

Review of Osmotic Pressure Driven Release of Proteins from Monolithic Devices

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Dedicated to the memory of Dr. Antoine Noujaim

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ABSTRACT – Purpose: Protein therapeutics are a rapidly growing drug class, with sales in 2004 in the area of \$US 34 billion. They are presently administered primarily by injection, although there is increasing recognition that many proteins would benefit from long-term, localized delivery. Such delivery represents a significant challenge due principally to protein stability concerns. Polymeric delivery systems which rely on osmotic pressure driven drug release may prove to be an effective formulation approach. This paper reviews the evolution of osmotic pressure drug release from polymers, with an emphasis on their potential for protein delivery. It is concluded that osmotic pressure driven release is promising for protein delivery, but there is still a need for *in vivo* demonstration of protein stability and delivery efficacy.

INTRODUCTION

Even before the sequencing of the entire genome, which has recently increased the interest in discovering new protein therapeutics, proteins were recognized as an important therapeutic drug class (1). The reasons for this are that protein therapeutics are highly specific in their action, and are expected to be less toxic than synthetically derived molecules and to behave more predictably *in vivo* (2). The number of new protein drugs gaining market approval has increased dramatically in the last decade, with 21 new FDA approvals in 2005 alone (3). They are also a significant market as they represented a 2004 market of \$US 34 billion, and this business is forecast to reach \$US 52 billion by 2010 (4).

Currently, the administration route of choice of protein drugs is parenteral, because of the

stability requirements of proteins, and the barriers to their absorption by other conventional routes such as oral, nasal, buccal, etc. (1,5). The specific function of a protein is determined by its three-dimensional conformation. A protein's conformation is dependent on relatively weak, noncovalent interactions such as hydrogen bonding, electrostatic interactions, disulfide linkages, hydrophobic interactions and van der Waals interactions. When the forces of these interactions are disrupted, the protein is likely to undergo a structural change (become denatured) and lose its biological activity. Proteins are susceptible to aggregation, denaturation and adsorption at interfaces, deamidation, isomerization, cleavage, oxidation, thiol disulfide exchange, and β -elimination in aqueous solutions (5). The major factors affecting these changes are mechanical forces such as shear, the presence of surfactants, buffers, ionic strength, the presence of oxidizers such as ions, radicals and peroxide, light, pH and temperature. Denaturation of the protein molecule may result in a loss of its activity or may make the protein immunogenic (6).

However, parenteral delivery may not be optimal or suitable for a number of protein drugs. Most therapeutic proteins have a short *in vivo* half-life (7) and, upon injection, are unevenly distributed in the interstitial fluid and unable to reach secluded organs or sites where the therapeutic effect is desired, and they may bind unselectively to cellular receptors and thus cause undesirable side-effects. Furthermore, many therapeutic proteins, for example, cytokines and growth factors, are produced locally to act on cells in the local environment. These proteins are active at very low concentrations and are required in the local tissue site for a prolonged period of time. Thus, administration regimens often consist of multiple injections, which poses problems with patient compliance and possible complications when administered in a non-clinical setting.

A long-term continuous and localized protein drug delivery depot could provide numerous and distinct advantages, both therapeutic and financial, for many therapeutic proteins, and in particular growth factors and cytokines.

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There is increasing appreciation in the pharmaceutical sector that sustained release approaches are necessary to improve the potential of these drugs and to differentiate products from competitors.

Various means of achieving localized delivery of bioactive proteins have been investigated and include the use of liposomes, polymer gels, and biodegradable microspheres. Liposomes are easily administered by injection, but, they produce relatively short drug release durations, inefficient drug loadings are achieved, and release rates are neither sustained nor controllable, with release typically occurring with a large burst of up to 75% of the initially loaded cytokine within the first eight hours (8). Polymer gels can be biocompatible and can be designed to be both pH and temperature responsive, so that varying and controllable release rates are possible. Nevertheless, release of proteins from gels are generally of short duration, typically less than 7 days, (9,10) and the presence of a large amount of water in the device may lead to protein stability problems before they are released from the device.

A more intensively examined formulation approach is encapsulation of the protein within biodegradable polymeric microspheres, and in particular poly(lactide-co-glycolide) (PLG) based microspheres. The microspheres are prepared such that the protein is distributed within the polymer as discrete solid particles. Such microspheres have been developed that are capable of delivering a virtually constant amount of an encapsulated protein (11-13). The protein is released in three phases: an initial burst, followed by diffusion controlled release, and eventually erosion controlled release. The initial burst is due to surface resident protein particles, while the diffusion controlled release is a result of dissolved protein diffusing through the water-filled pores and channels within the microspheres. To obtain a constant release rate from PLG microspheres, the diffusion phase must overlap with the erosion release phase such that new pores or channels are created. Polymeric microspheres have the advantages of not only providing a constant release, but of being easily injected to the target site, providing a long term release duration, consisting of proven biocompatible materials, having a reasonable shelf-life and degrading to completely bioresorbable

compounds.

There are a number of problems with these microspheres, however. For protein drugs such as cytokines that possess high physiological activity at low doses, the burst effect that often is obtained from these microspheres may result in undesired side effects. A larger problem with PLG microspheres as a delivery system is maintenance of protein stability. A typical protein encapsulation process involves using an oil-in water-in oil emulsification procedure in which the protein is dissolved in the aqueous phase. This preparation procedure has been found to result in protein denaturation due to interfacial contact of the protein with the solvent used to dissolve the PLG (14). Moreover, polymers such as PLG degrade by hydrolysis, and generate acidic oligomers and monomers. The presence of these acidic degradation products has been found to decrease the local pH at the surface of the polymer and in the pores and channels of the device (15,16). In fact, the pH at the centre of a PLG microsphere has been determined to be as low as from 1.5(17) to 1.8 (18). This reduction in the pH of the inner environment of the microspheres has been linked to inactivation and denaturation of many proteins within PLG microspheres prior to being released including: interleukin-1 α (19), interferon- γ (20), carbonic anhydrase (21), atriopeptin III (22), ovalbumin (23), protein C (24), trypsin and heparinase (25), interleukin-2 (26), and lysozyme (27). Attempts to overcome problems with decreasing pH during degradation in PLG microspheres have included the incorporation of basic salts into the matrix (28). However, a recent paper, wherein the micro-environmental pH of different size distributions of PLG microspheres was mapped, has demonstrated that the inclusion of a basic excipient does not prevent the internal pH of the microspheres from dropping below 5 over a 3 week period (29), and in fact can result in protein deamidation (30).

It can be appreciated that the numerous potential de-activating factors make formulating long-term, implantable protein delivery systems difficult. A delivery vehicle capable of encapsulating proteins with high and reproducible efficiency, while maintaining protein conformation and thus stability and *in vivo* efficacy, as well as providing predictable controlled release is needed.

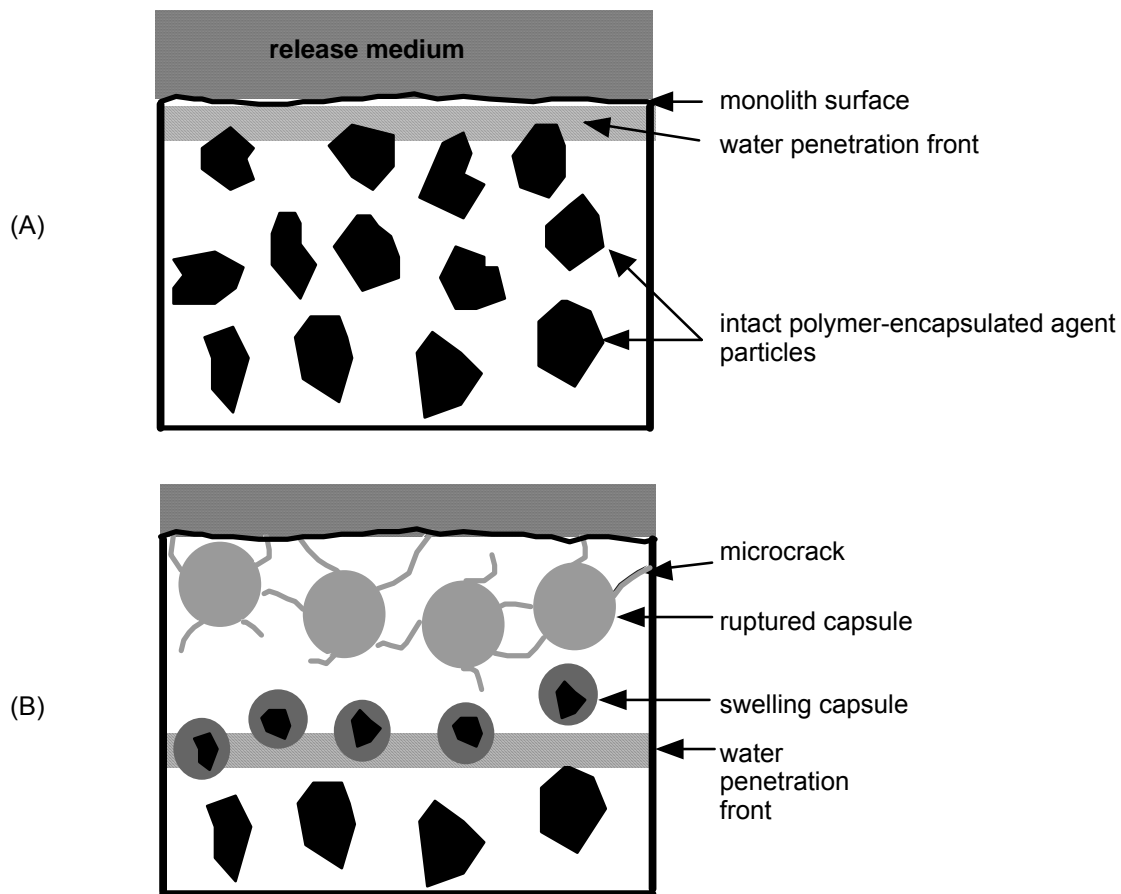


Figure 1. Schematic of the osmotic pressure release mechanism. A) Water vapor partitions into and diffuses through the polymer matrix. B) The presence of the three zones within the matrix: swollen and ruptured capsules with the presence of a microcrack network, swelling capsules that have not ruptured, and dry particles.

Osmotic Activity Driven Release

Instead of utilizing diffusion and/or polymer degradation as release mechanisms to generate a nearly constant release rate from a solid drug loaded polymer matrix, the aqueous osmotic activity of a compound has been used (31-42). This mechanism of achieving constant release was first reported for drug delivery from polymers in a patent filed in 1973 and issued in 1979 (39), although the potential for osmotic pressure driven release of water-soluble compounds from polymers was demonstrated by Marson in 1969, in his examination of the release of copper oxide from a paint film (43), and by Narkis and Narkis in 1976 who measured the extent of salt leaching from polymers such as poly(styrene) and poly(ethylene) (44)

Release Mechanism

Osmotic pressure driven drug release is considered

to proceed in the following manner (Figure 1) (42,45-47). Upon immersion of the device in an aqueous environment, water vapor partitions into and diffuses through the polymer until it encounters a polymer-surrounded drug particle (hereinafter referred to as a capsule). At the particle/polymer interface, the water phase separates and dissolves a portion of the solid particle to form a saturated solution. The water activity in the saturated solution is much less than that in the surrounding aqueous medium, and an activity gradient is established. Under the influence of this activity gradient, water is drawn into the capsule and the capsule swells, generating a pressure equal to the osmotic pressure of the dissolved solid. This pressure is resisted by the viscoelastic nature of the polymer. As the polymer is strained, energy is stored by polymer chain extension, bond bending or bond stretching.

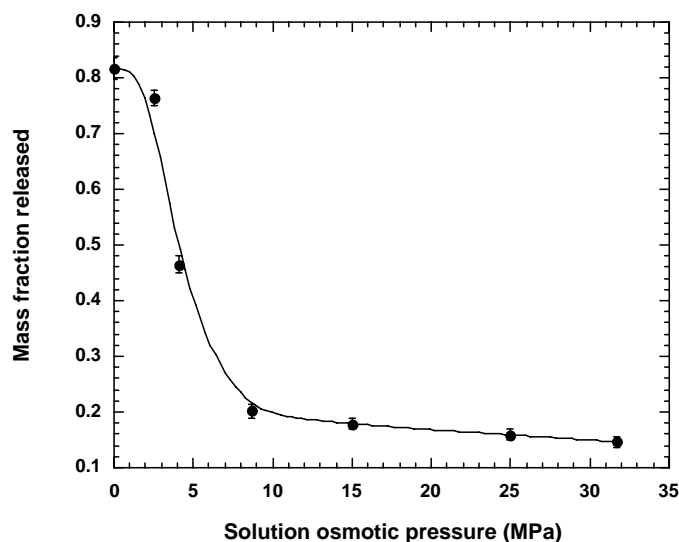


Figure 2. Total mass fraction of lysozyme released from EVA 40 microspheres versus the osmotic activity of the receiving solution (48). The microspheres had diameters of from 0.7 - 0.85 mm, a volumetric loading of lysozyme of 0.35, and the lysozyme particle size was < 53 μm .

This energy is dissipated if bond breakage or viscoelastic flow occurs. Bond breakage initiates crack formation in the polymer bulk. The crack formed connects the contents in the capsule to a pore network that ultimately extends to the surface of the device. The capsule contents are forced through the pore network under the pressure differential between the capsule and the external medium. This process occurs in a particle layer-by-particle layer manner throughout the device. If the intra-capsule osmotic pressure is insufficient to initiate crack formation, thermodynamic equilibrium is reached and the capsule contents are not released.

This release mechanism has been supported by experimental observation. Schirrer *et al.* (42) and Riggs *et al.* (46), prepared cylindrical samples of poly(dimethylsiloxane) rubber containing NaI and NH_4F , respectively. At specific times, Schirrer *et al.* sectioned the cylinders and examined their core structure, while Riggs *et al.* used ^1H NMR to determine the state of water within the rubbery matrix. These researchers independently found that three zones were present once salt release was established: 1) an outer layer, which was transparent, where the salt had been released, 2) an intermediate layer wherein the polymer-encased salt particle regions were swollen with water, and 3) an inner layer, which was dry and white. Further support for this release mechanism was obtained by

examining the total fraction of lysozyme released from poly(ethylene vinylacetate) microspheres immersed into saline solutions of varying osmotic activity. As the osmotic pressure of the saline solution increased, the total fraction of lysozyme initially loaded into the microspheres decreased (Figure 2) (48). The important factors controlling osmotically driven release are solute osmotic activity, the solute loading in the device, particle size, device geometry, and polymer properties such as hydraulic permeability, modulus and tear resistance (31,33,35,37,42,49,50). Increasing the osmotic activity of the solute, relative to the osmotic activity in the surrounding medium, results in an increase in release rate, for a given polymer, solute size and volumetric loading. Increasing polymer hydraulic permeability, modulus, or tear resistance results in a decrease in release rate. For brittle polymers, osmotic pressure induced microcrack formation is present, however, the release rate is not zero order. This was demonstrated by Zhang *et al.*, who examined the release of gentamicin sulfate from poly(D,L-lactide) coated poly(D,L-lactide) cylinders (51). Poly(D,L-lactide) has a glass transition temperature of 55-60 $^\circ\text{C}$, and so is brittle at body temperature and before significant degradation by hydrolysis occurs. These researchers showed that the release rate of gentamicin sulfate from the cylinders decreased as the osmolality of the external medium increased, yet, the kinetics of the release remained diffusional.

An increase in volume fraction of the particles produces a faster release, and as the particle size decreases, the release rate decreases. For slab geometries, the release rate is zero order for much of the release duration; at least until the final layer of particles has swollen and generated microcracks. However, for cylinders, a zero order release rate only can be approximated for up to a mass fraction released of 60% of the initially loaded particles (42,52), and for spheres, only for approximately a mass fraction released of 25% (48). This is because for cylinders and spheres, where release occurs predominantly in the radial direction, the total mass of particles per layer decreases as the penetrating water front moves through the device. Osmotic pressure driven release only dominates if the total volumetric loading of the particles in the polymer matrix is less than the percolation threshold (50). The percolation threshold is defined as the volume fraction of dispersed particles at which enough particles are touching so as to form a path spanning the thickness of the device. For most geometries, this percolation threshold value is a volume fraction of about 0.33 (53). Above the percolation threshold, release is no longer zero order, but, the release rate increases as the osmotic activity of the compound forming the particle increases (50), indicating that

the osmotic pressure mechanism is still present.

Mathematical Modeling

Mathematical models are often useful in the design of delivery systems, as they provide insight into the relative influence of parameters affecting the release rate and duration. Osmotic delivery from non-degradable polymers has been modeled, based on the layer-by-layer release mechanism described above. The mass of agent released by osmotic pressure induced polymer rupturing, m , with time, t , can be expressed as (36),

$$\frac{dm}{dt} = \frac{M_L}{t_b + t_p} \quad (1)$$

in which M_L is the mass of agent released per cross-sectional layer of the device, t_b is the time required to generate cracks due to capsule swelling, and t_p is the time during which solution is forced from a ruptured capsule. With the assumption that the time required to generate cracks is significantly greater than t_p , expressions for the release of agent from slabs (50), cylinders (52), and spherical (48) geometries have been developed:

for a slab:
$$\frac{m_t}{m_T} = 2\theta(1 - F_D) \left(\frac{x}{L} \right) \left(\frac{t}{t_b} \right) \quad (2)$$

for a cylinder:
$$\frac{m_t}{m_T} = \frac{2\theta(1 - F_D)}{1 + \frac{R}{x}} \left[\left(2 + \frac{x}{R} \right) \left(\frac{t}{t_b} \right) - \frac{x}{R} \left(\frac{t}{t_b} \right)^2 \right] \quad (3)$$

for a sphere:
$$\frac{m_t}{m_T} = \frac{\theta(1 - F_D)}{\left[a \left(\frac{R}{x} \right) - \frac{b}{2} \left(\frac{R}{x} \right)^2 + \frac{c}{3} \left(\frac{R}{x} \right)^3 \right]} \left[a \left(\frac{t}{t_b} \right) - \frac{b}{2} \left(\frac{t}{t_b} \right)^2 + \frac{c}{3} \left(\frac{t}{t_b} \right)^3 \right] \quad (4)$$

where:

m_t = the cumulative mass of agent released at time t

m_T = the total mass of agent initially loaded into the device

θ = the fraction of capsules per layer that rupture

F_D = the fraction of particles released by dissolution and diffusion

x = the thickness of a particle layer (i.e. the average wall thickness plus the average particle diameter)

t_b = the time required to induce a rupture in the capsule

L = the thickness of a slab

R = radius of sphere or cylinder

$a = 3(x/R) + 3(x/R)^2 + (x/R)^3$

$b = 6(x/R)^2 + 3(x/R)^3$

$c = 3(x/R)^3$

Use of the equations above requires a means of calculating t_b . Assuming that each capsule swells isotropically, t_b is given by,

$$t_b = \frac{r_o^2}{k_w} \int_1^{\lambda} h' \left(\Pi - \frac{E}{6} \left(5 - \frac{4}{\lambda} - \frac{1}{\lambda^4} \right) \right)^{-1} d\lambda \quad (5)$$

in which λ is the radial extension ratio of the swelling capsule, λ_c is the ultimate radial extension ratio of the swollen capsule upon crack formation, Π is the osmotic pressure of the dissolved solution within the capsule, E is the modulus of the polymer, r_o is the average particle radius, k_w is the water permeability of the polymer, and h' is a dimensionless parameter that represents the degree to which the thickness of the polymer wall surrounding the capsule has changed during water imbibition (absorption causing swelling) into the capsule. h' is given by,

$$h' = (\lambda^3 + \xi)^{1/3} - \lambda \quad (6)$$

$$\xi = \left(\frac{\bar{h}}{r_o} + 1 \right)^3 - 1 \quad (7)$$

and \bar{h} is the average distance between encapsulated particles. ξ can be considered a dimensionless structural parameter describing the initial particle distribution within the device. \bar{h} can be calculated using,

$$\bar{h} = \frac{d L \left(1 - \phi^{1/3} \right)}{L \phi^{1/3} + d} \quad (8)$$

where d is the particle diameter and L is the length of a cube of volume equal to the volume of the slab, cylinder, or sphere.

Other models have also been proposed. The first was derived by Wright *et al.*, who assumed that osmotic water imbibition into the capsules continues after cracks are formed, and that this water imbibition results in continued pumping of solution out of the ruptured capsules (36). This assumption was demonstrated to be incorrect by an analysis of the contributions of both diffusion and convection from a ruptured capsule (50). Schirrer *et al.* (42) also derived a model wherein they assumed

that the volumetric flux of water into the capsule during swelling was constant. It has recently been demonstrated that this assumption is also invalid (52).

Protein Delivery

Although osmotic delivery can be used for highly osmotically active drugs such as pilocarpine nitrate (33,39) or salts such as NaI (42) alone, proteins often do not possess sufficient osmotic activity to generate the pressure required to induce crack formation. To overcome this problem, Carelli, *et al.* (31) prepared particles containing both an osmotic agent (NaCl) and a protein (bovine serum albumin (BSA)), with mass fractions of BSA in the particles of 0.35 and 0.70. These particles were dispersed in a silicone elastomer at volumetric loadings up to 0.28, considered to be below the critical volumetric loading. They found that the BSA was released at a constant rate (Figure 3), and that NaCl was also released at a constant, but different, rate. Their results indicated that, in their systems, release was controlled by both diffusion and convection via an osmotic pressure driving force. Taking this approach further, Amsden and Cheng demonstrated that BSA and NaCl could be released at the same rate from poly(ethylene vinylacetate) slabs of 40% vinylacetate composition (EVA 40), if the amount of protein in protein/NaCl particles was reduced to less than 5 wt% (38). Moreover, proteins of varying molecular weight and properties could be released at the same rate, as the release was driven predominantly by the osmotic activity of the NaCl, and the protein was simply carried along in the capsule solution after the formation of microcracks. These approaches, however, suffer from the fact that highly concentrated solutions of NaCl are released into surrounding tissue, which is likely to result in significant irritation. More recently, Kajihara *et al.* have prepared poly(dimethylsiloxane) (PDMS) elastomers containing particles comprised of human serum albumin (HSA), interferon- α (IFN- α), with or without added excipients (34,35). The particle consisted predominantly of HSA, and two device configurations were examined: a cylinder, and a cylinder with a protein-free PDMS coating with ends uncovered. The particles were embedded in the biomedical grade silicone using a room temperature cure process over 3 days. This low temperature, and the lack of moisture, may prevent protein denaturation during product manufacture.

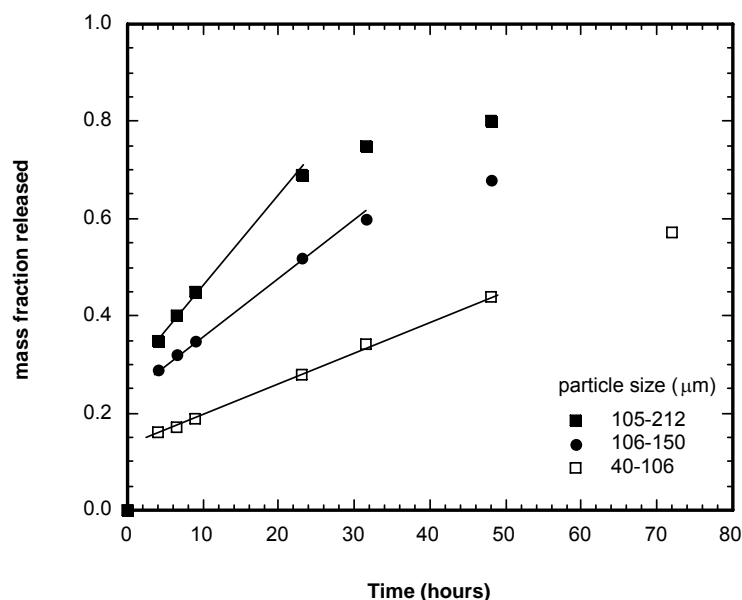


Figure 3. Mass fraction of BSA released from slabs (1 cm diameter, 0.3 cm height) of BSA:NaCl (35% BSA) particles embedded in poly(dimethylsiloxane) elastomer (particle volumetric loading of 0.28). The data are from Carelli *et al.*(31). The solid lines represent the region over which BSA release is approximately constant.

Release of IFN- α from the uncoated cylinders followed diffusionally controlled kinetics, while the coated cylinders exhibited prolonged periods of nearly constant release (Figure 4). Covering the cylinder radially with a polymer film effectively precludes radial release and converts the cylinder into a slab, which, as discussed, generates a more linear release profile. Inclusion of more osmotically active excipients into the particles, such as glycine, sodium glutamate, and NaCl, for the covered cylinders, produced release rates that increased in direct proportion to the osmotic activity of the dissolved particles. The osmotic pressure of a 0.5 g/ml solution of the dissolved particles. The osmotic pressure of a saturated solution of BSA, which has a molecular weight essentially equivalent to HSA (66 kDa), is approximately 12 atm (54). Previous work with EVA 40, showed that BSA was incapable of generating sufficient osmotic pressure to induce microcrack formation. The crosslinking conditions for the PDMS used by Kajihara *et al.*, therefore, likely produced a lower tensile strength than EVA 40. Kajihara *et al.* used addition-cured PDMS, which becomes stiffer and stronger as the crosslinking temperature is increased. The low crosslinking temperature used possibly resulted in a weak elastomer, although, Kajihara *et al.* did not provide mechanical property testing data.

Kajihara *et al.*, have also demonstrated the feasibility of the covered rod cylinder approach to deliver IFN- α to nude mice. The cylinders were implanted subcutaneously and IFN- α was detectable in the serum for 28 days (35). Additionally, IFN- α delivered from uncovered rods was shown to be effective in suppressing engrafted tumor growth in nude mice (34). This delivery device has also been implanted in sheep to deliver the antigen avidin, and demonstrated to induce antibody titres of greater magnitude and duration than soluble vaccines or the uncovered cylinder implant with adjuvant, but only if the adjuvant IL-1 β was included in the formulation (55). Thus, this osmotic pressure driven formulation appears to have potential as a protein delivery system. There are, however, some unresolved issues with this delivery system. Although IFN- α was detected in mice serum, and shown to induce an anti-tumor effect, the relative amounts of bioactive IFN- α were never measured. Also, PDMS is non-biodegradable, and so, if implanted, the polymer would eventually need to be explanted, necessitating an additional surgery. Moreover, due to its non-biodegradability, it is possible that not all of the incorporated protein in the device will be released, as some embedded particles may be surrounded by a polymer wall too thick to be cracked by the swelling pressure (37).

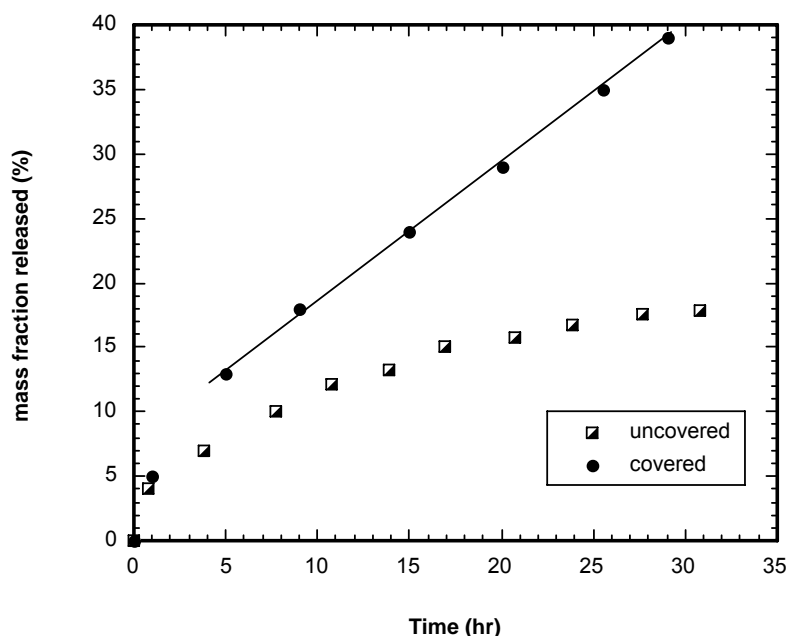


Figure 4. Data from Kajihara *et al.* for IFN- α release from uncovered (34) and covered (35) PDMS cylinders. The cylinders contained 30 w/w% particles, the particles had diameters between 53-150 μm , and each cylinder was 1 cm long. The uncovered cylinders had a diameter of 5 mm while the covered cylinders had a diameter of 1.9 mm.

An interesting possibility for improving the total fraction releasable, as well as a means of externally manipulating the release rate, has recently been proposed by Aschkenasy and Kost (56). By applying low-frequency ultrasound to salt loaded EVA 40 disks, they demonstrated that the release rate could be increased by a factor of from 30-500 (Figure 5). The mechanism provided to explain these results was cavitation pressure assisting in the rupturing of the polymer walls surrounding swelling, particle capsules. Although effective for low molecular weight salts, it remains to be demonstrated that this ultrasound approach will be effective for proteins, and that the pressures and localized heating that results from cavitation would not denature a protein within the device. Additionally, this approach still relies on the use of a non-degradable polymer that would ultimately need to be retrieved through an additional surgical procedure. An alternative strategy that has been tried is to use a biodegradable elastomer. To accomplish this, a photo-crosslinkable biodegradable elastomer was prepared from terminally acrylated *star*-poly(ϵ -caprolactone-*co*-D,L-lactide). This macromer can be rapidly crosslinked using an ultraviolet- or visible light-initiated free radical reaction. The mechanical

properties of the elastomer are readily alterable by varying the macromer molecular weight or composition. It is composed of monomers used to prepare polymers currently used in FDA approved devices, such as biodegradable sutures. The elastomer degrades *in vivo* primarily by hydrolysis, at a rate comparable to that observed *in vitro*. The mechanical properties of the elastomer are retained for approximately 4 weeks *in vivo*, after which the elastomer becomes weaker and softer (57).

This elastomer has been investigated in the delivery of three therapeutic proteins: interferon- γ (IFN- γ), interleukin-2 (IL-2), and vascular endothelial growth factor (VEGF) (58,59). These proteins were co-lyophilized with BSA and trehalose to form particles that were composed predominantly of BSA and trehalose. The lyophilized powder was ground using a mortar and pestle and sieved to yield particles of less than 145 μm diameter, and then mixed with 7800 g/mol or 2700 g/mol macromer dissolved in tetrahydrofuran. To make a slab shaped elastomer, the suspension of lyophilized powder distributed in the macromer solution was photo-polymerized in a rectangular mould (6x3x1 mm³). To form a cylindrical device, the suspension was photo-polymerized in a cylindrical glass mold

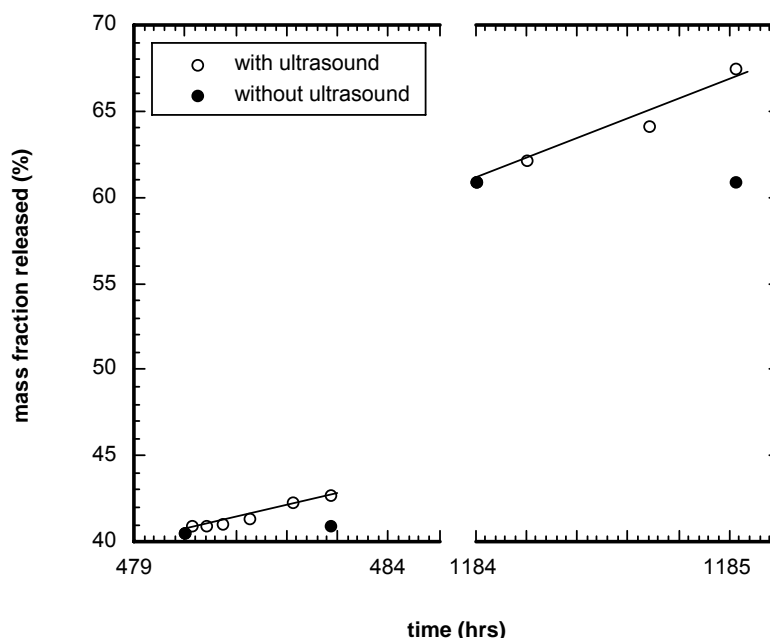


Figure 5. Ultrasound induced enhanced release from EVA 40 discs containing 35 w/w% NaCl (56). The intensity of sonic radiation was 4 W/cm² with a cycling frequency of 200 ms on and 800 ms off.

(diameter:length, 2:20 mm). Photopolymerization was performed by exposure to long-wave UV light (320 – 480 nm) at room temperature using a relative intensity of 100 mW/cm² for 2 min, followed by solvent evaporation. The amount of trehalose was fixed at 50% and 70% (v/v) of the lyophilized particles. The total volumetric loading of lyophilized particles in the final elastomer matrix was fixed at 10% (v/v) of the elastomer volume.

For the slabs, *in vitro* IFN- γ release into phosphate buffer was dependent on the trehalose content of the particle (Figure 6). Without trehalose in the device, IFN- γ release was slow and incomplete. As the amount of trehalose in the particle increased, the release rate increased and was constant for a substantial portion of the release duration. The release was complete within 4 weeks, and thus hydrolytic degradation of the polymer had little influence on the release mechanism since little mass or mechanical property changes were observed for this polymer over that time frame. Under the same formulation conditions, IL-2, IFN- γ and VEGF were released at essentially the same rate from cylinders (Figure 7), and the release was essentially constant for a significant portion of the release duration. Similar results were obtained for release from slabs. The release rate could be manipulated through the choice of macromer molecular weight

used in preparing the elastomer, increasing as the macromer molecular weight decreased (59), and thus as the modulus of the elastomer increased, as has been noted with PDMS (42).

Although a constant release rate over a portion of the release duration was demonstrated, it is important that the released protein retain its bioactivity. The bioactivities of VEGF, IFN- γ and IL-2 released from ELAST 7800 cylinders and recovered in the released media are shown in Figure 8. Despite the release rates of VEGF, IL-2 and IFN- γ being similar, their bioactivities differed. For all three proteins, over 70% of the protein released during the first week was bioactive; their bioactivities then decreased during the subsequent week. There was no major influence of trehalose loading concentration on the bioactivities of the three proteins. VEGF was more susceptible to protein deactivation than either IL-2 or IFN- γ ; over 70% of the VEGF released from ELAST 7800 rods was bioactive during the first 12 days, after which the bioactive fraction decreased to 73% on day 13 and to 40% by day 16. Furthermore, there was little effect of macromer molecular weight on protein bioactivity.

The source of the protein bioactivity was traced to a decrease in microenvironmental pH within the

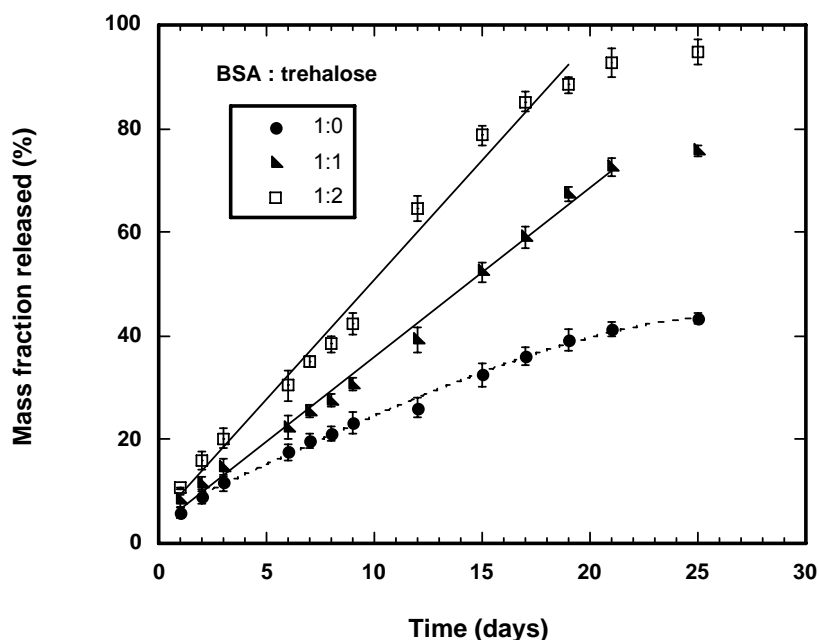


Figure 6. *In vitro* release profiles of IFN- α from biodegradable elastomers slabs prepared from 7800 g/mol macromer (73) showing the effect of increasing trehalose content on the release of IFN- α from the slabs.

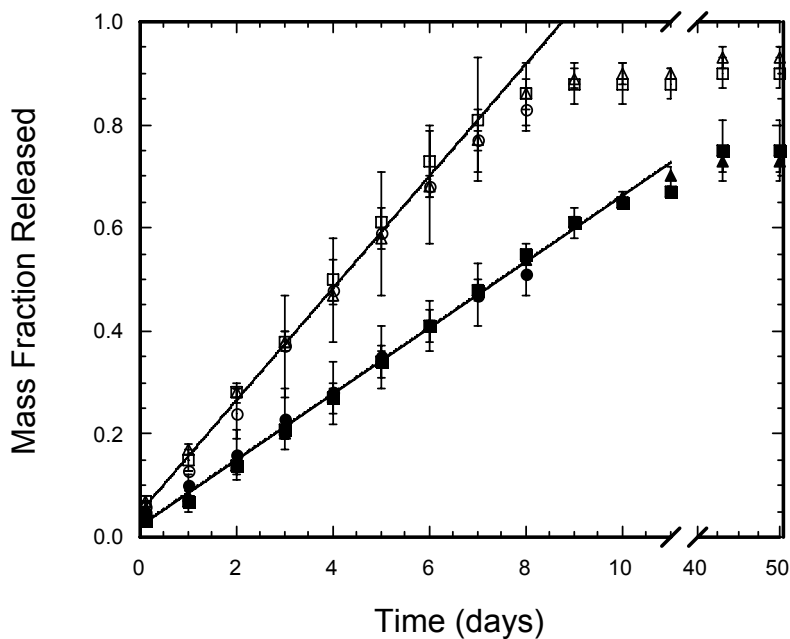


Figure 7. Release kinetics of VEGF (squares), IFN- α (triangles), and IL-2 (circles) from elastomers containing protein particles co-lyophilized with 50% (closed) and 70% (open) trehalose (59). The elastomers were prepared using 7800 g/mol macromer.

degrading devices; the pH within the releasing cylinders decreased to below 5 after only 11 days. IFN- γ does not aggregate in the pH range of 5 and 6 at temperatures up to 50°C (60) and the tertiary structure of IL-2 is stable across a pH range of 4 to 7 (61). VEGF, however, undergoes rapid deamidation of the Asn-10 residue on its C terminal side when the pH is below 7, resulting in a loss of up to 80% of its bioactivity (62). The mildly acidic environment inside the elastomer thus had a significant denaturant effect on VEGF, but not on IFN- γ or IL-2.

Despite not retaining complete protein bioactivity, the bioactivity results obtained represent an improvement over previously published bioactivity results for IFN- γ and IL-2. Yang and Cleland encapsulated IFN- γ in PLG microspheres, and showed that only 38% of the IFN- γ released within the first week was bioactive (20), and Sharma *et al.*, reported that only 40% of IL-2 released from nanospheres made of fumaric and sebacic acid was bioactive after one week of release (63). Thomas *et al.*, reported that the bioactivity of IL-2 released from double emulsion based PLG (50/50) formulations was around 80% for the first 5 days then rapidly dropped to less than 40% by day 10 (64). Using the osmotic delivery mechanism, protein bioactivity was greatly improved, due to the

protein being released before significant elastomer bulk degradation occurred.

Limitations and Future Directions

The osmotic pressure based delivery approach has potential for providing long-term and localized therapeutic protein delivery. There are, however, technical limitations to be overcome. Non-degradable polymers are not likely to gain significant patient compliance due to the need for their explanation. A biodegradable elastomer approach has merit, however, the polymers investigated to date may not be suitable for many proteins that are more acid-sensitive. The polymer composition must therefore be re-examined. This problem may possibly be solved by exchanging the D,L-lactide monomer of the star copolymer for trimethylene carbonate. Trimethylene carbonate undergoes ring-opening polymerization with ϵ -caprolactone (65-69), has a low glass transition temperature (65,70), does not degrade to form acidic products, and is biocompatible (71,72). Another area for future work is the curing process for the biodegradable elastomer. In our own photo-crosslinking approach, ultra-violet light has been used to initiate the cross-linking reaction. Ultra-violet radiation is known to denature proteins, and so visible light photo-crosslinking should be explored. Alternatively, thermoplastic elastomers

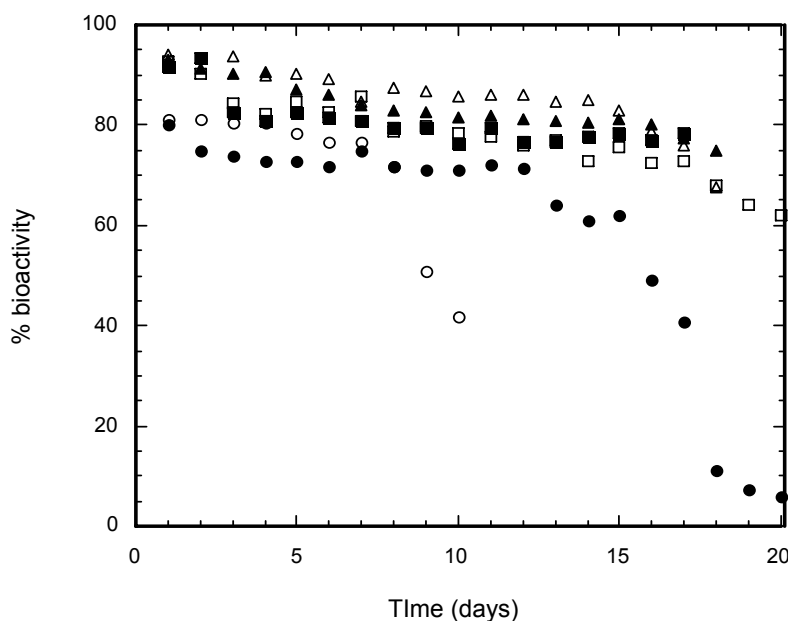


Figure 8. Bioactivity profiles of VEGF (circles), IL-2 (triangles) and IFN- γ (squares) from biodegradable elastomer cylinders prepared using a 7800 g/mol macromer (59). Each protein was co-lyophilized with 50% (open) and 70 w/w% (closed) trehalose.

could be used, which do not require curing. However, elasticity with these polymers require the formation of crystalline domains that degrade very slowly.

The mathematical models currently developed and presented above are only valid for a non-degradable polymer. Modeling release from a degradable elastomer would be much more complicated, as the time dependence of the change in modulus and water permeability of the elastomer must be taken into account. Nevertheless, the development of such a model may lead to insights into methods of better designing these delivery systems.

Despite the ability of osmotically controlled release to generate near constant release rates of protein drugs, there have been very limited studies on their *in vivo* performance. There have been no studies on the tissue response to the implanted devices during drug release and the influence it has on protein bioavailability, and no demonstration that the constant release obtained *in vitro* is reproduced *in vivo*. The *in vivo* data of Kajihara *et al.*, although promising, is incomplete. Moreover, the biodegradable elastomer system has not yet been demonstrated to be effective *in vivo*.

CONCLUSIONS

Protein therapeutics represent a large market and one that would benefit from effective polymeric delivery systems. Despite significant efforts to achieve this goal, formulations based on delivery systems such as liposomes, gels, and biodegradable polymer microspheres have proven unsuccessful at accomplishing both long term delivery and protein stability maintenance. As an alternative delivery approach, osmotic pressure induced rupturing of the polymer matrix is capable of extended periods of constant release while maintaining protein bioactivity. However, improvements in polymer design to avoid protein denaturation and demonstrations of efficacy of the osmotic pressure delivery approach *in vivo* are required before a device based on this approach is likely to reach the clinic.

REFERENCES

[1] Lee, V. Peptide and protein drug delivery systems. *Biopharm*, March 24-31, 1988.

[2] Frokjaer, S., Otzen, D.E. Protein drug stability: A formulation challenge. *Nature Reviews Drug Discovery*, 4: 298-306, 2005.

[3] Rader, R. *Biopharmaceutical Approvals Review*. Genetic and Engineering News, August 70-74, 2006.

[4] Pavlou, A.K., Reichert, J.M. Recombinant protein therapeutics - success rates, market trends and values to 2010. *Nature Biotechnology*, 22: 1513, 2004.

[5] Cleland, J.L., Powell, M.F., Shire, S.J. The development of stable protein formulations: a close look at protein aggregation, deamidation, and oxidation. *Critical Rev. Therap. Drug Carrier Sys.*, 10: 307-377, 1993.

[6] Lee, V.H.L. Changing Needs in Drug Delivery in the Era of Peptide and Protein Drugs, in *Peptide and Protein Drug Delivery*, V.H.L. Lee, Editor. 1991, Marcel Dekker: New York. p. 1-56.

[7] Langer, R. Bioavailability of macromolecular drugs and its control, in *Controlled Drug Bioavailability*. 1985. p. 307-363.

[8] van Slooten, M.L., Storm, G., Zoepfel, A., Kupcu, Z., Boerman, O., Crommelin, D.J.A., Wagner, E., Kircheis, R. Liposomes containing interferon-gamma as adjuvant in tumor cell vaccines. *Pharm. Res.*, 17: 42-48, 2000.

[9] Yu, H., Grainger, D.W. Modified release of hydrophilic, hydrophobic and peptide agents from ionized amphiphilic gel networks. *J. Control. Release*, 34: 117-127, 1995.

[10] Fujioka, K., Takada, Y., Sato, S., Miyata, T. Novel delivery system for proteins using collagen as a carrier material: the minipellet. *J. Control. Release*, 33: 307-315, 1995.

[11] Takada, S., Uda, Y., Toguchi, H., Ogawa, Y. Preparation and characterization of copoly(d,l-lactic/glycolic acid) microparticles for sustained release of thyrotropin releasing hormone by double nozzle spray drying method. *J. Control. Release*, 32: 79-85, 1994.

[12] Mehta, R.C., Thanoo, B.C., Deluca, P.P. Peptide containing microspheres from low molecular weight and hydrophilic poly(d,l-lactide-co-glycolide). *J. Control. Release*, 41: 249-257, 1996.

[13] Sah, H., Toddywala, R., Chien, Y.W. Continuous release of proteins from biodegradable microcapsules and in-vivo evaluation of their potential as a vaccine adjuvant. *J Control Release*, 35: 137-144, 1995.

[14] van de Weert, M., Hennink, W.E., Jiskoot, W. Protein instability in poly(lactic-co-glycolic acid) microparticles. *Pharm. Res.*, 17: 1159-1167, 2000.

[15] Gopferich, A., Gref, R., Minamitake, Y., Shieh, L., Alonso, M.J., Tabata, Y., Langer, R. Drug Delivery From Bioerodible Polymers: Systemic and Intravenous Administration, in *Formulation and*

- Delivery of Proteins and Peptides, J.L. Cleland and R. Langer, Editors. 1994, ACS: Washington.
- [16] Mader, K., Gallez, B., Liu, K.J., Swartz, H.M. Non-invasive in vivo characterization of release processes in biodegradable polymers by low-frequency electron paramagnetic resonance spectroscopy. *Biomaterials*, 17: 457-461, 1996.
- [17] Fu, K., Pack, D.W., Klibanov, A.M., Langer, R. Visual evidence of acidic environment within degrading poly(lactic-co-glycolic acid) (PLGA) microspheres. *Pharm. Res.*, 17: 100-106, 2000.
- [18]. Shenderova, A., Burke, T.G., Schwendeman, S.P. Evidence for an acidic microclimate in PLGA microspheres, in *Proceed. Int'l. Symp. Control. Rel. Bioact. Mater.* 1998, Controlled Release Society: Las Vegas. p. 265-266.
- [19] Chen, L., Apte, R.N., Cohen, S. Characterization of PLGA microspheres for the controlled delivery of IL-1alpha for tumor immunotherapy. *J. Control. Release*, 43: 261-272, 1997.
- [20]. Yang, J., Cleland, J.L. Factors affecting the in vitro release of recombinant human interferon-g from PLGA microspheres. *J. Pharm. Sci.*, 86: 908-914, 1997.
- [21] Park, T.G., Lu, W., Crotts, G. Importance of in vitro experimental conditions on protein release kinetics, stability and polymer degradation in protein encapsulated poly(D,L-lactic acid-co-glycolic acid) microspheres. *J. Control. Release*, 33: 211-222, 1995.
- [22] Johnson, R.E., Lanaski, L.A., Gupta, V., Griffin, M. J., Gaud, H.T., Needham, T.E., Zia, H. Stability of atriopeptin-III in poly(D,L-Lactide-co-glycolide) microspheres. *J. Control. Release*, 17: 61-67, 1991.
- [23] Takahata, H., Lavelle, E.C., Coombes, A.G.A., Davis, S.S. The distribution of protein associated with poly(DL-lactide co-glycolide) microparticles and its degradation in simulated body fluids. *J. Control. Release*, 50: 237-246, 1998.
- [24] Zambaux, M.F., Bonneaux, F., Gref, R., Dellacherie, E., Vigneron, C. Preparation and characterization of protein C-loaded PLA nanoparticles. *J. Control. Release*, 60: 179-188, 1999.
- [25] Tabata, Y., Gutta, S., Langer, R. Controlled delivery systems for proteins using polyanhydride microspheres. *Pharmaceutical Research*, 10: 487-496, 1993.
- [26] Hora, M.S., Rana, R.K., Nunberg, J.H., Tice, T.R., Gilley, R.M., Hudson, M.E. Controlled release of interleukin-2 from biodegradable microspheres. *Biotechnology*, 8: 755-758, 1990.
- [27] Aubert-Pouessel, A., Bibby, D.C., Venier-Julienne, M.-C., Hindre, F., Benoit, J.-P. A novel in vitro delivery system for assessing the biological integrity of protein upon release from PLGA microspheres. *Pharm. Res.*, 19: 046-1051, 2002.
- [28] Zhu, G.Z., Mallery, S.R., Schwendeman, S.P. Stabilization of proteins encapsulated in injectable poly (lactide-co-glycolide). *Nature Biotechnology*, 18: 52-57, 2000.
- [29] Li, L., Schwendeman, S.P. Mapping neutral microclimate pH in PLGA microspheres. *J. Control. Release*, 101: 163-173, 2005.
- [30] Houchin, M., Neuenswander, S., Topp, E. Effect of excipients on PLGA film degradation and the stability of an incorporated peptide. *J. Control. Release*, accepted, 2006.
- [31] Carelli, V., Di Colo, G., Guerrini, C., Nannipieri, E. Drug release from silicone elastomer through controlled polymer cracking: An extension to macromolecular drugs. *Int. J. Pharm.*, 50: 81-188, 1989.
- [32] Di Colo, G. Controlled drug release from implantable matrices based on hydrophobic polymers. *Biomaterials*, 13: 850-856, 1992.
- [33]. Gale, R., Chandrasekaran, S.K., Swanson, D., Wright, J. Use of osmotically active therapeutic agents in monolithic systems. *J. Membrane Sci.*, 7: 319-331, 1980.
- [34] Kajihara, M., Sugie, T., Mizuno, M., Tamura, N., Sano, A., Fujioka, K., Kashiwazaki, Y., Yamaoka, T., Sugawara, S., Urabe, Y. Development of a new drug delivery system for protein drugs using silicone (I). *J. Control. Release*, 66: 49-61, 2000.
- [35] Kajihara, M., Sugie, T., Hojo, T., Maeda, H., Sano, A., Fujioka, K., Sugawara, S., Urabe, Y. Development of a new drug delivery system for protein drugs using silicone (II). *J. Control. Release*, 73: 279-291, 2001.
- [36] Wright, J., Chandrasekaran, S.K., Gale, R., Swanson, D. Model for the release of osmotically active agents from monolithic polymeric matrices. *AIChE Symp. Ser.*, 77(206), 1981.
- [37] Amsden, B. G., Cheng, Y.L. Enhanced fraction releasable above percolation threshold from monoliths containing osmotic excipients. *J. Control. Release*, 31: 21-32, 1994.
- [38] Amsden, B.G., Cheng, Y.L. A generic protein delivery system based on osmotically rupturable monoliths. *J. Control. Release*, 33: 99-105, 1995.
- [39] Michaels, A.S., Guillod, M.S. Osmotic bursting drug delivery device, US patent, 4,117,256 (1979).
- [40] McGinity, J.W., Hunke, L.A., Combs, A.B. Effect of water-soluble carriers on morphine release from a silicone polymer. *J. Pharm. Sci.*, 68: 662-664, 1979.
- [41] Golomb, G., Fisher, P., Rahamim, E. The relationship between drug release rate, particle size and swelling of silicone matrices. *J. Control. Release*, 12: 121-132, 1990.
- [42] Schirrer, R., Thepin, P., Torres, G. Water absorption, swelling, rupture and salt release in salt-

- silicone rubber compounds. *J. Mater. Sci.*, 27: 3424-3434, 1992.
- [43] Marson, F. Anti-fouling paints: I. Theoretical approach to leaching of soluble pigments from insoluble paint vehicles. *J. Appl. Chem.*, 19: 93-99, 1969.
- [44] Narkis, N., Narkis, M. Slow release of water-soluble salts from polymers. *J. Appl. Polym. Sci.*, 20: 3431-3436, 1976.
- [45] Fedors, R.F. Osmotic effects in water absorption by polymers. *Polymer*, 21: 207-212, 1980.
- [46] Riggs, P.D., Kinches, P., Braden, M., Patel, M.P. Nuclear magnetic imaging of an osmotic water uptake and delivery process. *Biomaterials*, 22: 419-427, 2001.
- [47] Marson, F. Antifouling paints II. A more detailed examination of the effect of pigment volume concentration. *J. Appl. Chem. Biotech.*, 24: 515-527, 1974.
- [48] Amsden, B. Osmotically activated protein release from electrostatically generated polymer microbeads. *AIChE J.*, 42: 3253-3266, 1996.
- [49] Carelli, V., Dicolo, G., Nannipieri, E. Factors in zero-order release of clonidine hydrochloride from monolithic polydimethylsiloxane matrices. *Int. J. Pharm.*, 35: 21-28, 1987.
- [50] Amsden, B.G., Cheng, Y.L., Goosen, M.F.A. A mechanistic study of the release of osmotic agents from polymeric monoliths. *J. Control. Release*, 30: 45-56, 1994.
- [51] Zhang, X., Wyss, U., Pichora, D., Goosen, M.F.A. A mechanistic study of antibiotic release from biodegradable poly(D,L-lactide) cylinders. *J. Control. Release*, 31: 129-144, 1994.
- [52] Amsden, B. A model for osmotic pressure driven release from cylindrical rubbery polymer matrices. *J. Control. Release*, 93: 249-258, 2003.
- [53] Siegel, R., Kost, J., Langer, R. Mechanistic studies of macromolecular drug release from macroporous polymers. I. Experiments and preliminary theory concerning completeness of drug release. *J. Control. Release*, 8: 223-236, 1989.
- [54] Vilker, V.L., Colton, C.K., Smith, K.A. The osmotic pressure of concentrated protein solutions: Effect of concentration and pH in saline solutions of Bovine Serum Albumin. *J. Coll. Int. Sci.*, 79: 548-566, 1981.
- [55] Kemp, J.M., Kajihara, M., Nagahara, S., Sano, A., Brandon, M., Lofthouse, S. Continuous antigen delivery from controlled release implants induces significant and anamnestic immune responses. *Vaccine*, 20: 1089, 2002.
- [56] Aschkenasy, C., Kost, J. On-demand release by ultrasound from osmotically swollen hydrophobic matrices. *J. Control. Release*, 110: 58, 2005.
- [57] Amsden, B.G., Tse, M.Y., Turner, N.D., Knight, D.K., Pang, S.C. In vivo degradation behavior of photo-cross-linked star-poly(epsilon-caprolactone-co-D,L-lactide) elastomers. *Biomacromolecules*, 7: 65, 2006.
- [58] Gu, F., Neufeld, R.J., Amsden, B. Osmotic driven release kinetics of bioactive therapeutic proteins from a biodegradable elastomer are linear, constant, similar and adjustable. *Pharm. Res.*, 23: 782-789, 2006.
- [59] Gu, F., Neufeld, R.J., Amsden, B. Sustained release of bioactive therapeutic proteins from a biodegradable elastomeric device. *J. Control. Release*, accepted.
- [60] Mulkerrin, M., Wetzel, R. pH-Dependence of the reversible and irreversible thermal-denaturation of gamma-interferons. *Biochemistry-US.*, 28: 6556-6561, 1989.
- [61] Ricci, M.S., Sarkar, C.A., Fallon, E.M., Lauffenburger, D.A., Brems, D.N. pH Dependence of structural stability of interleukin-2 and granulocyte colony-stimulating factor. *Protein Science*, 12: 1030-1038, 2003.
- [62] Goolcharran, C., Cleland, J.L., Keck, R., Jones, A. J.S., Borchardt, R.T. Comparison of the rates of deamidation, diketopiperazine formation, and oxidation in recombinant human vascular endothelial growth factor and model peptides. *AAPS Pharmsci.*, 2, 2000.
- [63] Sharma, A., Sharma, U.S. Liposomes in drug delivery: progress and limitations. *Int. J. Pharm.*, 154: 123-140, 1997.
- [64] Thomas, T., Kohane, D., Wang, A., Langer, R. Microparticulate formulations for the controlled release of interleukin-2. *J. Pharm. Sci.*, 93: 1100-1109, 2004.
- [65] Zhu, K.J., Hendren, R.W., Jensen, K., Pitt, C.G. Synthesis, properties, and biodegradation of poly(1,3-trimethylene carbonate). *Macromolecules*, 24: 1736-1740, 1991.
- [66] Ruckenstein, E., Yuan, Y.M. Molten ring-open copolymerization of L-lactide and cyclic trimethylene carbonate. *J. Appl. Polym. Sci.*, 69: 1429-1434, 1998.
- [67] Grijpma, D.W., Pennings, A.J. (Co)Polymers of L-Lactide. 1. Synthesis, Thermal-Properties and Hydrolytic Degradation. *Macromol. Chem. Phys.*, 195: 1633-1647, 1994.
- [68] Jie, C., Zhu, K.J., Yang, S.L. Preparation, characterization and biodegradable characteristics of poly(1,3-trimethylene carbonate-co-glycolide). *Polymer Int.*, 41: 369-375, 1996.
- [69] Jie, C., Zhu, K.J. Preparation, characterization and biodegradable characteristics of poly(D,L-lactide-co-1,3-trimethylene carbonate). *Polymer Int.*, 42: 373-379, 1997.
- [70] Pego, A.P., Poot, A.A., Grijpma, D.W., Feijen, J. Copolymers of trimethylene carbonate and epsilon-caprolactone for porous nerve guides: Synthesis and

- properties. *J. Biomater. Sci. Polym. Edn.*, 12: 35-53, 2001.
- [71] Cai, J., Zhu, K.J., Yang, S.L. Surface biodegradable copolymers - poly(D,L-lactide-co-1-methyl-1,3-trimethylene carbonate) and poly(D,L-lactide-co-2,2-dimethyl-1,3-trimethylene carbonate): preparation, characterization and biodegradation characteristics in vivo. *Polymer*, 39: 4409-4415, 1998.
- [72] Pego, A.P., Van Luyn, M.J.A., Brouwer, L.A., van Wachem, P.B., Poot, A.A., Grijpma, D.W., Feijen, J. In vivo behavior of poly(1,3-trimethylene carbonate) and copolymers of 1,3-trimethylene carbonate with D,L-lactide or epsilon-caprolactone: Degradation and tissue response. *J. Biomed. Mater. Res. A*, 67A: 1044-1054, 2003.
- [73] Gu, F., Younes, H.M., El-Kadi, A.O.S., Neufeld, R. J., Amsden, B.G. Sustained interferon-gamma delivery from a photocrosslinked biodegradable elastomer. *J. Control. Release*, 102: 607-617, 2005.