degradation of liposomes in the GI tract is reported to be one reason for the failure to observe oral activity (Chiang and Weiner, 1987; Woodley and Prescott, 1988). Most studies of the encapsulation of macromolecules in liposomes have had the objective of improved cellular uptake and tissue targeting (Gregoriadis, 1985) rather than sustained release. An exception is the entrapment of liposomes in a collagen gel (Weiner et al., 1985). The extent of entrapment was improved by modifying the liposome surface with fibronectin. Release of insulin and growth hormone for 5 and 14 days, respectively, was achieved after i.m. or s.c. injection. Another variation of the standard liposomal formulation is microencapsulation of proteins entrapped in liposomes. Wheatley et al. (1985) described the coating of liposomes with alginate, followed by poly-D-lysine then polyvinylamine, and a final coat of alginate. The kinetics of release of myoglobin from such capsules were bimodal, giving rise to the possibility of either pulsed or delayed drug release. In a further development of this approach, phospholipases were included in the formulation to control the degradation of the liposomes (Kibat et al., 1989). Slow release of the protein was followed by more rapid release after a time interval which decreased with the amount of added lipase.

The i.m. injection of liposomes for the sustained release of calcitonin is the subject of a patent application (Yao-Young, 1987).

Four patent applications claim the use of oil suspensions to prolong the delivery of somatotro-

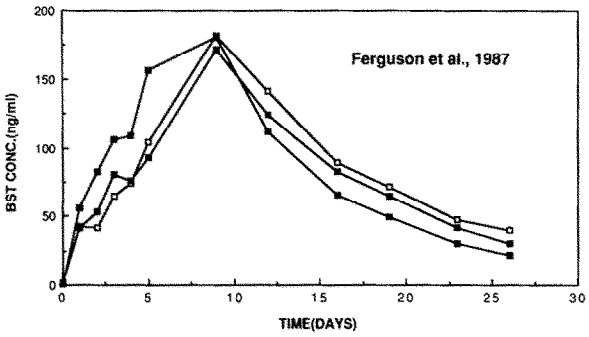

Fig. 13. Somatotropin blood levels in three cows after injection of a suspension of bovine somatotropin (BST) in peanut oil thickened with beeswax (Ferguson et al., 1987).

pins to food animals (Mitchell, 1986; Ferguson et al., 1987; Martin, 1987; Steber et al., 1987). Both thickening of the oil with wax and the use of micronized protein were considered to be important factors in prolonging the delivery time. Delivery of bovine somatotropins in cows for 28 days was achieved (Ferguson et al., 1988), the delivery rate rising to a maximum after 14 days and declining thereafter (Fig. 13).

Biodegradable Polymer Systems

Biodegradable polymers may be subdivided into two basic classes: those in which the degradation occurs primarily at the surface (surface erodibles) and those undergoing bulk degradation. The biodegradable hydrogels and microspheres described in preceding sections are examples of the latter category. Polyorthoesters, prepared by the general procedure shown in Fig. 14, are examples of a surface-erodible system which has been used for the delivery of a variety of lower molecular weight drugs (Heller, 1985). Recently, Heller et al. have demonstrated that polyorthoesters may be used to deliver higher molecular weight drugs such as LHRH analogs (Heller et al., 1986) and insulin (Linhardt et al., 1983; Heller et al., 1987). The rate of surface erosion of polyorthoesters is controlled in part by the lipophilicity of the polymer composition, and the crosslink density. Increasing the crosslink density also serves to eliminate diffusional loss of a drug. Since the hydrolysis of the orthoester group is acid-catalyzed, the inclusion of basic additives such as $Mg(OH)_2$ is used to suppress bulk hydrolysis and limit erosion to the surface. Inclusion of an acidic species, 9,10-dihydroxystearic acid, as a monomer may be used to increase the rate of surface erosion of the matrix and the rate of peptide release. In a different system, an orthoester was prepared with a tertiary amino group in the polymer chain. This created an orthoester which was very sensitive to changes in the internal pH of the polymer matrix, and was the basis of a self-regulated insulin delivery system (vide infra).

Polyanhydrides, developed by the Langer group (Linhardt et al., 1983; Rosen et al., 1983; Leong et al., 1985, 1986a,b, 1987; Domb and Langer, 1987; Mathiowitz and Langer, 1987; Chasin et al., 1988; Mathiowitz et al., 1988; Langer and Domb, 1988) represent a second major class of surface-erodible polymers. Hydrolysis of the anhydride link is inhibited by acid; as a result, bulk erosion of the polymer is autosuppressed by the acidity of the

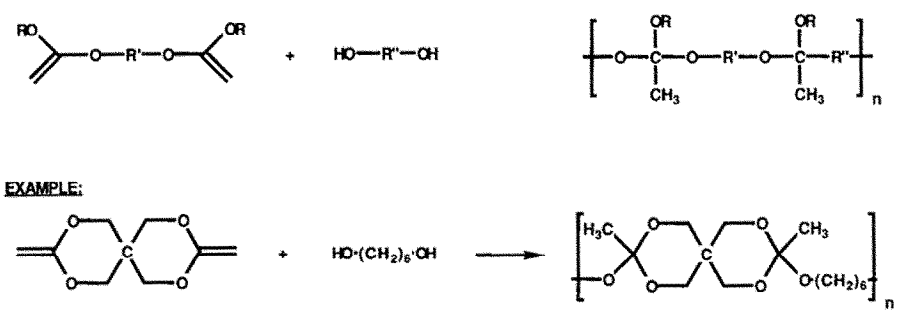

Fig. 14. General method of synthesis of polyorthoesters, and a specific example (after Heller, 1985).

carboxylic products of the hydrolytic process (Domb and Langer, 1987). A series of chemical structures, with varying hydrophilicity, has been screened and a correlation between polymer structure/lipophilicity and the rate of surface erosion established (Leong et al., 1986b; Chasin et al., 1988). A method of obtaining high molecular weight polyanhydrides was also developed (Langer and Domb, 1988). Injection molding and compression molding techniques have been used to incorporate the drug in the polymer. The former method afforded the more homogeneous morphology but required higher temperatures and caused some reaction of the amine group of drugs with the anhydride link (Leong et al., 1986a); it is therefore less suitable for protein formulation. Mathiowitz et al. (1988) reported the use of polyanhydride spheres (50–1000 μm) to deliver insulin and myoglobin, as well as lower molecular weight dyes. Spheres were prepared by mixing the drug and melted polymer, suspending in a hot non-miscible solvent, and cooling until solid. After screening a number of polyanhydrides, the copolymer of sebacic anhydride and bis(*p*-carboxyphenoxy)-propane anhydride was identified as having the requisite material properties and rate of hydrolytic degradation. Incorporation of the drug affected the rate of surface erosion of the polymer. The rate of insulin release was not constant in vitro, but suppression of glucose levels in diabetic rats was demonstrated over a period of several days. Sonic acceleration of both the rates of drug release and polymer erosion was reported in another study (Leong et al., 1986b).

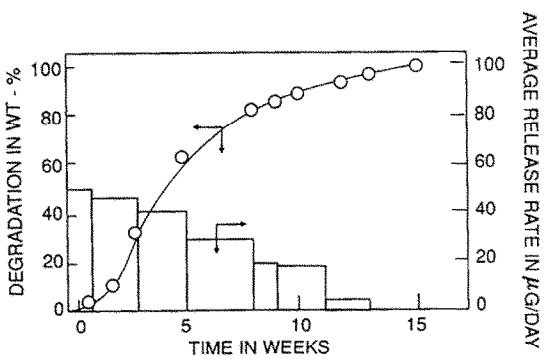

Fig. 15. In vivo degradation of hot-pressed pure PLA and the average daily dose of an LHRH agonist released in vivo from the hot-pressed PLA formulation. $M_n = 2200$.

Other examples of bulk erosion systems are known. Asano et al. (1985) prepared a hot-pressed rod (1 cm × 2 mm o.d.) from powdered low molecular weight poly(DL-lactic acid) and the agonist [D-Leu^6,Pro^9,NHEt]LHRH. The two components were mixed at 70 °C, and then compressed within a Teflon tube. The release of the peptide and the extent of polymer weight loss were determined by recovery of samples at prescribed time intervals after implantation in the dorsal region of rats. Both the rates of polymer erosion and peptide release decreased with time over a 15 week period, at which point no polymer was recoverable (Fig. 15).

Furr and Hutchinson (1985) reported the biological effects of sustained delivery of [D-Ser(tBu)^6, AzaGly^{10}]LHRH (Zoladex, ICI) compounded with a 1 : 1 copolymer of DL-lactic acid and glycolic acid and implanted subdermally as a rod (1 mm × 3–6 mm). This formulation, which was totally biodegradable, was designed to deliver the peptide for at least 28 days. Both polymer erosion and drug diffusion through drug-created pores in the polymer matrix contributed to the release rate. The rate could be controlled by the choice of copolymer composition and molecular weight and, because of the duality of the release mechanism, could be made bimodal (Hutchinson and Furr, 1985).

Saffran et al. (1986) devised a method of oral administration of insulin based on the localized biodegradation of azo compounds by indigenous microflora in the large intestine. Non-gelled copolymers of styrene and hydroxyethyl methacrylate, crosslinked with 1 or 2% divinylazobenzene, were prepared by radical polymerization. Films of the polymer were shown to lose strength and undergo surface erosion when incubated with human feces. When gelatin capsules containing either vasopressin or insulin were coated with the azo polymer and deposited in the stomach of rats, significant biological responses (antidiuresis and hypoglycemia) were observed.

Porous Membrane Systems

A distinction can be made between porous membranes and other polymeric membranes

covered by this review. Porous membranes are defined as having stable pores, the size and distribution of which do not depend on the hydration of hydrophilic polymer sequences (hydrogels) or the presence of hydrophilic drugs (self-diffusion systems). Porous cellulosic membranes have a long history of use as dialysis membranes (Brock, 1983). In 1971, Colton et al. (1971) characterized the permeabilities of commercial, modified, and laboratory-cast porous cellulose membranes, using 15 water-soluble solutes with molecular weights ranging from 58 000 to 68 000. The rates of diffusion of the solutes decreased as their size increased. A log-log plot (cf. Eqn. 1) of the membrane resistance (reciprocal of permeability) vs. the drug molecular weight was linear for 7 to 9 solutes, with only heparin and polyethylene glycol failing to conform. A semilog plot of the ratio, D_m/D_w, of the diffusion coefficients in the membrane and in water vs. the molecular radius of the solute was also linear (Fig. 16). These correlations permited prediction of the permeabilities of other solutes.

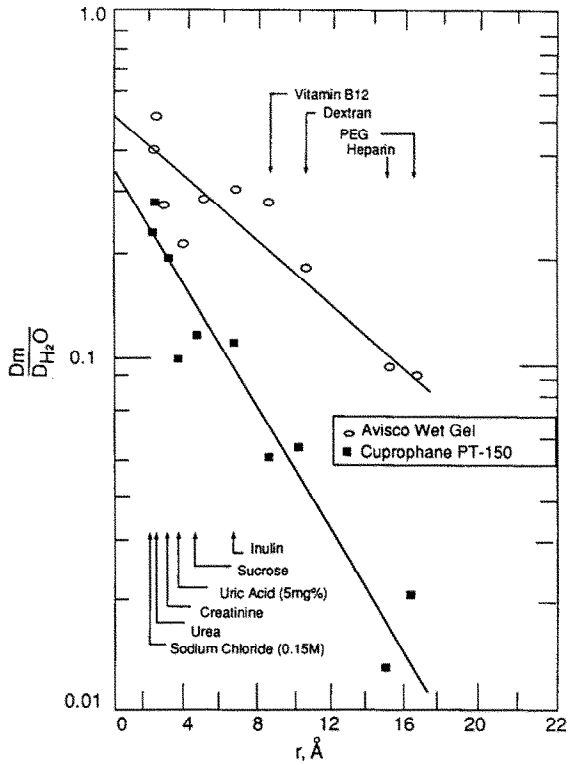

Fig. 16. Diffusion coefficient reduction in porous cellulosic membranes as a function of characteristic molecular radius of solute (Colton et al., 1971).

Boer and Kruisbrink proposed the use of microporous polypropylene tubing (Accurel^R) for the controlled delivery of peptides and proteins (Kruisbrink and Boer, 1984; Boer et al., 1984; Boer and Kruisbrink, 1987). The commercial tubing was loaded with an aqueous solution of vasopressin (VP), sealed at each end, and coated with a collodion membrane (> 60 μm). Pseudo-zero order release (1–100 ng/day) and not the expected first-order kinetics of release was achieved for months in vitro. This kinetic result was attributed to reversible binding of the peptide to the polypropylene surface, which served to maintain a constant activity source within the aqueous interior. The collodion coating prevented proteins entering the device in vivo, and displacing the VP from the polypropylene surface. A miniaturized device, (3 mm^3), introduced into the lateral ventricle of rat brain, produced steady, enhanced levels of VP in the CNS for at least 1 week. Delivery of oxytocin, α-MSH, and methionine-enkephalin was also reported.

Sato and Kim (1984) obtained porous films of a 1:1 copolymer of DL-lactic acid and glycolic acid by casting the polymers from a methanol-chloroform solution. Membranes of this biodegradable polymer were more permeable to glucose and insulin than several porous cellulosic and hydrogel membranes evaluated during the same study. These biodegradable membranes were used as part of an autoregulated insulin delivery system now under development (Cohen et al., 1984).

Schindler used a pore-forming additive to create a reservoir device with a porous membrane suitable for macromolecule delivery. He then demonstrated its use with LHRH analogs (Pitt et al., 1987; Schindler, 1987). In this method, a biodegradable polyester such as polylactic acid or poly(ϵ-caprolactone) is melt extruded as a blend with an inert additive. The resulting tubing, typically 1–2 mm outer diameter, is extracted with a solvent to remove the additive and, in the process, to create a porous wall (Fig. 17). The porosity of the wall is governed by such experimental variables as the choice and amount of additive, the

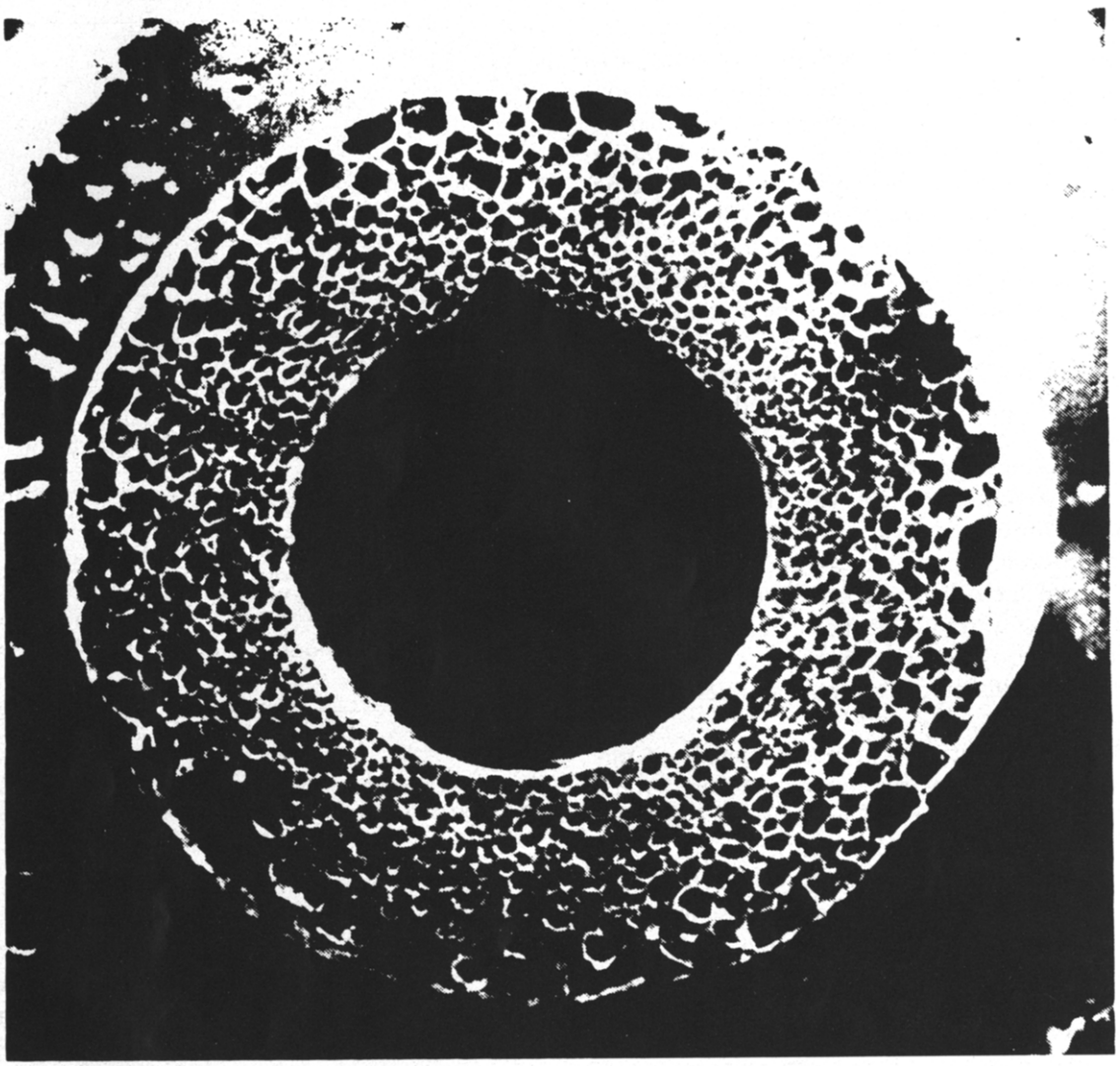

Fig. 17. Scanning electron micrograph of cross-section of a porous capsule of poly(ε-caprolactone) prepared by solvent extraction of an inert additive; magnification ×15. (Micrograph, courtesy of P. Ingram.)

temperature, and the solvent used for extraction. The tubing is suitable for preparation of reservoir (capsule) delivery systems that may be implanted subdermally. The rate of release of macromolecules is dependent on the porosity of the tube wall, and values ranging between 20 and 400 μg per day for [D-Trp^6,desGly^{10}]LHRH ethylamide have been achieved (Fig. 18). These capsules are equally useful for the delivery of higher molecular weight proteins.

Eenink et al. (1985) described the preparation of porous tubing (1 mm o.d.) by coagulation spinning of polymer solutions. The polymers employed were poly-L-lactic acid and a copolymer of γ-ethyl-L-glutamate and γ-piperonyl-L-glutamate, both of which are biodegradable. The porosity of the tube walls was controlled by the choice of solvents for dissolution and coagulation of the tubing, and by inclusion of additives such as monomeric L-lactide and polyvinylpyrrolidone in the polymer solution. The porosity of the tubing was determined by measurement of the rate of

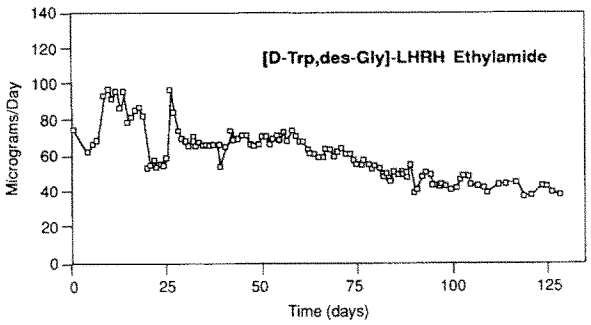

Fig. 18. In vitro rate of release of [D-Trp^6,des-Gly^{10}]LHRH ethylamide from a porous capsule of the type shown in Fig. 17. (Data courtesy of A. Schindler.)

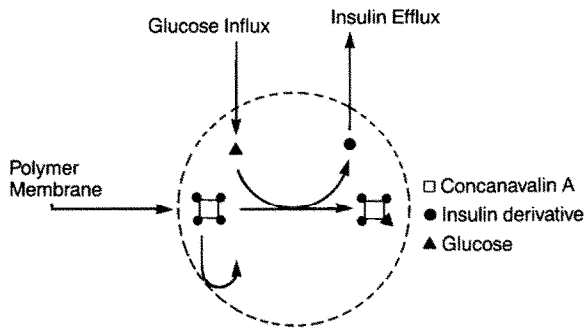

Fig. 19. Schematic of an insulin delivery system consisting of a membrane which selectively retains Con A and its glycosylated insulin complex, while permitting rapid permeation of glucose and glycosylated insulin (after Jeong et al., 1984).

release of the dye cresyl violet acetate (M_w 321) from capsules prepared using the tubing. The application to higher molecular weight species has not been reported, but should be feasible.

A porous alumino-calcium-phosphorus oxide ceramic (ALCAP) is reported (Bajpai, 1985; Bajpai and Benghuzzi, 1988) to be capable of controlled release of a variety of drugs, including testosterone, gossypol, GRH, BSA, bovine γ-globulin, insulin, and chymotrypsinogen. The ceramic can be compounded with polylactic acid and formulated as a hollow cylinder. The release from such cylinders in vitro is determined by the size of the calcined particles.

Self-Regulated Protein Delivery Systems

Self-regulated systems have been the subject of recent reviews (Heller, 1983, 1989; Pitt, 1986). Brownlee and Cerami (1979, 1983) devised a self-regulated insulin delivery system based on the observation that glucose and glycosylated insulins bind strongly and competitively to concanavalin A (Con A), a high molecular weight plant protein (M_w 108 000) with an affinity for carbohydrate residues. The use of this equilibrium (Eqn. 6),

$$\text{glucose} + \text{insulin}^*\text{-Con A} \leftrightarrow \text{glucose-Con A} + \text{insulin}^* \quad (\text{insulin}^* = \text{glycosylated insulin}) \qquad (6)$$

in combination with a porous membrane that is permeable to insulin (M_w 7 000) and glucose, but retains the larger Con A-insulin complex, provides the elements of self-regulation (Fig. 19). The equilibrium constant could be modified by the choice of the sugar conjugate without significant loss of insulin bioactivity. This is of importance if one is to minimize or inhibit displacement of the equilibrium (Eqn. 6) to the right under hyperglycemic conditions and stimulate liberation of insulin under hypoglycemic conditions.

Kim et al. elaborated this system, with studies of the equilibrium constants and biological activities of a range of glycosylated derivatives of insulin, and the characterization of porous membranes to construct an implantable device with the requisite permselectivity. Porous PGLA, cellulose and pHEMA were shown to be useful membranes. Functionality in pancreatectomized dogs was demonstrated (Jeong et al., 1984; Kim et al., 1984; Sato and Kim, 1984; Sato et al., 1984a).

The majority of the other self-regulated systems under development are insulin delivery systems based on the chemistry of the glucose oxidase (GO)-glucose reaction (Eqn. 7):

$$C_6H_{12}O_6 + GO \rightarrow \text{gluconic acid} + H_2O_2 \qquad (7)$$

The reduction in pH associated with the formation of gluconic acid has been used to increase the permeability of membranes to insulin, triggering or enhancing the rate of delivery of the protein in response to an increase in glucose concentration.

For example, Horbett, Ratner and their co-workers immobilized GO in a copolymer of diethylaminoethyl methacrylate (DEAM) and HEMA, crosslinked with tetramethylene glycol dimethacrylate (Horbett et al., 1983; 1984; Albin et al., 1985; Kost et al., 1985c; Ratner and Horbett, 1985). When exposed to 400 mg% of glucose, protonation of the amino groups in the membrane at the lower pH and the associated Donnan equilibrium effect increased the permeability of a membrane to insulin 2.4–5-fold. A similar approach by Ishihara et al. (1984a) used a sandwich membrane. Here GO was immobilized in a crosslinked polyacrylamide gel, which was combined with a pH-sensitive membrane derived from DEAM and 2-hydroxypropyl methacrylate. The water content of the pH-sensitive membrane increased from 30 to 60%, and the insulin permeability from 0.5×10^{-7} to 4×10^{-7} cm^2 s^{-1}, when the pH of the external phase decreased from 7.4 to 6.5. Kinetic and theoretical studies suggested that body fluids do not permeate these pH-sensitive membranes quickly enough to suppress the pH changes created by the glucose-GO reaction (Albin et al., 1987).

Ishihara and Matsui (1986) evaluated varying copolymer ratios of *p*-trimethylsilylstyrene (hydrophobic), HEMA (hydrophilic) and DEAM (pH-responsive) as a means of adjusting the hydrophilicity of the membrane, and hence its permeability to insulin. The water content changed from 30 to 50%, and the permeability from 5.5×10^{-9} to 2.3×10^{-7} cm^2 s^{-1} (a 50-fold increase), when the pH was changed from 6.3 to 6.1 (Fig. 20). The proportion of the monomers also influenced the mechanical strength of the membrane.

The strategy of reversible pH-dependent hydrogel swelling was proposed as a means of driving a glucose-responsive mechanical insulin pump after initial studies showed that an osmotic pump controlled by water permeation through a pH-sensitive membrane was not practical (Siegel and Firestone, 1988).

Fischel-Ghodsian et al. (1988) chose an alternative pH-based approach to self-regulated insulin delivery, and used the pH change of the glucose-GO reaction to alter the solubility of insulin in the

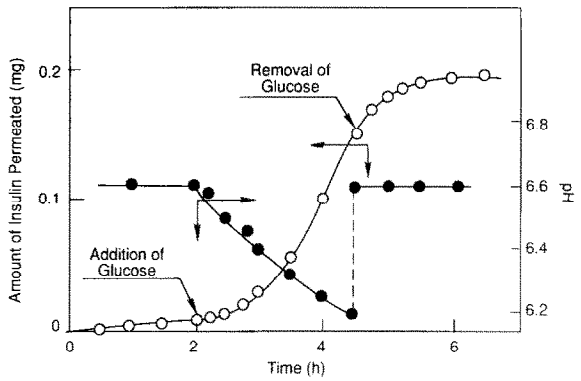

Fig. 20. The effect of addition and removal of glucose on the local pH and the insulin permeability of a membrane containing glucose oxidase. The membrane was prepared from a copolymer of *p*-trimethylsilylstyrene, HEMA and DEAM (Ishihara and Matsui, 1986).

local environment. Because the solubility of insulin (with an isoelectric point of 5.4) increases as the pH decreases from physiological pH, the feasibility of the concept was demonstrated using trilysyl insulin. This physiologically active derivative of insulin has an isoelectric point of 7.4 and becomes more soluble as the pH decreases. Repetitive exposure of a monolithic EVA device containing trilysyl insulin and immobilized glucose oxidase to buffered glucose solutions over several weeks caused insulin release to increase reversibly during each exposure. Polymer-implanted diabetic rats infused with glucose solutions showed a significant increase in insulin concentration in 30 min, an effect not observed in three different sets of control rats.

The other product of Eqn. 7, hydrogen peroxide, has been used to trigger (irreversibly) a change in the hydrophilicity of a membrane derived from polystyrene substituted in the *para* position of the aromatic ring with a dihydropyridine moiety (Ishihara et al., 1983). Oxidation converted this group to a charged pyridinium salt. Only a 50% increase in permeability of the membrane to insulin was produced by this transformation.

Changes in pH have been used to trigger other classes of delivery systems. For example, Seti and Tirrell (1984) showed that acidification of aqueous solutions of polyethacrylic acid (PEA) causes a

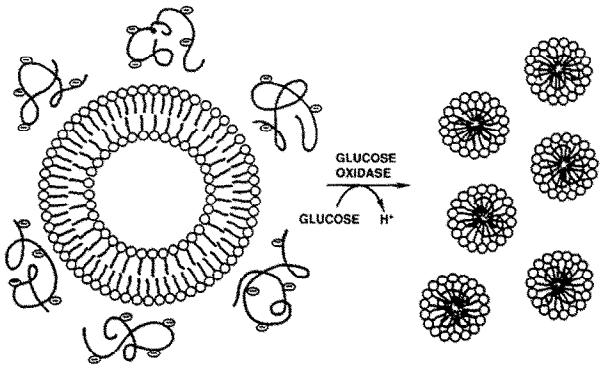

Fig. 21. The concept of a glucose-triggered insulin delivery system based on the observation that bilayer vesicles prepared from phosphatidylcholines and phosphatidylglycerols collapse to a globular form and discharge their contents when exposed to the unionized form of poly(ethacrylic acid) (Devlin and Tirrell, 1986).

conformational transition to a globular structure. The globular form interacts strongly with bilayer vesicles prepared from phosphatidylcholines and phosphatidylglycerols, causing collapse of the bilayer structure and concomitant discharge of the internal content. It was demonstrated that this process (Fig. 21) could be induced by the pH change of the glucose-GO reaction and, as such, provides the essential components of a glucose-triggered delivery system for insulin (Devlin and Tirrell, 1986).

Heller and co-workers (Heller et al., 1985, 1989; Heller, 1989) used the pH change of Eqn. 7 to drive the surface erosion of polyorthoesters. Preparation of polyorthoesters containing a tertiary amine function in the main chain and crosslinked with triethanolamine afforded a matrix for which the hydrolysis of the orthoester bond was suppressed by the basic amino functions. Protonation of the amino groups at the surface of the polymer eliminated the inhibition and reversibly initiated surface erosion and insulin release.

Similar self-regulated and triggered systems have been used for delivery of low molecular weight drugs (Heller, 1983, 1989; Pitt, 1986).

Finally, self-regulated delivery systems based on the encapsulation of living cells should be mentioned. In this case, the primary objective of the materials scientist is to develop a biocompatible environment for the cells which permits access by small nutrient molecules and diffusional release of insulin, but prevents permeation of higher molecular weight endogenous proteins. Several insulin systems derived from microencapsulation of pancreatic islets have been described. Alginate (Lim and Sun, 1980; Goosen et al., 1985; Sun and O'Shea, 1985), agarose (Sefton et al., 1987), and acrylate membranes (Nilsson et al., 1983) have all been successfully employed. Cell encapsulation and protein release are being explored by the biotechnology industry as a means of fluidized bed manufacture of proteins.

Conclusions

It is evident from this review of the literature that a number of approaches to the controlled subdermal administration of peptides and proteins are possible, and that the low diffusivity of high molecular weight proteins is not unduly restrictive. It is also true that none of the approaches has satisfactorily solved problems of protein instability when the required payload dictates a high concentration of peptide. Proteins which aggregate in concentrated (> 5%) aqueous solution, e.g. somatotropins, cannot be formulated in hydrogels or in self-diffusion systems. Biodegradable polymers such as polyesters and polyorthoesters absorb sufficient water that they are unable to prevent the protein forming a concentrated aqueous solution in situ. The only simple method of pulsed subdermal drug delivery is sonic stimulation of diffusion or biodegradation (Kost et al., 1986). Methods of self-regulated delivery, responsive to biological needs, are still in the exploratory stage of development. The foreign body response, with tissue encapsulation of the implant and enhanced proteolytic degradation in the region of the implant, are recognized but largely unquantitated problems (Davis, 1974; Anderson et al., 1981; Goosen et al., 1982).

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