ELSEVIER

International Journal of Pharmaceutics 116 (1995) 1–9

international journal of pharmaceutics

Invited Review

# Recent advances on the use of biodegradable microparticles and nanoparticles in controlled drug delivery

Lisa Brannon-Peppas

*Biogel Technology, Inc., 9521 Valparaiso Court, Indianapolis, IN 46268, USA*

Received 16 June 1994; modified version received 3 October 1994; accepted 5 October 1994

**Abstract**

The possibility of using biodegradable polymers as drug carriers was brought to the attention of many scientists when bioresorbable sutures entered the market two decades ago. Since that time, researchers in pharmacy, chemical engineering, and other disciplines have striven to design biodegradable polymers with desired degradation mechanisms and mechanical properties. Biodegradable polymers have advantages over other carrier systems in that they need not be surgically removed when drug delivery is completed and that they can provide direct drug delivery to the systemic circulation. The drug and polymer may be combined in a number of different ways depending upon the application of interest. Microparticulate formulations have the widest applicability to the widest variety of formulation needs: oral delivery, intramuscular injection, subcutaneous injection, and targeted delivery. This review addresses recent work utilizing biodegradable polymers for controlled drug delivery, focusing on micro- and nanoparticulate delivery systems containing poly(lactic acid), poly(glycolic acid) or their copolymers.

*Keywords:* Controlled release; Biodegradable polymer; Microparticle; Nanoparticle

## 1. Introduction

The most widely used and studied class of biodegradable polymers is the polyesters, including poly(lactic acid), poly(glycolic acid), and their copolymers. Poly(glycolic acid) (henceforth referred to as PGA) was first marketed in 1970 as a biodegradable suture and poly(lactic acid) (henceforth referred to as PLA) was investigated as a drug delivery material as early as 1971. By varying the monomer ratios in the polymer processing and by varying the processing conditions, the resulting polymer can exhibit drug release capabilities for months or even years (Lewis, 1990). The degree of crystallinity has a significant effect on the rate of degradation. These polymers, which have been prepared as films, microparticles, rods, and other forms, display a bulk erosion hydrolysis.

Biodegradable polymers have long been of interest in controlled release technology because of the ability of these polymers to be reabsorbed by the body. This alleviates the need for removal, often surgically, of a drug release device. Knowledge and skill in the field of biodegradable polymer technology is progressing rapidly enough that

0378-5173/95/$09.50 
*SSDI* 0378-5173(94)00324-6

researchers have at their disposal a substantial number of degradable polymers with a range of degradation rates. Not only may researchers use a single polymer, copolymer, or blend, but they may also use a combination of polymers.

## 2. Drug delivery systems using biodegradable microparticles and nanoparticles of poly(lactic acid) and poly(lactic-co-glycolic acid)

There are a large number of research groups, worldwide, examining poly(lactic-co-glycolic) acids (PLA/PGA), especially in the form of microparticles and nanoparticles, for use in controlled drug delivery systems. Most researchers utilize a solvent evaporation technique, or modification thereof, to prepare microparticles or nanoparticles of PLA/PGA (Grandfils et al., 1992; Ike et al., 1992; Yamakawa et al., 1992; Alonso et al., 1993; Fawaz et al., 1993; Iwata and McGinity, 1993; Niwa et al., 1993; Scholas et al., 1993; Verrecchia et al., 1993; Yamaguchi and Anderson, 1993; Zhifang et al., 1993). Other particles are prepared by grinding of larger slabs (Mauduit et al., 1993a,b,c) or by a salting-out process (Allemann et al., 1993).

### *2.1. Microparticulate systems*

In order to analyze the in vivo biocompatibility of microspheres of Medisorb 65/35 PLA/PGA, Yamaguchi and Anderson (1993) injected microparticles into the back of the side of rats and monitored their response for 150 days. They observed only mild inflammation and unimpaired wound healing throughout the study. At 150 days, the microparticles completely degraded with minimal inflammatory response. The conclusion was that PLA/PGA polymers were good biocompatible materials from implantation through complete degradation. The injection vehicles used were aqueous dextran solutions at 262 mg microparticles/2 ml or 87 mg microparticles/ml. It was also observed that microspheres of a size 1–20 $\mu$m were phagocytosed, as determined by their being surrounded by macrophages and without foreign body giant cell attachment. In vitro work has also shown that microparticles less than 12 $\mu$m in diameter are phagocytosable by macrophages.

In a series of papers, Mauduit et al. (1993a,b,c) studied the release a local antibiotic, gentamicin sulfate, from both microparticles and films of amorphous and semicrystalline poly(lactic acid)s. Microparticles prepared from amorphous materials showed a burst of drug release, followed by 2 months of sustained release. Microparticles prepared from semicrystalline PLA, in contrast, released all of the drug within 6 h, a result only of morphological differences between the microparticles, not differences in biodegradation rates. In this case, the microparticles from amorphous PLA were formed by mixing gentamicin sulfate with a polymer/acetone solution and allowing the acetone to evaporate, then grinding the remaining polymer/antibiotic mixture. Chloroform was used as the solvent for preparation of microparticles from semi-crystalline PLA. The semi-crystalline polymer preparation technique produced porous microparticles, probably due to variations in the solvent evaporation rate. It is therefore important to control the solvent evaporation rate in all microparticle preparation techniques so as to have reproducible particle morphology.

Further studies (Mauduit et al., 1993c) evaluated the differences between the release of gentamicin base and gentamicin sulfate, with the findings that not only were there differences in physical appearance between the two types of formulations (those with gentamicin sulfate were waxy whereas those with gentamicin base were more solid) but also differences in release behavior. The microparticles containing gentamicin base released nearly 50% of their drug in a burst at the beginning of the experiment, and essentially no additional drug for the remaining 24 days of the study. Those microparticles prepared with gentamicin sulfate released at a high but steady rate for the first 4 days, then at a substantially lower rate for the remaining 21 days of the study. To further elucidate these behaviors, a third study involved preparation of films of gentamicin sulfate with PLAs of various types. It was noted that the degradation rate of the PLA depended upon the presence of gentamicin. Inter-

actions between the carboxylic end groups and the antibiotics were thought to be the cause.

Other drug delivery studies with various PLA/PGA and PLA microspheres included release of norethisterone (Zhifang et al., 1993) which showed drug release over 96 h in vitro and from intramuscular injections in rats for 45 days in vivo. Release of a neurotensin analogue (Yamakawa et al., 1992) in vitro showed a 20% burst of release, followed by release for 1 month with PLA of molecular weight (Mol. Wt) 2000. However, with PLAs of Mol. Wt 4000 and 6000, the in vitro release demonstrated a smaller burst, followed by a lag time of 2–3 weeks with no significant release, then release for at least 5 more weeks. Studies with the anti-cancer drug cisplatin (Ike et al., 1992) showed in vitro release for 30–57 days, and in vivo for 21–42 days from microparticles prepared from PLA of Mol. Wt 12000.

A recent study compared the immune response due to intraperitoneal or subcutaneous administration of microparticles of PLA/PGA containing ovalbumin, which is a poor immunogen (O'Hagan et al., 1991) The microparticles elicited a significantly greater response than unencapsulated ovalbumin. The microparticles were prepared by solvent evaporation technique and had an average particle size of approx. 5 $\mu$m. It was found that particles of less than 6–7 $\mu$m diameter are effectively phagocytosed by various macrophage populations which can allow delivery of entrapped drugs intracellularly to the cells responsible for immune response initiation. The preliminary results from this study showed that PLA/PGA microparticles are an effective antigen delivery system that can induce potent primary and secondary immune responses.

Biodegradable microspheres have also been studied as an embolic material (Grandfils et al., 1992). Microparticles of PLA, again prepared by a solvent evaporation procedure, were injected intravenously and were designed to stop blood flow in areas surrounding tumors before surgery to reduce hemorrhagic complications during surgery. Particles of 100–160 $\mu$m diameter, injected at a concentration of 5 mg/ml, were found to be successful in this endeavor because their size is appropriate to reach the precapillary arterioles.

### *2.2. Hybrid microparticulate systems: microparticles in implants and films*

While biodegradable microparticles have proven to be useful in a wide range of controlled drug delivery applications, our research group has investigated opportunities for utilizing biodegradable microparticles in composite or hybrid systems with other biodegradable or nondegradable systems (Brannon-Peppas, 1992, 1994; Brannon-Peppas et al., 1994). The release rates and profiles of both hydrophilic drugs (gentamicin sulfate) and hydrophobic drugs ($\beta$-estradiol) have been shown to be significantly changed when the biodegradable microparticles containing these drugs are incorporated into silicone (nondegradable) or gelatin (degradable) films. These studies utilized low molecular weight PLA/PGA polymers of ratios 50:50, 65:35 and 75:25.

The drug release from the microparticles within the silicone films does not exhibit the initial high burst of release in vitro from free microparticles. However, except for systems with high drug loadings ($\geq 40\%$) and high microparticle loadings ($\geq 30\%$), the majority of the gentamicin sulfate was not released from these formulations. These microparticle-silicone composite systems are currently under investigation for delivery of proteins and peptides which would not otherwise be able to be released from traditional solid silicone implants. Incorporation of microparticles into gelatin films only slightly decreased the initial burst of drug release, but greatly increased the length of time of the drug release.

### *2.3. Systems prepared by modification of traditional solvent evaporation techniques*

In order to evaluate the possibility of using serum albumin instead of poly(vinyl alcohol) as a stabilizer during the preparation of biodegradable microparticles of poly(lactic acid), Verrecchia et al. (1993) measured both the bound and free serum albumin present on the surface of

PLA nanoparticles prepared by a solvent evaporation process. It was found that the amount of adsorption is directly related to the surface area of the particles. A part of the serum albumin was irreversibly bound, and a portion was reversibly bound. The researchers do not suggest that any portion of the serum albumin is physically entrapped within the nanoparticles, but 35–45% of the albumin is permanently bound to the particles.

Tetanus vaccines, prepared with both 50:50 PLA/PGA and PLA, were prepared as microparticles using modified solvent evaporation and solvent extraction procedures (Alonso et al., 1993). Depending upon whether the tetanus toxoid was incorporated into the microparticles as a solid or as an aqueous solution, the release behavior differed slightly. Lower molecular weight polymers showed bursts of 25–35% release in the first day, then slower release. Higher molecular weight polymers (50:50 PLA/PGA, Mol. Wt 100 000) released less than 10% of the drug in the first day, but also had only released 25% of the total loaded amount after 27 days. The advantage of a solvent extraction method described is that microspheres are formed within 30 min as opposed to several hours with traditional solvent evaporation techniques.

A multiple-emulsion solvent evaporation technique was used to prepare conventional and multi-phase PLA/PGA microspheres containing water-soluble compounds (Iwata and McGinity, 1993). Because of the high water solubility of some drugs, traditional solvent evaporation methods to prepare microparticles with these drugs yield very low drug loading efficiencies because of drug partitioning into the aqueous phase from the polymer phase. This new method yielded particles with many distinct zones of drug within a main polymer matrix. The conventional method yielded a more homogeneous mixture of polymer and drug throughout the microparticle. For release of brilliant blue and chlorpheniramine maleate, this study did not show any burst of drug release for either type of formulation, yet the multi-phase microspheres consistently showed a larger amount of drug released that did the corresponding conventional microparticles.

### 2.4. Nanoparticulate systems

While microparticles have been prepared using PLA and PLA/PGA for many years, nanoparticles of these materials are fairly new and are the result of modifications of existing preparation techniques and the realization that sub-micron particles could find utility in particular drug targeting applications. It has been found that PLA nanoparticles injected intravenously are taken up by cells of the mononuclear phagocyte system, mainly the Kuppfer cells (Fawaz et al., 1993). This may naturally concentrate these particles close to liver parenchymal cells and facilitate biliary clearance and enterohepatic circulation. In general, such nanoparticles are rapidly cleared from the blood and are concentrated in the liver, spleen and blood marrow.

PLA nanoparticles containing savoxepine, a new neuroleptic drug, were prepared by a reversible salting-out process (Allemann et al., 1993). A cross-flow filtration technique utilizing magnesium chloride hexahydrate and magnesium acetate tetrahydrate as salting-out agents resulted in a 90% entrapment of the drug, in a process which takes only 3 h to prepare one batch of nanoparticles.

Nanospheres of PLA/PGA (75:25) of diameters less than 200 nm have also been prepared especially for site-specific delivery (Scholas et al., 1993). Biodistribution of injected colloidal carriers is highly dependent upon their size and their surface properties. For example, for targeted administration to the lung, particles should be several microns in diameter. If particles are larger than 250–300 nm they will be captured by filtration in the spleen.

Nanospheres containing indomethacin or 5-fluorouracil (as model water-insoluble and water-soluble drugs) were prepared using a modified solvent evaporation technique with a high-speed homogenizer. The resulting particles were found to be 400–600 nm in diameter (Niwa et al., 1993) This study found that the release of indomethacin into phosphate-buffered saline was highly dependent upon the polymer molecular weight, with faster release from the nanoparticles prepared from the polymer with the lowest

molecular weight (Mol. Wt 12279 vs 66671 vs 127598).

## 3. Protein and peptide delivery from PLA/PGA particulate systems

Efforts are also continuing on delivery of proteins and peptides from biodegradable microparticles (Sanders et al., 1985; Hora et al., 1990; Heya et al., 1991). Controlled release of interleukin-2 and variations from 50:50 PLA/PGA microspheres, on the order of 50–200 $\mu$m in diameter, showed a burst of release, followed by a period of extremely low release for days 6–15, ending with nearly constant release through 30 days (Hora et al., 1990). Thyrotropin-releasing hormone (TRH) release was studied from microparticles prepared from 75:25 PLA/PGA and 100% PLA (Heya et al., 1991). The microparticles were prepared using a modified solvent evaporation system based upon a water/oil/water emulsion system and were approx. 50 $\mu$m in diameter. The injection vehicle used was an aqueous solution containing 1% sodium carboxymethylcellulose and 0.5% Tween 80. Lower molecular weight polymers gave larger bursts of release of TRH and faster overall release (Mol. Wt 5000, 6000, 8000 and 11000). Drug release was faster than the polymer weight loss, indicating that the drug diffused through the channels formed in the polymer at advanced stages of degradation. Release of nafarelin (LHRH analogue) was found to follow a triphasic pattern with an initial burst, then a low rate of release, followed by a higher rate of release in some early work carried out on this system (Sanders et al., 1985) Microparticles of 50:50 PLA/PGA with 10–50 $\mu$m diameters showed in vitro release for up to 40 days and in vivo activity for even up to 70 days. A similar system is now marketed worldwide and is known as the Lupron depot.

## 4. Delivery of anti-cancer agents from PLA/PGA particulate systems

Some studies have addressed the encapsulation of anti-cancer agents into PLA and PLA/PGA microparticles (Wada et al., 1988a,b). Aclacinomycin (or aclarubicin hydrochloride) is an anti-cancer agent which has many undesirable side effects such as nausea, vomiting, anorexia, leukocytopenia and thrombocytopenic toxicities. It would be desirable to target delivery of this drug to only cancerous tissues. Microspheres were prepared by traditional solvent evaporation methods using PLA in a variety of molecular weights (3600, 4000, 4800, 7200 and 10000) (Wada et al., 1988a) The drug release rate was found to be strongly dependent upon both molecular weight and drug loading rate. Microparticles of higher molecular weight polymers showed release in vitro for more than 35 days. Adriamycin and cisplatin, other anti-cancer drugs, have also been encapsulated into PLA and PLA/PGA by traditional solvent evaporation methods (Wada et al., 1988b) These formulations were prepared with quite high drug loadings of 50–70 wt%, with in vitro release only lasting for up to 17 days.

## 5. Delivery of vaccines from PLA/PGA particulate systems

Another advantage of biodegradable drug delivery systems is that they can be designed to deliver vaccines in a number of pulses from a single injection of microencapsulated drug. A significant effort, in concert with the World Health Organization, is in place in a number of institutions to develop vaccine delivery systems for developing countries. For example, biodegradable microspheres of PLA/PGA 50:50 with only 1% of the toxoid vaccine of staphylococcal enterotoxin B (SEB) dramatically increased the circulating IgG anti-toxin toxoid when compared to free toxoid (Eldridge et al., 1991). This study used a mixture of microspheres of 1–10 $\mu$m and 20–50 $\mu$m. This mixture gave an initial release from the smaller microspheres and a later release from the population of microspheres with the larger size. They were administered by intraperitoneal injection in mice. Higher antibody levels were seen for at least 90 days upon a single injection of the mixed population of microspheres. The response of the microparticles of size less than 10 $\mu$m after

injection appears to be both a depot effect as well as because of the rapid phagocytosis of the microspheres by antigen-presenting accessory cells such as T cells. Other efforts include delevopment of oral delivery systems based upon biodegradable microparticles (Eldridge et al., 1989). Orally administered microspheres, containing SEB toxoid, of 1–10 $\mu$m are taken up by Peyer's patch lymphoid tissue of the gut, and those of size 5–10 $\mu$m can remain there for up to 35 days, providing controlled release.

Some studies have also been conducted using the diphtheria toxoid delivered with PLA microparticles of Mol. Wt 49 000 (Singh et al., 1991). These formulations have shown release up to 75 days in vivo, comparable to traditional treatment requiring three injections as opposed to a single effective injection with microparticle formulation. The microparticles prepared by a variation of the solvent evaporation method, were implanted subcutaneously and were of the size 30–100 $\mu$m.

## 6. Other forms of PLA / PGA used for drug delivery

Recent work has been reported with tablets or slabs of PLA/PGA (Omelczuk and McGinity, 1992; Asano et al., 1993; Fischel-Ghodsian and Newton, 1993; Pistner et al., 1993; Zhang et al., 1993) including analysis of drug release kinetics from slabs with and without surrounding rate-limiting membranes (Fischel-Ghodsian and Newton, 1993) and the effect of the polymer glass transition and molecular weight of the drug release behavior of theophylline (Omelczuk and McGinity, 1992). Release of albumin from rods prepared from PLA/acetone suspensions which were then coated with pure PLA was found to be most strongly dependent upon the geometry of the system (rod length compared to cross-sectional area) and drug loading (Zhang et al., 1993) Melt-pressed formulations of calcitonin with PLA showed an initial burst of drug release and complete release within 3 days for polymers of Mol. Wt 1400, and 24 days for Mol. Wt 4400 (Asano et al., 1993). A long-term degradation study of PLA in vivo was conducted using rods and blocks of high molecular weight PLA (120 000, 200 000 and 429 000) (rods $25 \times 3 \times 2$ mm, blocks $3 \times 3 \times 2$ mm) which were implanted into the dorsal muscle of rats (Pistner et al., 1993). The lower specimens were totally degraded with 1 year and the moderate molecular weight specimens nearly degraded in 2 years. All polylactides were incorporated well, forming a collagenous fibrous layer without tissue irritation.

Vert et al. (1992) have evaluated the biocompatibility of biodegradable polymers and the mechanism of drug release from them (Shah et al., 1992). It has been found that the initial degradation of the polyesters is hydrolytic, and that during the latter stages, enzymatic degradation may also take place (Vert et al., 1992). The release of drug from these degrading polymers is a combination of diffusion and degradation, which each mechanism predominating at a specific time in the degradation of the polymer (Shah et al., 1992). Initial drug release may be in large part due to diffusion, depending upon the drug that is releasing. The increase in release rate often seen with drug release from biodegradable microparticles is due to the kinetics of the polymer weight loss after bulk hydrolysis has progressed to a point where there is measurable weight loss from the polymer instead of simply a decrease in the average molecular weight as occurs early in the biodegradation process.

## 7. PLA / PEG and PLA / PEO block copolymers for drug delivery

Some work has also been performed to combine poly(ethylene glycol) and poly(lactide-co-glycolide)s in the same drug delivery formulation. The research groups that have addressed this opportunity have usually taken the route of preparing block copolymers of PLA and PEG or PEO (Cohn and Younes, 1988; Zhu et al., 1990). Much of this work has been spurred by the fact that PEG is non-toxic and has been cleared by the US Food and Drug Administration for internal use in the human body (Zhu et al., 1990). Release in vitro of norethisterone (30 wt% drug) from copolymers of PLA and PEG showed no

greater a burst of release than from PLA alone and a significantly higher rate of release, when compared to PLA alone, from that point on.

Block copolymers of PLA and PEO have been investigated with the aim of developing a new family of biodegradable polymers (Cohn and Younes, 1988). These new polymers ranged from 20 to 84% PLA and the PEO chains were of Mol. Wt 600–6000 (which in reality qualify them as PLA/PEG copolymers). The incorporation of the PEO into the polymer yielded a highly hydrophilic material, with equilibrium water contents higher than 60%. For hydrolytically sensitive drugs such as proteins, this type of material is usually not desirable because the stability of the drug may often be compromised when in such a water-filled environment. PLA/PGA polymers are excellent for protein and peptide release because of their hydrophobic nature which serves to protect the drugs from the fluid in the in vivo environment and thus increase their stability as well as control their release. These new copolymers were successful, however, in combining the material strength and hydrophobicity of the PLA with the elasticity and hydrophilicity of the PEO but no drug delivery data was presented.

Studies have shown that subcutaneously administered nanoparticles are taken up by the lymph nodes (Trubetskoy et al., 1993). PEG attached to individual drug molecules has been shown to significantly increase the drug circulation time because of the masking effect of the PEG and its resulting lack of uptake into the reticulo-endothelial system (Katre, 1993). PEG is the most extensively studied method for extending circulating half-life of proteins (Davis et al., 1991) PEG is a linear, uncharged, hydrophilic, nonimmunogenic molecule that has been used to modify a large number of compounds, including trypsin, superoxide dismutase, catalase, adenosine deaminase, bovine serum albumin, asparaginase, uricase, lipase, hemoglobin, interleukin-2 and arginase. At least six PEG-enzyme conjugates have reached clinical trials: PEG-adenosine deaminase (for severe combined immunodeficiency disease, SCID); PEG-antigen E (for ragweed hay fever); PEG-asparaginase (for acute lymphoblastic, lymphocytic and undifferentiated leukemias and for lymphomas); PEG-honeybee venom (for reperfusion injury associated with organ transplantation); and PEG-uricase (for hyperuricema associated with chemotherapy or gout).

For use in magnetic resonance imaging contrasting agents, liposomes were studied which were unmodified as well as surface modified with dextran or PEG. PEG-modified liposomes showed an imaging efficiency of more than twice that of the other liposomes. PEG-coated 'stealth' nanospheres have been prepared by using diblock copolymers of PLA/PGA and PEG (Mol. Wt 350–20 000) (Gref et al., 1993). During preparation of the nanoparticles, using solvent evaporation techniques, the PEG segment migrates to the surface of the particles, leaving it 'covered' with a PEG layer. Particles thus prepared were on the order of 200 nm in diameter. After injection of plain or modified nanospheres (using Indium radioactivity tracking) 5 min after injection 40% of the unmodified nanoparticles were found in liver and 15% in blood. Modified nanoparticles, however, showed 15% in the liver and 60% in the blood with a significantly improved circulating time. Plain nanospheres completely disappeared from blood in 4 h, but at that same time approx. 30% of modified nanospheres were still circulating. Delivery of antisense oligonucleotides using an injectable polymer composed of PEG chains with a degradable oligo-lactide segment and reactive acrylate segment on each end has also been studied recently (Hill-West and Hubbell, 1993).

## 8. Conclusions

Biodegradable microparticles have one of the greatest ranges of utility in controlled release of any formulation yet studied. They can be utilized in injectable formulations, oral formulations, bioadhesive systems, and as the principal release-controlling component of degradable and non-degradable implants and films. The future opportunities for the in vivo use of PLA/PGA polymers as biodegradable microparticles are being well examined by researchers worldwide, with advances in the field being made continuously. While the potential for PLA/PGA formulations

is evident, researchers must also remember the possibilities for combining the desirable characteristics of these delivery systems with other materials, natural and synthetic, to yield controlled drug delivery formulations for an even wider range of applications.

## References


Allemann, E., Doelker, E. and Gurny, R., Drug loaded poly(lactic acid) nanoparticles produced by a reversible salting-out process: purification of an injectable dosage form. *Eur. J. Pharm. Biopharm.*, 39 (1993) 13–18.

Alonso, N.J., Cohen, S., Park, T.G., Gupta, R.K., Siber, G.R. and Langer, R., Determinants of release rate of tetanus vaccine from polyester microspheres. *Pharm. Res.*, 10 (1993) 945–953.

Asano, M., Yoshida, M., Omichi, H., Mashimo, T., Okabe, K., Yuasa, H., Yamanaka, H., Morimoto, S. and Sakakibara, H., Biodegradable poly(DL-lactic acid) formulations in a calcitonin delivery system. *Biomaterials*, 14 (1993) 797–799.

Brannon-Peppas, L., Controlled release of $\beta$-estradiol from biodegradable microparticles within a silicone matrix. *J. Biomater. Sci., Polymer Ed.*, 5 (1994) 339–351.

Brannon-Peppas, L., Design and mathematical analysis of controlled release from microsphere-containing polymeric implants. *J. Controlled Release*, 20 (1992) 201–208.

Brannon-Peppas, L., Grosvenor, A.L. and Smith, B.S., Drug delivery from biodegradable microparticles within degradable and nondegradable films. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 21 (1994).

Cohn, D. and Younes, H., Biodegradable PEO/PLA block copolymers. *J. Biomed. Mater. Res.*, 22 (1988) 993–1009.

Davis, F.F., Kazo, G.M., Nucci, M.L. and Abuchowski, A., Reduction of immunogenicity and extension of circulating half-life of peptides and proteins. In Lee, V.H.L. (Ed.), *Peptide and Protein Drug Delivery*, Dekker, New York, 1991, pp. 831–864.

Eldridge, J.H., Gilley, R.M., Staas, J.K., Moldoveanu, Z., Meulbroak, J.A. and Tice, T.R., Biodegradable microspheres: vaccine delivery system, for oral immunization. *Curr. Top. Microbiol. Immun.*, 146 (1989) 59–66.

Eldridge, J.H., Staas, J.K., Meulbroek, J.A., McGhee, J.R., Tice, T.R. and Gilley, R.M., Biodegradable microspheres as a vaccine delivery system. *Mol. Immun.*, 28 (1991) 287–294.

Fawaz, F., Bonini, F., Guyot, M., Lagueny, A.-M., Fessi, H. and Devissaguet, J.-P., Influence of poly(DL-lactide) nanocapsules on the biliary clearance and enterohepatic circulation of indomethacin in the rabbit. *Pharm. Res.*, 10 (1993) 750–756.

Fischel-Ghodsian, F. and Newton, J.M., Analysis of drug release kinetics from degradable polymeric devices. *J. Drug Targeting*, 1 (1993) 51–57.

Grandfils, C., Flandroy, P., Nihant, N., Barbette, S., Jerome, R., Teyssie, P. and Thibaut, A., Preparation of poly (D,L) lactide microspheres by emulsion-solvent evaporation, and their clinical applications as a convenient embolic material. *J. Biomed. Mater. Res.*, 26 (1992) 467–479.

Gref, R., Minamitake, Y., Peracchia, M.T., Trubetskoy, V., Milshteyn, A., Sinkule, J., Torchilin, V. and Langer, R., Biodegradable PEG-coated stealth nanospheres. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 20 (1993) 131–132.

Heya, T., Okada, H., Ogawa, Y and Toguchi, H., Factors influencing the profiles of TRH release from copoly(dl-lactic/glycolic acid) microspheres. *Int. J. Pharm.*, 71 (1991) 199–205.

Hill-West, J.L. and Hubbell, J.A., Delivery of antisense oligonucleotides from photopolymerized hydrogels. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 20 (1993) 248–249.

Hora, M.S., Rana, R.K., Nunberg, J.H., Tice, T.R., Gilley, R.M. and Hudson, M.E., Controlled release of interleukin-2 from biodegradable microspheres. *Bio / Technology*, 9 (1990) 755–758.

Ike, O., Shimizu, Y., Wada, R., Hyon, S.-H. and Ikada, Y., Controlled cisplatin delivery system using poly(DL-lactic acid). *Biomaterials*, 13 (1992) 230–234.

Iwata, M. and McGinity, J.W., Dissolution, stability, and morphological properties of conventional and multiphase poly(DL-lactic-co-glycolic acid) microspheres containing water-soluble compounds. *Pharm. Res.*, 10 (1993) 1219–1227.

Katre, N.V., The conjugation of proteins with polyethylene glycol and other polymers: altering properties of proteins to enhance their therapeutic potential. *Adv. Drug Del. Rev.*, 10 (1993) 91–114.

Lewis, D.H., Controlled release of bioactive agents from lactide/glycolide polymers. In Chasin, M. and Langer, R. (Eds), *Biodegradable Polymers as Drug Delivery Systems*, Dekker, New York, 1990, pp. 1–41.

Mauduit, J., Bukh, N. and Vert, M., Gentamicin/poly(lactic acid) blends aimed at sustained release local antibiotic therapy administered per-operatively: I. The case of gentamicin base and gentamicin sulfate in poly(DL-lactic acid) oligomers. *J. Controlled Release*, 23 (1993a) 209–220.

Mauduit, J., Bukh, N. and Vert, M., Gentamycin/poly(lactic acid) blends aimed at sustained release local antibiotic therapy administered per-operatively: II. The case of gentamicin sulfate in high molecular weight poly(DL-lactic acid) and poly(L-lactic acid). *J. Controlled Release*, 23 (1993b), 221–230.

Mauduit, J., Bukh, N. and Vert, M., Gentamycin/poly(lactic acid) blends aimed at sustained release local antibiotic therapy administered per-operatively: III. The case of gentamicin sulfate films prepared from high and low molecular weight poly(DL-lactic acids). *J. Controlled Release*, 25 (1993c) 43–49.

Niwa, T., Takeuchi, H., Hino, T., Kunou, N. and Kawashima, Y., Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with DL-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *J. Controlled Release*, 25 (1993) 89–98.

O'Hagan, D.T., Rahman, D., McGee, J.P., Jeffery, H., Davies, M.C., Williams, P., Davis, S.S. and Challacombe, S.J., Biodegradable microparticles as controlled release antigen delivery systems. *Immunology*, 73 (1991) 239–242.

Omelczuk, M.O. and McGinity, J.W., The influence of polymer glass transition temperature and molecular weight on drug release from tablets containing poly(DL-lactic acid). *Pharm. Res.*, 9 (1992) 26–32.

Pistner, H., Gutwald, R., Ordung, R., Reuther, J. and Muhling, J., Poly(L-lactide): a long-term degradation study in vivo. *Biomaterials*, 14 (1993) 671–677.

Sanders, L.M., McRae, G.I., Vitale, K.M. and Kell, B.A., Controlled delivery of an LHRH analogue from biodegradable injectable microspheres. *J. Controlled Release*, 2 (1985) 187–195.

Scholas, P.D., Coombes, A.G.G., Illum, L., Davis, S.S., Vert, M. and Davies, M.C., The preparation of sub-200 nm poly(lactide-co-glycolide) microspheres for site-specific drug delivery. *J. Controlled Release*, 25 (1993) 145–153.

Shah, S.S., Cha, Y. and Pitt, C.G., Poly(glycolic acid-co-DL-lactic acid): diffusion or degradation controlled drug delivery. *J. Controlled Release*, 18 (1992) 261–270.

Singh, M., Singh, A. and Talwar, G.P., Controlled delivery of diphtheria toxoid using biodegradable poly(DL-lactide) microcapsules. *Pharm. Res.*, 8 (1991) 958–961.

Trubetskoy, V.S., Cannillo, J.A., Milshteyn, A., Torchilin, V.P. and Wolf, G.L., Controlled delivery of Gd-containing liposomes to lymph nodes: surface modification may enhance MRI contrast properties. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 20 (1993) 123–124.

Verrecchia, T., Huve, P., Bazile, D., Veillard, M., Spenlehauer, G. and Couvreur, P., Adsorption/desorption of human serum albumin at the surface of poly(lactic acid) nanoparticles prepared by a solvent evaporation process. *J. Biomed. Mater. Res.*, 27 (1993) 1019–1028.

Vert, M., Li, S.M., Spenlehauer, G. and Guerin, P., Bioresorbability and biocompatibility of aliphatic polyesters. *J. Mater. Sci., Mater. Med.*, 3 (1992) 432–446.

Wada, R., Hyon, S.-H., Ikada, Y., Nakao, Y., Yoshikawa, H. and Muranishi, S., Lactic acid oligomer microspheres containing an anticancer agent for selective lymphatic delivery: I. In vitro studies. *J. Bioact. Compat. Polym.*, 3 (1988b) 126–136.

Wada, R., Tabata, Y., Hyon, S.-H. and Ikada, Y., Preparation of poly(lactic acid) microspheres containing anti-cancer drugs. *Bull. Inst. Chem. Res., Kyoto Univ.*, 66 (1988a) 241–250.

Yamaguchi, K. and Anderson, J.M., In vivo biocompatibility studies of medisorb 65/36 DL-lactide/glycolide copolymer microspheres. *J. Controlled Release*, 24 (1993) 81–93.

Yamakawa, I., Tsushima, Y., Machida, R. and Watanabe, S., In vitro and in vivo release of poly(DL-lactic acid) microspheres containing neurotensin analogue prepared by novel oil-in-water solvent evaporation method. *J. Pharm. Sci.*, 81 (1992) 808–811.

Zhang, X., Wyss, U.P., Pichora, D., Amsden, B. and Goosen, M.F.A., Controlled release of albumin from biodegradable poly(DL-lactide) cylinders. *J. Controlled Release*, 25 (1993) 61–69.

Zhifang, Z., Mingxing, Z., Shenghao, W., Fang, L. and Wenzhao, S., Preparation and evaluation in vitro and vivo of copoly(lactic/glycolic) acid microspheres containing norethisterone. *Biomater., Artif. Cells Immob. Biotech.*, 21 (1993) 71–84.

Zhu, K.J., Xiangzhou, L. and Shilin, Y., Preparation, characterization and properties of polylactide (PLA)-poly(ethylene glycol) (PEG) copolymers: a potential drug carrier. *J. Appl. Polym. Sci.*, 39 (1990) 1–9.