PREPARATION AND CHARACTERIZATION OF PACLITAXEL-LOADED
POLY(D,L-LACTIDE-CO-GLYCOLIDE) MICROSPHERES FOR INTRA-
ARTICULAR INJECTION

by

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ABSTRACT

Paclitaxel has been shown to cause significant regression of existing rheumatoid arthritis and to prevent the induction of collagen-induced arthritis (CIA) in animal models. Paclitaxel suppresses arthritis because rapidly proliferating inflammatory pannus cells in the joint are susceptible to the phase-specific cytotoxic effects of paclitaxel.

Intra-articular therapy using anti-inflammatory steroids is used for patients in whom rheumatoid arthritis manifests itself in only a limited number of joints. The objective of the research was to prepare and characterize paclitaxel-loaded microspheres using lactide:glycolide (LA:GA) polymers, which might potentially be suitable for the intra-articular delivery of paclitaxel in arthritis.

Paclitaxel-loaded poly(\(d, l\)-lactide-co-glycolide) (PLG) microspheres were prepared using the solvent evaporation method. PLG polymers having different compositions of lactide and glycolide as well as having different molecular weights with the same lactide and glycolide composition were chosen to study the influences of these factors on the paclitaxel release rate. The effects of paclitaxel loading in the polymer matrix and the sizes of the microspheres on the paclitaxel \textit{in vitro} release behavior were also assessed.

Paclitaxel was loaded into PLG microspheres with encapsulation efficiencies of over 90% due to the hydrophobicity of the drug. Differential scanning calorimetry (DSC) thermograms indicated that the glass transition temperatures increased with an increase in paclitaxel loading in the PLG matrices, which was believed to be due to an interaction involving the formation of hydrogen bonds between paclitaxel and PLG polymers. X-ray diffraction data showed only the presence of an amorphous matrix, with no evidence by
either X-ray diffraction or DSC, of crystalline paclitaxel present in the microspheres matrix.

Degradation studies of both control and paclitaxel-loaded microspheres in phosphate buffered saline (PBS) containing albumin at 37°C showed that the molecular weights of PLG microspheres with a 50:50 lactide:glycolide composition decreased rapidly with time. The molecular weights of PLG microspheres with higher lactide content (> 50 mole% of lactide) did not decrease significantly until after 3 weeks of incubation in PBS-albumin.

The release profiles of paclitaxel from all PLG microsphere formulations showed a burst phase of release, followed by a phase of relatively steady release. The burst phase was caused by rapid release of paclitaxel from the superficial surface layers of the microspheres. The release rates of paclitaxel from PLG50:50 microspheres were influenced by paclitaxel loading and molecular weights of the PLG50:50 polymers. Increased loading and decreased molecular weight led to faster paclitaxel release rates. PLG microspheres prepared from polymers with LA:GA ratios of 85:15, 75:25 and 65:35 showed that the LA:GA compositions had minimal effect on paclitaxel release rates. The two size ranges of microspheres showed minimal effects on the rates of paclitaxel releases from the microspheres.
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<tr>
<td>CIA</td>
<td>Collagen-induced arthritis</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane or methylene chloride</td>
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<td>Oil in water</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PD</td>
<td>Polydispersity</td>
</tr>
<tr>
<td>PLG</td>
<td>Poly(d, l-lactide-co-glycolide)</td>
</tr>
<tr>
<td>PLG100:0</td>
<td>Poly(d, l-lactide) with 100% (mole%) lactide</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>Poly(d, l-lactide-co-glycolide) with 50% (mole%) lactide</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>PLG65:35</td>
<td>Poly($d,l$-lactide-co-glycolide) with 65% (mole%) lactide</td>
</tr>
<tr>
<td>PLG75:25</td>
<td>Poly($d,l$-lactide-co-glycolide) with 75% (mole%) lactide</td>
</tr>
<tr>
<td>PLG85:15</td>
<td>Poly($d,l$-lactide-co-glycolide) with 85% (mole%) lactide</td>
</tr>
<tr>
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<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
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<td>Paclitaxel</td>
</tr>
<tr>
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<td>Tetrahydrofuran</td>
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<tr>
<td>X-ray</td>
<td>X-ray diffractometry</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
</tr>
</tbody>
</table>
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1. INTRODUCTION

Microspheres are fine spherical particles (diameter less than 1,000μm). Upon encapsulation with a drug, microspheres can be divided into two types: (1) homogenous or monolithic microspheres in which drug is dissolved or dispersed throughout the polymer matrix; (2) reservoir type microspheres in which the polymer matrix surrounds a drug core.

Microsphere drug delivery systems using various kinds of biodegradable polymers have been studied extensively for over 30 years. Long-term controlled releases of growth hormones (Cleland et al., 1997), narcotic antagonists (Pitt et al., 1981), peptides (Mehta et al., 1994), local anesthetics (Wakiyama et al., 1982) and anticancer agents (Burt et al., 1995) have been investigated to eliminate the inconvenience of repeated injection and for targeted delivery to the site of action.

Paclitaxel has been shown to result in the significant regression of existing rheumatoid arthritis (RA) and to prevent the induction of collagen-induced arthritis in animal models (Brahn et al., 1994; Oliver et al., 1994). Intra-articular therapy using anti-inflammatory steroids is used for patients in whom RA manifests itself in only a limited number of joints. However, the treatment of RA by direct injection of drugs into the joint cavity is limited by the rapid clearance of the drugs from the joint into the blood (Emkey et al., 1996).

In this work, paclitaxel was encapsulated into a biodegradable polymer, poly(d,l-lactide-co-glycolide) (PLG). Paclitaxel-loaded PLG microspheres in different size ranges, potentially suitable for intra-articular injection, were prepared and characterized. PLG polymers having different compositions of lactide (LA) and glycolide (GA), as well as
having different molecular weights with the same LA and GA composition, were chosen to study the influence of these factors on the paclitaxel release profiles. The effect of paclitaxel loading in the polymer matrix and the size ranges of the microspheres on *in vitro* paclitaxel release rates were also assessed.

The degradation characteristics of control and paclitaxel-loaded microspheres were determined. Scanning electron microscope (SEM) and differential scanning calorimetry (DSC) were used to determine the changes in surface morphologies and glass transition temperatures of the PLG microspheres with or without loading with paclitaxel and before and after incubation in PBS-albumin. Fourier transform infra-red spectroscopy (FTIR) was used to investigate a possible paclitaxel-polymer interaction.
2. BACKGROUND

2.1. Paclitaxel

2.1.1. Source

Paclitaxel is a natural diterpenoid extracted from the bark of *Taxus brevifolia* (the Pacific yew). Its efficacy against human cancers was first discovered by Monroe Wall and his collaborators (Wani *et al.*, 1971). Paclitaxel used to be mainly produced by semi-synthetic methods from renewable sources such as bark, wood, and dried needles of the yew tree using several different approaches (Magri *et al.*, 1986 & 1988; Denis *et al.*, 1990 and Palomo *et al.*, 1990). A final partial synthetic method was reported by Holton (1990) and was later modified by Ojima *et al.* (1991) to significantly increase the yield of paclitaxel. This offered the best overall strategy for the preparation of paclitaxel from baccatin III and was adopted by Bristol-Myers Squibb (Kingston, 1991). Total synthesis of paclitaxel was also achieved by Nocolaou *et al.* (1994) and Holton *et al.* (1994), although this will not be a practical supply source due to the cost.

2.1.2. Chemistry of paclitaxel

The structure of paclitaxel, as shown in Figure 1, is composed of a baccatin III, a typical taxane structure and an ester linked side chain attached at the C-13 position of baccatin III (Lythgoe *et al.*, 1968). Structurally, paclitaxel is differentiated from most other taxane diterpenoids by its ester side chain at C-13 and its oxetane ring (Kingston, 1991). The 2'-OH and 3'-phenyl at side chain (2'R-3'S) are the absolute stereochemical requirements for good binding. The presence of the 4,5-oxetane is necessary for activity but it is not clear whether it is involved in binding or serves as a "conformational lock" on the preferred binding conformation (Suffness, 1994B).
Figure 1  Chemical structure of paclitaxel (numbers assigned to the carbons in the structure according to IUPAC nomenclature) (The Merck Index, 1996).
The chemical structure and structure-activity relationships of paclitaxel have been reviewed and summarized by Kingston (1991). The most important feature of paclitaxel's structure with respect to activity is the C-13 side chain. Deletion of the side chain gives baccatin III, which shows a 1700-fold decrease in anti-mitotic activity (Lataste et al., 1984; Miller et al., 1981). Any modification on baccatin III has been shown to lead to less effectiveness in tumor inhibition (Wani et al., 1971; Miller et al., 1981; Kingston et al., 1982 and Lataste et al., 1984).

2.1.3. Crystal structure

X-ray crystallographic data have been reported for paclitaxel precipitated from a dioxane, water and xylene cosolvent (Mastropaolo et al., 1995). Gao et al. (1995) also reported the crystal structures of a paclitaxel solvate and paclitaxel analog (2-debenzoyl, 2-acetoxy paclitaxel).

2.1.4. Solubility and stability

Paclitaxel's solubility in various aqueous vehicles and organic solvents such as polyethylene glycol 400, ethanol, isopropanol, soybean oil, acetonitrile and methylene chloride were summarized by Adams et al. (1993). Data from different investigators showed paclitaxel solubility in water at 37°C was ~30 μg/mL (Swindell et al., 1991; Ringel et al., 1991), ~0.7 μg/mL (Mathew et al., 1992), and 6 μg/mL (Tarr et al., 1987). Recent evidence suggested that the differences in solubility might be due to the presence of different hydrate and anhydrous forms of paclitaxel (Perrone et al., 1996; Liggins et al., 1997).

Dordunoo and Burt (1996) reported that paclitaxel degraded in an aqueous phosphate buffered saline with albumin (PBS-albumin, pH = 7.3). Degradation followed
pseudo-first order kinetics and the major degradation products were baccatin III, 10-deacetylbaccatin III and their 7-epi isomers (baccatin V and 7-epi-10-deacetylbaccatin III). They also found that degradation of paclitaxel in aqueous buffers was pH dependent and paclitaxel was relatively stable in the pH range of 3 to 5.

The degradation products of paclitaxel in methanol at a pH of 9 were found to be similar to those from PBS-albumin (Lataste et al., 1984; Ringel et al., 1987).

2.2. Paclitaxel in rheumatoid arthritis

2.2.1. Rheumatoid arthritis (RA)

RA is a chronic and relapsing inflammatory disease of unknown cause. RA affects the wrist and hand joints, elbows, shoulders, neck, jaw, hips, knees, ankles, and feet in a symmetrical pattern. It tends to persist over prolonged periods of time, and the inflamed joints eventually can become damaged (Figure 2) (Harris, 1997). Harris (1990 & 1997), Hollingsworth et al. (1967) and Lafyatis et al. (1989) have described RA as being characterized by a marked thickening of synovial membrane and the formation of villus projections. The villus projections then extend into the joint space and there is extensive synoviocyte proliferation (or multilayering of the synoviocyte lining) and infiltration of the synovial membrane with white blood cells (macrophages, lymphocytes). The tissue formed as a result of this process is called pannus and eventually it grows to fill the joint space. The pannus develops an extensive network of new blood vessels through the process of angiogenesis. Releases of digestive enzymes (such as collagenase and stromelysin) and other mediators of the inflammatory process from the cells of the pannus tissue lead to the progressive destruction of the cartilage.
tissue. The pannus invades the articular cartilage leading to erosions and fragmentation of
the cartilage tissue.

2.2.2. Mechanism of action of paclitaxel

Paclitaxel induces cytotoxicity by a unique mechanism of action. Paclitaxel
affects the fiber-like cell structures called microtubules, which play an important role in
cell division and other cell functions. A large number of microtubules are formed at the
start of cell division. As cell division comes to an end, these microtubules are normally
broken down. However, paclitaxel stabilizes microtubules and inhibits their
depolymerization back to tubulin (Schiff et al., 1979; Howitz et al., 1982 & 1992 and
Suffness, 1994A). Horwitz and co-workers largely elucidated the molecular
pharmacology of paclitaxel, demonstrating that paclitaxel bound preferentially to
microtubules rather than free tubulin subunits. The binding of paclitaxel to polymerized
tubulin is reversible (Schiff et al., 1979 & 1980; Parness et al., 1981 and Manfredi et al.,
1984). Microtubules formed in the presence of paclitaxel are dysfunctional, causing the
interruption of normal cell functions, including mitosis (Schiff et al., 1980; Caplow et al.,
1994).

In clinical studies, paclitaxel has been demonstrated to be efficacious against
many cancers. A 24-hour continuous infusion of paclitaxel (180 to 250 mg/m) gave 20% 
complete response and 95% partial response in ovarian cancer patients (Einzig et al.,
1992; Milas et al., 1995). Single agent therapy using paclitaxel gave a response rate of
62% in breast cancer patients (Arbuck et al., 1994; O'Shaughnessy et al., 1994 and Zoli
et al., 1995). In vitro studies have indicated that paclitaxel possesses significant potency
against brain tumors (Cahan et al., 1994) and bladder cancer (Rangel et al., 1994).
Figure 2  Illustration of normal and inflammatory joints (adapted from Harris 1997).
2.2.3. Preclinical effectiveness of paclitaxel in rheumatoid arthritis

Hui et al. (1997) has demonstrated that paclitaxel is capable of inhibiting synovial cell proliferation at low concentrations (10^{-8}M) and is cytotoxic to proliferating synoviocytes at higher concentrations (10^{-7}M). In contrast, paclitaxel at high concentrations (10^{-5}M) has no effect on chondrocyte cell function or viability. Paclitaxel spares non-proliferating synoviocytes and chondrocytes, but is selectively toxic to proliferating synoviocytes which lead to the development of a locally invasive and destructive pannus tissue.

Brahn et al. (1994) used Louvain rats immunized with type II collagen (day 0) to induce arthritis. Paclitaxel was administered beginning on day 2 (prevention protocol) or at the onset of arthritis on day 9 (in either a high-dose or low-dose suppression protocol). Paclitaxel resulted in significant regression of existing collagen-induced arthritis and completely prevented the induction of collagen-induced arthritis (CIA). Paclitaxel is particularly cytotoxic for cells undergoing mitosis (Lopes et al., 1993). By interfering with normal microtubule function, paclitaxel inhibits cell mitosis (Milas et al., 1995), migration and suppresses intracellular transport (Rowinsky et al., 1990). Hence, paclitaxel can suppress arthritis because rapidly proliferating, inflammatory pannus cells in RA are more susceptible to paclitaxel’s phase-specific cytotoxic effects (Oliver et al., 1994). Oliver and Brahn (1994) suggested that paclitaxel also acted as a phase-specific anti-angiogenesis agent in rheumatoid arthritis.
2.3. **Intra-articular therapy**

2.3.1. **Side effects of paclitaxel systemic therapy**

Paclitaxel is poorly soluble in water and other common vehicles used for parenteral administration. Cremophor-EL (polyethoxylated castor oil) with 50% dehydrated alcohol (Grem et al., 1987) has been selected as a vehicle for clinical administration of paclitaxel to cancer patients. A 24-hour infusion schedule with a wide range of paclitaxel dosages (135 to 250 mg/m²) was proposed after the completion of phase I studies (Onetto et al., 1993; Jamis-Dow et al., 1993). However, during phase I and phase II trials, serious side effects, such as hypersensitivity reactions, thrombocytopenia, anemia, arthralgias and myalgias, cardiac effects, alopecia, and gastrointestinal effects (Finley et al., 1994), were reported. The hypersensitivity reactions and cardiotoxicity seemed to be independent of dose (Rowinsky et al., 1992) and were due to the Cremophor EL vehicle used in the formulation (Adams et al., 1993; Rowinsky et al., 1993).

2.3.2. **Intra-articular therapy**

Intra-articular therapy is used for patients in whom the rheumatoid arthritis manifests itself in only a limited number of joints as the systemic therapy will expose the patients to the risk of adverse reactions (Cawley et al., 1969; Itoh, 1992).

The treatment of arthritis by direct injection of drugs into the joint cavity is limited by the rapid clearance of the drugs from the joint into the blood (Bird, 1979; Emkey et al., 1996). Using a radio isotope-labeled albumin, Owen et al. (1994) demonstrated that once injected into the synovial cavity, the drugs first diffused into the cartilage, and then were taken up by the cells in the synovium. Finally, the drugs passed
through the synovium into the synovial capillaries and then into the systemic circulation (Weiss et al., 1983), or into the lymphatic system. Hunneyball (1986) concluded that the currently available drugs, such as methotrexate (Hoffmeister, 1983) and hydrocortisone, had very short-term (3-4 days) effects in the joints post injection. Microcrystalline steroids such as triamcinolone hexacetonide (TH) possess a relatively prolonged anti-inflammatory effect in the joints (See, 1998) because drug release is controlled by the rate of crystal dissolution. The efficacy and duration of action of the microcrystalline steroid preparation may last up to several weeks (Bird, 1979).

2.3.3. Problems associated with intra-articular therapy

Hunneyball (1986) and Mow et al. (1991) summarized the principal problems associated with local injection therapy as follows:

- The drug is released into the systemic circulation with consequent production of adverse reactions.
- Deleterious effects of the drug on cells in the cartilage, ligaments, and tendons due to significant amounts of drugs present in the joints.
- Local flare reaction due to the physical nature of drug and the risk of infection in the joint with frequent injections.

Because of these factors, the recommendation is not to inject the joints more frequently than once every three months (Hunneyball, 1986).

2.3.4. Controlled release intra-articular drug delivery systems

De Silva et al. (1979) and Dingle et al. (1978) reported the use of a liposomal cortisol palmitate delivery system for rheumatoid arthritis. Intra-articular administration of this liposomal preparation, at a dose equivalent to 2 mg of hydrocortisone, produced a
therapeutic effect that was maximal at 48 hours but returned to the pre-injection state after 14 days. Liposomes have also been used to deliver other steroids (Shaw et al., 1979) or methotrexate (Foong et al., 1993) for intra-articular injection, but the anti-inflammatory effect can only be sustained for 3-4 days.

2.3.5. Controlled release microspheres for arthritis

Polymeric microspheres have been investigated for their tolerability in joints and their suitability for delivering anti-inflammatory drugs to the joints. Cavalier et al. (1986) and Ratcliffe et al. (1984A & 1984B) used polylactic acid (PLA), polybutylcyanoacrylate (PBCA), gelatin (PG), and albumin (PA) microspheres as carriers to deliver anti-inflammatory drugs to rabbit knee joints. However, in vivo biocompatibility tests with synovial tissues indicated that PLA, PBCA and PG microspheres with sizes of 1-10μm caused joint inflammation. PA microspheres with sizes of 1-10μm were well tolerated by the tissues. However, PA microspheres disintegrated rapidly, which resulted in short-lived clinical effects (Ratcliffe et al., 1987).

The use of biodegradable poly(d,l-lactide-co-glycolide) (PLG) microspheres with a composition of 85:15 d,l-lactide:glycolide as a 90-day delivery device has been reported in a US patent by Tice et al. (1985). An undisclosed anti-inflammatory agent encapsulated up to 75% (w/w) in the biodegradable PLG85:15 microspheres, was administered by intra-muscular injection into the area surrounding the inflamed joints. A continuous release of the anti-inflammatory agent into the joints was achieved.
2.4. Polymer chemistry

2.4.1. Structure

Flory (1953) defined polymers as long-chain molecules of very high molecular weights. Depending on their structure, polymers can be divided into two categories, homopolymers and copolymers. A homopolymer is a polymer consisting of many repeating units or so-called structural units, and these repeating units may contain one or more chemically different structures in a regular sequence. A copolymer, however, is a polymer consisting of two or more chemically different units in an irregular sequence (Rabek, 1980).

2.4.1.1. Configuration and conformation

Polymer configuration refers to the organization of the repeat units along the chain. Configurational isomerism involves the different arrangements of atoms and substituents in a chain that can be inter-converted only by the breakage and reformation of primary chemical bonds (Flory, 1953). Configurational isomers (Jenkins, 1972) include structural isomers (such as branched/cross-linked polymers, sequence distributions in copolymers, and head-tail isomers) and steric isomers (cis-trans and tetrahedral such as atactic, isotactic and syndiotactic isomers).

Polymer conformation (Flory, 1953) refers to the different arrangements of atoms and substituents of the polymer chain brought about by internal rotation about single bonds. Examples of different polymer conformations (Jenkins, 1972) include the fully extended zigzag, helical, folded chain, and random coils. Polymers can have many possible conformations. For example, a polyethylene without any side chains may possess at least $3^{2N-3}$ relatively stable conformations (N is the degree of polymerization).
2.4.1.2. Morphology and models of polymer structures

Polymer morphology describes the arrangement, form and structure of polymer chains in crystalline and amorphous regions (Rabek, 1980). Compared to small molecules, polymer morphology is quite complex due to the long chain structures and chain entanglement (Flory, 1953).

Polymers are generally considered to be either amorphous or semi-crystalline. The concept of the “Fringed Micelle Model” was first proposed by Herrmann (1930) to describe the nature of semi-crystalline polymers (Figure 3A). In the “Fringed Micelle Model”, crystallites are interspersed in an amorphous matrix. Some polymer chains contribute to both the amorphous and crystalline regions in the polymer matrix. Amorphous and crystalline regions co-exist and are not separated by distinct boundaries. However, the “Fringed Micelle Model” fails to explain the observation that polymer single crystals are generally 100 Å in thickness and are much shorter than the length of the polymer chains. Keller (1957) later used a “Chain Folded Model” (Figure 3B) to show how polymer chains could fold and form a single crystal without changing the bond angle. In this model, lamellae are formed by adjacent re-entry of a single polymer chain. In contrast, Flory (1962) proposed a random reentry model or “Switchboard Model” (Figure 3C). He believed a single polymer chain could enter the same lamellae or different lamellae randomly at different location. The discovery of inter-lamellar links between poly(ethylene) lamellae by Keith et al. (1966) supported the switchboard model.
Figure 3   Fringed-micelle, folded chain and switchboard models
(adapted from Rabek, 1980).
Amorphous polymers do not exhibit either a crystalline X-ray diffraction pattern or a first order melting transition (Rabek, 1980). Irregularity in the polymer chain, chain entanglement, branching, and cross-linking can prevent polymer crystallization when polymers are cooled from the liquid state. However, some semicrystalline polymers can behave as amorphous polymers if they either have a very slow crystallization rate (such as polycarbonate and polyethylene terephthalate) or only become crystalline at very low temperatures (such as natural rubber at -25°C). Flory (1953) believed that amorphous polymers did not have any long-term and short-term order in the matrix and could be described by his “Single-phase Random-coil Model” (Figure 4A). This model gives an excellent explanation of the structures of elastomers, but fails to interpret the nodule structures in many amorphous polymers and the much higher X-ray diffraction intensity in molten polymers. Yeh (1972) later solved this problem by introducing a “Folded-chain Fringed Micellar Grain Model” (Figure 4B). According to his model, amorphous polymers can be divided into “grain” (G) and “intergrain” (IG) regions and the “grain” is composed of “grain boundary” (GB) and “ordered domain” (OD).
A Single-phase random-coil model

B Folded-chain fringed micellar grain model

Figure 4 Single-phase random-coil and folded-chain fringed micellar grain models (G: grain; IG: intergrain; GB: grain boundary and OD: ordered domain) (adapted from Sperling, 1986).
2.4.2. Molecular weight

Physicochemical properties of polymers are determined not only by their chemical structures but also by their molecular weights. Polymers consist of molecules which have a distribution of molecular weights instead of possessing a single molecular weight like small molecules (Flory, 1953). Several important molecular weight parameters are used to describe polymer systems. The most common ones are the number-average molecular weight ($M_n$), which is defined as the total weight of all solute species divided by the total number of moles present (equation 1) and the weight-average molecular weight ($M_w$), which is defined in equation (2):

$$M_n = \frac{\sum N_i M_i}{\sum N_i} = \frac{\sum W_i}{\sum W_i / M_i}$$

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum W_i M_i}{\sum W_i}$$

Where: $M_i$, $N_i$ and $W_i$ are the molecular weight, number of moles, and weight of the $i$th solute species, respectively. Both $M_n$ and $M_w$ are unique values for a given polymer. $M_n$ is most affected by the lower molecular weight fraction, whereas $M_w$ is most affected by the higher fraction. Therefore $M_w$ is always greater than $M_n$ (Figure 5).

A single number or index has also been used to describe the molecular weight distribution of a polymer. The extent of variability in molecular weight is determined by the polydispersity (PD), which can be calculated from the following equation:

$$PD = \frac{M_w}{M_n}$$

Equation 3
Figure 5 Molecular weight distribution of a polymer (Rabek, 1980).
2.4.3. **Biodegradable polymers**

A variety of synthetic polymers have been reported to degrade and erode in mammals. The degradation products of these polymers can be eliminated from the body by either metabolism or renal filtration. Polymers that undergo degradation in a biological environment, through either simple chemical reactions or enzyme-catalyzed reactions, are designated as biodegradable polymers (Vert et al., 1991). These polymers can function as a matrix to control the drug release by polymer hydration and degradation, which is then followed by polymer bulk erosion and elimination of the degradation products from the body (Park et al., 1996).

A great number of biodegradable polymers have been developed and evaluated for drug delivery, but the most widely investigated biodegradable polymeric systems are the lactide:glycolide polymers.

2.4.4. **Glass transition**

2.4.4.1. **Glass transition temperature (T<sub>g</sub>)**

The glass transition is the change of an amorphous polymer or region from a hard, brittle, glassy state to a soft, flexible, rubbery state or vice versa (Figure 6). The temperature of this transition is called the glass transition temperature (T<sub>g</sub>) (Turi, 1997). Below the glass transition, chain segments are frozen in fixed positions. A diffusion-rearrangement of the segmental position is also less probable. With increasing temperature, the amplitude of segmental vibrations increases. In the transition-state, chain segments have sufficient energy to overcome the secondary bonding forces. Chain segments or chain loops may perform rotational and translational motions and the polymer becomes flexible (Flory, 1953).
The glass transition temperature is well marked in amorphous polymers. In semi-crystalline polymers, it is less conspicuous because it only occurs in the non-crystalline regions.

2.4.4.2. Factors influencing glass transition temperature

A decrease in chain flexibility due to rigid and bulky side groups and intermolecular bonds such as cross-linking or hydrogen bonds will lead to an increase in \( T_g \). Factors leading to a decrease in \( T_g \) include end group effect (branching) and presence of low molecular weight compounds, such as water, solvent and plasticizers (Rabek, 1980). In semicrystalline polymers, \( T_g \) increases proportionally with the degree of crystallinity, because the crystallites tend to reinforce or stiffen the structure.

The \( T_g \) of most copolymers falls between the glass transition temperatures of their individual polymers. An amorphous random copolymer exhibits a single \( T_g \) which can be predicted by:

\[
T_g = \frac{KW_1T_{g1} + W_2T_{g2}}{KW_1 + W_2}
\]

Equation 4

Where \( T_{g1} \) and \( T_{g2} \) are the glass transition temperatures of two homopolymers. \( W_1 \) and \( W_2 \) are the weight fractions of two homopolymers and \( K \) is a constant given by:

\[
K = (\alpha_r - \alpha_g)_2 / (\alpha_r - \alpha_g)_1
\]

Equation 5

Where \( \alpha_1 \) and \( \alpha_2 \) are the thermal expansion coefficients of the homopolymers, the subscript \( r \) referring to the rubber state and the subscript \( g \) referring to the glassy state.

The molecular weight dependence of the glass transition has been defined by the Fox-Flory equation (Fox et al., 1950):

\[
T_g = T_{g(\infty)} - KM_n^{-1}
\]

Equation 6

21
Where $T_{g(\infty)}$ is the limiting high-molecular weight value of $T_g$ and K is a constant related to free volume.

Figure 6  DSC thermogram for a polymer illustrating the different, possible thermal events (Rabek, 1980).
2.5. Poly(\(d,l\)-lactide-co-glycolide) (PLG)

2.5.1. Structure and synthesis

Low molecular weight PLG (usually less than 10,000 g/mole) is prepared from the direct copolycondensation of \((d,l)\) lactic acid and glycolic acid, with or without a catalyst such as antimony, or tin at 180°C and 4 mmHg. PLG with higher molecular weight is manufactured by the ring opening melt-polycondensation of lactide and glycolide, the cyclic dimers of lactic acid and glycolic acid respectively, at higher temperatures (220°C and 0.1 mmHg) in the presence of the same kinds of catalysts (Figure 7). This process gives a higher molecular weight ranging from 20 to 250 kg/mole (Gilding et al., 1979; Reed et al., 1981).

Unlike glycolide, which contains only symmetric atoms, lactide has two asymmetric carbon atoms and is thus a chiral molecule that exists as two optical isomers or enantiomers, \(d\)-lactide and \(l\)-lactide and a racemic \(d,l\)-lactide. The lactide isomers give rise to four morphologically distinct polymers: poly(\(d\)-lactide) and poly(\(l\)-lactide), which are two stereoregular polymers; and poly(meso-lactide) and poly(\(d,l\)-lactide), which are racemic polymers. Polymers derived solely from \(d\)-lactide, \(l\)-lactide and glycolide are semicrystalline. The homopolymers of \(d,l\)-lactide and copolymers of \(d,l\)-lactide and glycolide are all amorphous polymers (Gilding et al., 1979; Fukuzaki et al., 1991).
Figure 7  Synthetic pathway for PLG polymers and chemical structure of stannous 2-ethyl-hexanoate catalyst (Zhang et al., 1994).
2.5.2. *Drug delivery systems (DDS) based on PLG*

2.5.2.1. *Microspheres*

PLG microspheres have been successfully used in the formulation of many pharmaceutical agents as summarized by Illum *et al.* (1982); Holland *et al.* (1986 & 1992) and Lewis (1990). Upon encapsulation of a drug, microspheres can become a monolithic type microspheres in which the drug is dissolved or dispersed throughout the polymer matrix or a reservoir-type microspheres in which the polymer matrix coats the drug particle.

2.5.2.2. *Biocompatibility*

Cutright *et al.* (1971) and Craig *et al.* (1975) first demonstrated the biocompatibility of PLG using sutures. When implanted into rats, these polymer sutures induced a mild local inflammatory reaction, infiltration of macrophages, giant cells and fibroblasts. The sutures were surrounded by a thin layer of connective tissue until they were completely absorbed. Visscher *et al.* (1985) also reported the tissue response after intra-muscular injection of PLG microspheres containing lysine-8-vasopressin and described the morphologic changes in the microspheres based on histological observations. In the case of a device with a one-month life-span, a minimal inflammatory reaction characterized by infiltration of lymphocytes, plasma cells, histocytes, and acute myositis was observed soon after injection. By day 63, degradation and erosion of the microspheres were extensive and the minimal chronic cell response had almost completely resolved (Visscher *et al.*, 1985). Spenlehauer *et. al.* (1989) also observed that at about 6 weeks after implantation, the absorption of microspheres was almost completed. The intensity of the inflammatory response had decreased and only remnants of microspheres could be
seen. Kou et al. (1997) reported that PLG rods and their breakdown products were well tolerated by the brain tissue upon implantation into rat brains. In general, the tissue response to PLG is a very localized inflammation consisting of macrophages, foreign body giant cells, and capillary infiltration and it is generally accepted that PLG polymers are biocompatible with living tissue (Maulding, 1987).

2.5.2.3. Mechanism of in vitro degradation and erosion

According to traditional definitions, only chemical agents can cause chemical degradation of polymers and biodegradation is caused by enzymes, bacteria, and fungi. Graham and Wood (1982) defined biodegradation as the process in which polymers degraded after a period of time to soluble species, which were readily removed from the \textit{in vivo} site and excreted by the body.

We use the term “degradation” or “biodegradation” to describe the chain scission process throughout the polymer matrix. During degradation, polymer chains are cleaved to form oligomers and finally to form monomers. Degradation may not involve any mass loss during the process (Gopferich, 1996). Erosion, on the other hand, is the process of conversion of an initially water-insoluble material to a water-soluble material with an accompanying mass loss (Heller, 1980 & 1985). Erosion emphasizes the loss of material owing to monomers and oligomers leaving the polymer surface (surface erosion) or matrix (bulk erosion) (Tamada, \textit{et al.}, 1993).

PLG polymers are a class of biodegradable polymers suitable for drug delivery systems since they are biocompatible with living tissue and the non-toxic degradation products are readily removed from the site of application (Holland \textit{et al.}, 1992). PLG is
degraded via a labile ester bond. It also has suitable mechanical properties and can be sterilized with limited impairment of properties.

The degradation of PLG is a random process which is characterized by rupture of the PLG main-chain. PLG used in drug delivery systems is generally degraded by hydrolysis. Water molecules diffuse into the amorphous polymer matrix, followed by random hydrolytic cleavage of ester bonds to produce oligomers. Erosion can commence with the degradation process (Heller, 1984).

In many cases, the release rate of the encapsulated drug depends on drug diffusion rate and the rate of degradation and erosion of PLG polymers (Lewis, 1990). During degradation, the molecular weight of the polymer steadily decreases, but the matrix can remain in its original shape and retain mass until a critical molecular weight, when bulk erosion begins (Iwata et al., 1993).

2.5.2.4. Factors affecting PLG degradation

Factors affecting the degradation and erosion of PLG include pH, molecular weight, and temperature (Reed et al., 1981; Vert et al., 1991). A discontinuity of mass loss for PLG is showed at the glass transition temperature ($T_g$) in which the mass loss above $T_g$ is greater than that below $T_g$, because water uptake and hydrolysis occur more readily when the temperature is above $T_g$. The molecular weights of the PLG also profoundly affect the degradation kinetics. The degradation rate is decreased for lower molecular weight PLG polymer (Reed et al., 1981).

PLG polymers possess different compositions of lactide and glycolide, which provide for different life-spans in living tissue. The half-life of PLG polymers with various compositions of lactide:glycolide, molecular weights and crystallinity, generally
ranged from 2 weeks to 6 months. An amorphous poly(\(l\)-lactide-co-glycolide) with 70:30 mole\% and weight average molecular weights of 16,900-41,300 g/mole showed 65\% mass loss after 10 weeks of implantation (Fukuzaki et al., 1991). In in vivo studies, Maulding (1987) and Visscher et al. (1985) showed that a 50:50 mole\% PLG with a number-average molecular weight of 9,500 g/mole was almost completely resorbed in tissue by day 63.

2.5.2.5. **Mechanism of drug release from PLG matrix**

*In vitro* drug release from PLG microspheres generally follows a triphasic profile (Sanders et al., 1986). Initially, a burst phase is observed over the first few days due to the release of drug from the surface of the microspheres and the matrix located near the surface. There is a period of slower release when the degradation medium diffuses into the polymer matrix. Degradation begins and at the same time, the drug diffuses slowly out of the matrix.

The third phase, a secondary burst phase of drug release, occurs when the bulk erosion of polymer matrix begins. In some cases, the third phase does not appear due to significant amount of drug being released in a short period of time, leading to a biphasic release profile.

2.5.2.6. **Factors affecting drug release from PLG matrix**

Factors affecting the degradation and erosion of PLG microspheres also affect the release rates of drug from the polymer matrix. Physical properties of polymers, such as the molecular weight and the composition (the ratio of the more hydrophobic lactide fraction to the less hydrophobic glycolide fraction), have a major influence on the drug
release since polymer degradation and erosion are dependent on these factors (Sanders et al., 1986; O'Hagan et al., 1994 and Ruiz et al., 1990).

The sizes of the microspheres may be important in the early phase of drug release. Since smaller microspheres have a larger surface area, the release rate may be faster.

The molecular weight and structure of drugs encapsulated into the polymer matrix also play a role in release kinetics. The diffusivity of larger molecules through a polymer matrix is lower (Holland et al., 1986) and therefore slower drug release from the matrix is expected. Increasing drug loading by creating a network of pores within the matrix provides more pathways for drug release. An extensive pore network will facilitate rapid elution of the drug from the matrix (Takada et al., 1994).

2.5.3. **Enzymatic degradation**

The role of enzymatic attack in the degradation of PLG is less defined. Poor *in vivo - in vitro* correlation in PLG degradation rates has often been taken as evidence of enzymatic involvement. For polymers used below their glass transition temperature ($T_g$), enzymatic degradation is not thought to be a major factor during matrix breakdown. Enzymes may play a more significant role in PLG degradation above the polymer $T_g$ (Holland et al., 1992).

2.5.4. **Effects of sterilization on microspheres**

Presently, the most expedient method for sterilizing moisture and heat sensitive substances such as PLG is Co-60 gamma irradiation. Prior studies demonstrated that gamma irradiation of PLG induced dose-dependent chain scission and molecular weight loss (Hausberger et al., 1995). However, it has been reported that the degradation due to irradiation caused no significant change in the initial drug release rate in the dose range
up to 2.5 Mrad. The decrease in glass transition temperature caused by irradiation is not large enough to affect the initial release rate (Yoshioka, et al., 1995).

2.5.5. Paclitaxel-loaded PLG microspheres

In this work, paclitaxel-loaded microsphere formulations have been developed for intra-articular delivery of paclitaxel based on PLG. Formulations of paclitaxel-loaded microspheres suitable for intra-articular administration have not previously been developed. Furthermore, the size of microspheres suitable for intra-articular injection has not been determined.

PLG microspheres containing paclitaxel were prepared using the solvent evaporation technique. Microspheres were extensively characterized and in vitro release rates of paclitaxel determined. The effects of different molecular weights of PLG polymers and differences in lactide:glycolide compositions in the polymers on the physicochemical characteristics and release properties of the microspheres were assessed.
2.6. Objectives

The overall objective of the research was to prepare and characterize paclitaxel loaded microspheres using the biodegradable lactide:glycolide polymers, which might potentially be suitable for the intra-articular delivery of paclitaxel in rheumatoid arthritis.

Specific Aims

The specific aims of the proposed research were to:

- Prepare and characterize paclitaxel-loaded microspheres using different molecular weights and lactide:glycolide ratios of PLG polymers.
- Determine the degradation behavior of the polymers.
- Determine the *in vitro* release rates of paclitaxel from PLG microspheres.
3. EXPERIMENTAL

3.1. Materials & supplies

3.1.1. Lactide:glycolide polymers

PLG with different inherent viscosities (I.V.) and lactide:glycolide compositions were purchased from Birmingham Polymers Inc. (Birmingham, AB). Polymers were stored in a desiccator filled with nitrogen at room temperature as required.

3.1.2. Paclitaxel

Paclitaxel was purchased from Hauser Co. (Boulder, CO) and stored in a refrigerator at -4°C as required.

3.1.3. Chemicals & solvents

Dichloromethane (DCM), HPLC grade, Fisher Scientific (Nepeon, ON).

Poly(vinyl) alcohol (PVA), M.W.= 13,000-23,000 g/mole, 98% hydrolyzed, Aldrich (Milwaukee, WI).

Methanol, chloroform, and acetonitrile, HPLC grade, all from Fisher Scientific (Fairlawn, New Jersey).

Polysorbate 80, Sigma (St. Louis, MO).

Potassium bromide (KBr), FTIR grade, from Sigma (St. Louis, MO).

Bovine serum albumin Fraktion-V (Boehringer Mannheim, German).

Sodium phosphate mono-hydrogen (Na$_2$HPO$_4$) and sodium dihydrogen orthophosphate (NaH$_2$PO$_4$H$_2$O), all from BDH Inc. (Toronto, ON).

Sodium chloride (NaCl), Acros Organics, (Belgium, New Jersey).

Acetone, analytical grade, Fisher Scientific (Fairlawn, New Jersey).

Ethanol, analytical grade, Fisher Scientific (Fairlawn, New Jersey).
DSC indium standard (99.99%), Sigma (St. Louis, MO).

Chloroform-D (99.8%) from Cambridge Isotope Laboratory (MA).

Distilled water.

*Broad polystyrene (PS) standards with molecular weights of 2,000, 4,000, 9,000, 17,500, 30,000, 50,000, 100,000 and 170,600 g/mole from Pressure Chemical Company.*

### 3.1.4. Buffers, mobile phases and PVA solution

Phosphate buffered saline with 0.4 g/L albumin (pH = 7.3) was prepared with
2.60 g of sodium phosphate mono-hydrogen (Na2HPO4), 0.32 g of sodium dihydrogen orthophosphate (NaH2PO4 H2O), 8.22 g of sodium chloride (NaCl), and 0.4 g of albumin. All above chemicals were then dissolved in 1.0 L distilled water.

HPLC mobile phase was prepared by mixing 50 mL of methanol, 370 mL of distilled water, and 580 mL of acetonitrile. All solvents were HPLC grade.

PVA solution (5.0% (w/v)) was prepared with 50 g of PVA dissolved in 1.0 L distilled water at a temperature of no higher than 75°C.

### 3.1.5. GPC analysis

All GPC standards and polymer sample solutions were prepared by dissolving the corresponding polymer in chloroform (HPLC grade). For GPC standards and polymer samples with molecular weights less than 20,000 g/mole, a 0.20% (w/v) solution was used. For GPC standards and polymer samples with molecular weights greater than or equal to 20,000 g/mole, a 0.10% (w/v) solution was used. Pure chloroform (HPLC grade) was used as GPC mobile phase.
3.1.6. Glassware

Release studies used Pyrex® brand test tubes (15 mL) with Teflon®-lined screw-cap lids. Beakers (250 mL) and graduated cylinders used for manufacturing microspheres were also Pyrex® brand. Scintillation vials (20 mL) with polypropylene-lined screw-cap lids were used to store microspheres. All glassware was purchased from Fisher Scientific (Toronto, ON).

3.2. Equipment

3.2.1. Apparatus for microsphere manufacture

The apparatus for microsphere manufacturing consisted of a Dyna-Mix overhead stirrer with a speed controller (model-143, Fisher Scientific, Rockford, IL). A 4-blade impeller with shaft length = 203 mm and shaft diameter = 6.4 mm (each blade was a 90° sector which was 15° inclined from horizontal) was immersed in a 250-mL beaker containing the PVA solution which was emulsified by stirring (Figure 8).

3.2.2. High performance liquid chromatography (HPLC)

Chromatographic analyses of paclitaxel were performed using a Shimadzu system (Tokyo, Japan), equipped with a Shimadzu SIL-9A autosampler (Tokyo, Japan), a Shimadzu model SPD-6A detector (Tokyo, Japan), a Shimadzu Chromatopac C-R3A integrator (Tokyo, Japan), and a Beckman 110A pump from Beckman Instruments Inc. (Palo alto, CA). The analytical column was a C-18 reverse phase column from Polymer Sciences Inc. (Boston, MA).

3.2.3. Gel permeation chromatography (GPC)

GPC analyses of polymers were performed using a Shimadzu system (Tokyo, Japan), equipped with a Shimadzu SIL-9A autosampler (Tokyo, Japan), a Shimadzu
refractive index detector RID-6A (Tokyo, Japan), a Shimadzu Chromatopac C-R601 integrator (Tokyo, Japan), and a Shimadzu LC-10AD pump (Tokyo, Japan). The GPC column was a PLgel column with a nominal pore size of 10^4 Å, bead size of 5 μm and 7.5 x 300 mm in dimensions (Polymer Laboratory, Boston, MA).

3.2.4. Differential scanning calorimetry (DSC)

Thermal analysis was performed using a differential scanning calorimeter, model 910S from Dupont Instruments Inc. (New Castle, DL). The thermal analysis system was controlled by an IBM compatible computer loaded with a thermal analyst software from TA Instruments. Aluminum sample pans and lids were from Rheometric Scientific (Piscataway, NJ) and were sealed with a crimper (Dupont, model 900878-901).

3.2.5. Scanning electron microscopy (SEM)

Microsphere morphology was determined using a Hitachi S-2300 scanning electron microscope (Tokyo, Japan). Samples were coated with gold-palladium using a Hummer sputter coater (Technics, Alexandra, VA).

3.2.6. Fourier transform infra-red spectroscopy (FTIR)

FTIR measurements were conducted using a Fourier transform infra-red spectrometer (Bomem Inc., Quebec). KBr sample discs were compressed using a FTIR KBr sample die (Wilmad, NJ).

3.2.7. Particle size analysis

The size distributions of microspheres were measured with a Coulter LS130 laser scattering particle size analyser (Coulter Scientific, Hialeah, FL) with Coulter LS130 version 1.53 computer software.
3.2.8. **Polymer compositions**

The molar compositions of lactide:glycolide polymers were measured using nuclear magnetic resonance (NMR), Bruker Instrument AC-200 (Bruker, Germany).

3.2.9. **X-ray powder diffraction**

Rigaku Geigerflex X-ray diffractometer (Tokyo, Japan) was used to determine polymer and paclitaxel X-ray powder diffraction patterns.

3.2.10. **Centrifuges**

A centrifuge, model GPR (Beckman Instruments Inc., Palo Alto, CA) was used during microsphere manufacture. A high-speed centrifuge, model GS-6 (Beckman Instruments Inc., Palo Alto, CA) was used for *in vitro* paclitaxel release studies.

3.2.11. **Incubator and ovens**

An isotemp incubator (Fisher Scientific, Fairlawn, NJ) and a culture tube rotator with a rotation speed control unit (VWR, Toronto, ON) were used for microsphere release studies. Napco vacuum oven model 5831 (Precision Scientific, Chicago, IL) equipped with an Emerson vacuum pump model SA55NXGTE4870 (Emerson Motor, St. Louis, MO) was used to dry paclitaxel and KBr for FTIR studies.

3.2.12. **Other equipment**

Olympus optical microscope model BH-2 (Olympus Optical, Japan).

Contax 35 mm camera, model 167MT (Kyocera Corp., Tokyo, Japan).

Mettler balances model PJ300 (Mettler Instruments, Zurich, Switzerland).

Vortexer (VWR, Bohemia, NY).

Reacti-Therm III heating/stirring module (Pierce Inc. Rockford, IL).

Refrigerator & freezer (Caltel Scientific, Richmond, BC).
A: Dyna-Mix overhead stirrer with a speed controller

B: 4-blade impeller

Figure 8 Illustration of microsphere fabrication (A: Dyna-Mix overhead stirrer with a speed controller and B: 4-blades overhead impeller).
3.3. Methods

3.3.1. Preparation of microspheres

Microspheres in the size range of 1-20μm were prepared using the solvent evaporation method (Jeffery et al., 1991; O’Hagan et al., 1994). A total weight of 0.50 g of polymer and paclitaxel were dissolved in 10 mL of DCM. The weights of paclitaxel used ranged from 5 mg to 100 mg, depending on the paclitaxel loading in the polymer matrix.

A 5% (w/v) PVA aqueous solution (100 mL) was added into a 250-mL beaker using a graduated cylinder. The PVA solution was stirred with an overhead impeller at 900 ± 50 rpm at room temperature. The paclitaxel/polymer solution was slowly added into the PVA emulsion. After 2.5 - 3 hours, the suspension of microspheres was centrifuged at 1000xg for 10 minutes with a bench top centrifuge. The supernatant was removed by suction and the microspheres were washed four times with distilled water and then centrifuged. The washed microspheres were transferred into a 20-mL scintillation vial and air-dried overnight. The dried microspheres were stored at room temperature in a desiccator for further drying. Control microspheres (paclitaxel-free) were prepared the same way as described above.

Microspheres with size ranges between 20-100μm were prepared by altering the PVA concentration and stirring rate. The paclitaxel/polymer solution was slowly added into 100 mL 2.5% (w/v) PVA aqueous solution and stirred with an overhead impeller stirrer at 550 ± 50 rpm at room temperature. After 2.5 - 3 hours, the suspension of microspheres was centrifuged at 170 x g for 10 minutes. The supernatant was removed and the microspheres were washed four times with distilled water. The washed
microspheres were then air-dried overnight and stored in a desiccator at room temperature for further drying. Control microspheres (paclitaxel-free) were also prepared the same way as described above. The yields of all microspheres were calculated.

3.3.2. **Characterization of microspheres**

3.3.2.1. **Nuclear magnetic resonance**

The molar compositions of the polymers were characterized by $^1$H-NMR spectroscopy. Briefly, 6-10 mg of each pure polymer was placed in a seven-inch round bottom NMR tube (I.D. = 5 mm) and a 4.5 cm height of high purity CDCl$_3$ solvent was added into the tube to completely dissolve the samples. The spectra were recorded on a Bruker AC-200 (200 MHz) NMR spectrometer using tetramethylsilane (TMS) as an internal reference.

3.3.2.2. **Measurement of molecular weight by gel permeation chromatography**

Polystyrene (PS) standards or polymer sample solutions for GPC testing were obtained by dissolving 1.0 to 2.5 mg of each corresponding polymer in 1.0 mL of chloroform at room temperature. Each sample solution was injected into the GPC equipped with a PLgel column (nominal pore size of $10^4$ Å) through a loop injector (20 μL). The instrument was stabilized at least one hour before measurement and the temperature was controlled at 40°C throughout the testing. The solvent flow rate was 1.0 mL/min. The calibration curve of polystyrene standards was obtained by plotting logarithms of the molecular weights of polystyrene standards versus the retention time.
3.3.2.3.  Determination of sizes and morphology of microspheres

A.  Optical microscope

For dried microspheres, 5 mg of microspheres was reconstituted with 0.5 mL of distilled water and 0.5 mL of 1% of polysorbate 80 solution. One drop of reconstituted microsphere suspension was placed on the glass slide and photographed.

B.  Laser scattering

The microsphere size distributions were determined by Coulter LS130. Approximately 5 mg of microspheres were resuspended in 2.0 mL of 1.0% polysorbate 80 solution. When a background scan on distilled water was completed, 1.0 mL of the above microsphere suspension was added into the sample cell (obscuration reading should be between 7 and 11%). The sample was measured by two 60-seconds scans. A volume% against particle size distribution was obtained along with sample mean ± standard deviation.

C.  Scanning electron microscope

The microsphere samples were sprayed on to the conductive adhesive film on the sample holders. The sample holders were then placed into the sample coater connected with a high vacuum. When a vacuum of $10^{-5}$ torr was reached, argon gas was turned on and a high voltage was applied to provide a current of about 25 mA. Samples were coated with gold-palladium for 3 minutes (about 100 Å in thickness). A second coating of the microsphere samples was done at an angle of 45°. After coating, samples were placed into the SEM (Hitachi model S-2300) to observe the surface morphology at 25 mA and less than 10 kV (with most favorable condition at 5-7kV). Pictures were taken using a computer interface (PCI3.0).
3.3.2.4. X-ray diffraction

Powder X-ray diffraction analysis on control and paclitaxel-loaded microspheres was conducted with a Rigaku Geigerflex powder X-ray diffractometer. Between 0.7-1.0 g of microsphere sample was pressed firmly in the X-ray sample frame. An empty sample frame was used when doing the background scan. All samples were scanned from 5 to 50° (2θ) at a rate of 2°/min. Pure paclitaxel as received from the manufacturer was scanned in the same way. The X-ray source was Cu Kα1 radiation (λ = 1.5418Å) and was generated at 40 kV and 20 mA.

3.3.2.5. Thermal analysis

The glass transition temperatures (Tg) of microspheres with or without paclitaxel loading were measured with a Dupont model 910S differential scanning calorimeter. About 4.50 ± 0.20 mg samples were accurately weighed in a DSC pan and then sealed with a DSC lid with a Dupont sample crimper. An empty pan and lid was also crimped and used as a reference pan. All samples were analyzed at a heating rate of 10°C/min with N2 gas flowing at a rate of 40 mL/min through the sample cell.

The DSC was calibrated with indium each time before running samples. Indium was weighed (4.4 mg) and sealed in the DSC pan and run under the same conditions as the samples. The standard melting temperature for indium is 156.7°C.

3.3.2.6. Fourier transform infra-red spectroscopy (FTIR)

FTIR was used to investigate a possible interaction between the paclitaxel and PLG polymers. Briefly, KBr was heated to 40°C and carefully ground under an infrared lamp to avoid condensation of atmospheric moisture. The KBr was dried at 105°C for 1 hour under a nitrogen atmosphere and then stored in a vacuum oven at room temperature.
Both KBr powder and paclitaxel were dried in the oven at room temperature and at reduced pressure (20 mmHg) overnight. PLG microspheres (2.00 ± 0.02 mg), with or without paclitaxel, were mixed with 200 ± 1.0 mg of ground KBr. Then 150 ± 2.0 mg of mixed samples was immediately placed in a KBr die. The samples were pressed under initial pressure (2 tons) for 15 minutes and then under high pressure (8-10 tons) for another 15 minutes to form a transparent KBr disc (with a diameter of 13 mm and 0.3 mm in thickness). The sample discs were loaded in an FTIR sample disc holder. A background scan was done to calculate the adsorption due to air and then the samples were scanned between 400 - 4000 cm\(^{-1}\) with a resolution of 2 cm\(^{-1}\). The final spectrum was obtained by subtracting the background adsorption.

To obtain a spectrum of a physical mixture, 0.20 ± 0.02 mg of paclitaxel and 1.80 ± 0.02 mg of microspheres (paclitaxel-free) were mixed with 200 ± 1.0 mg of ground KBr. Sample discs were prepared and tested as described above.

A pure paclitaxel FTIR spectrum was obtained using the same conditions as the microsphere samples. Briefly, 1.00 ± 0.02 mg of dried paclitaxel was mixed with 200 ± 1.0 mg of pre-dried KBr powder. Sample discs were prepared and tested as described above.

3.3.3. Total content of paclitaxel in microspheres

3.3.3.1. Determination of total content of paclitaxel in microspheres

The assay for measuring total content of paclitaxel in microspheres was based on the method of Burt et al. (1995) with slight modification. Approximately 2 mg of paclitaxel-loaded microspheres, accurately weighed, were dissolved in 1 mL of DCM in a test tube and the resulting solution was transferred to a 100-mL volumetric flask. Most of
the DCM was allowed to evaporate at room temperature and 100 mL of acetonitrile was then added to the flask. A portion of the ACN solution (5 mL) was then added to a clean test tube and the residual DCM was further evaporated by vortexing for 2 minutes. The resulting solution was centrifuged and 20 μL of each sample was injected into the HPLC. The samples were analyzed for paclitaxel using HPLC with a mobile phase of water:methanol:ACN (37:5:58) at a flow rate of 1.0 mL/min and UV detection at 232 nm (Burt et al., 1995). The paclitaxel concentration was calculated from the standard calibration curve. Paclitaxel encapsulation efficiency was based on the original sample weights.

3.3.3.2. Recovery of paclitaxel from microspheres

To determine the amount of paclitaxel recovered from the above procedure, 5.0 mL of a paclitaxel standard solution was transferred to a test tube. The ACN was allowed to evaporate completely. Approximately 2 mg of control (no drug) microspheres, accurately weighed, were placed into the tube and dissolved in 1 mL DCM and the resulting solution was transferred to a 100-mL volumetric flask. Paclitaxel was analyzed as described above. The amount of paclitaxel recovered from the procedure was calculated.

3.3.4. Paclitaxel release studies

3.3.4.1. Paclitaxel standard curves used for HPLC analysis

Paclitaxel standard solutions were analyzed before measuring each set of samples. Paclitaxel standard solutions with concentrations ranging from 0.1 to 50 μg/mL were prepared by diluting a stock paclitaxel solution which was obtained by dissolving about 10 mg of paclitaxel (accurately weighed) in acetonitrile in a 200-mL volumetric flask.
Until use, the solution was stored in a 4°C refrigerator. The standard curve was obtained by plotting the peak areas against the paclitaxel concentrations.

3.3.4.2. Determination of chemical stability of paclitaxel in acetonitrile, PBS-albumin, distilled water and methanol

Paclitaxel standard solutions were prepared as before and analyzed at weeks 1, 2, 3, and 4, followed by once every month up to 6 months. Paclitaxel standard solution (0.1 mL) was transferred into a 2-mL HPLC vial, the ACN evaporated completely in the fumehood and 1.0 mL of methanol, distilled water or PBS-albumin was added to each tube. All vials were vortexed for 2 minutes. At each time interval (t = 0 and every 24 hours up to 7 days), the methanol sample was injected directly into the HPLC. The other samples were prepared by adding 0.5 mL of DCM to extract the paclitaxel. Samples were vortexed for 1 minute, centrifuged at 1000×g for 10 minutes and the supernatant discarded. The DCM solution was dried completely under a stream of nitrogen. ACN:H₂O (50:50) (1.0 mL) was added into each tube. Samples were vortexed for 1 minute and analyzed by HPLC as described previously.

3.3.4.3. Determination of in vitro release profiles of paclitaxel from microspheres

Microspheres (2.5 mg, accurately weighed) were placed into a 15-mL tube and 13 mL of 0.01 M PBS-albumin were added (N = 4). The tubes were tumbled in the incubator at constant temperature (37°C). At given time intervals, the tubes were centrifuged (550×g, 5 minutes) and 12 mL of supernatant solution were withdrawn. The microspheres were resuspended in 12 mL of PBS-albumin replacement buffer and placed back in the incubator. To the supernatants was added 1.0 mL DCM. The tubes were vigorously shaken, allowed to settle for 15 minutes and centrifuged at 1000×g for 10 minutes.
Paclitaxel was extracted into the DCM and the supernatant was withdrawn. The DCM was evaporated to dryness under a stream of nitrogen in the heating block (45°C). The tubes were reconstituted with 1 mL 50% acetonitrile in distilled water. The solutions were transferred to 1.5-mL Eppendorf tubes and centrifuged at 8000×g for 10 minutes.

The samples were analyzed for paclitaxel using HPLC with a mobile phase of water:methanol:ACN (37:5:58) at a flow rate of 1.0 mL/min and UV detection at 232 nm (Burt et al., 1995).

The cumulative amounts of paclitaxel released were plotted as a function of time in order to obtain the paclitaxel in vitro release profiles.

3.3.4.4. Recovery of paclitaxel from PBS-albumin

Paclitaxel standard solution (500 μL) was added to a tube. ACN was allowed to evaporate completely in a fume-hood at room temperature. PBS-albumin (12 mL) was added into each tube and vortexed for one minute. DCM (1.0 mL) was added to each tube, vigorously shaken for 10 seconds and then allowed to settle for 15 minutes. The tubes were centrifuged at 1000×g for 10 minutes and the supernatant removed. The samples were dried under a stream of nitrogen gas at 45°C for 30 minutes, reconstituted with 1 mL of ACN:H₂O (50:50) and vortexed for 1 minute. The solutions were transferred to 1.5-mL Eppendorf tubes and centrifuged at 8000×g for 10 minutes. The supernatants were then transferred to HPLC vials and analyzed by HPLC.

3.3.5. Microsphere degradation studies

PLG microspheres (10 mg) with or without paclitaxel loading were placed in a 50-mL tube filled with 50 mL of 0.01 M phosphate buffered saline (PBS) containing albumin (0.4g/L) and tumbled in the incubator at 37°C. The microspheres were sampled
at different time intervals. The buffer was changed either every day for paclitaxel-loaded samples or alternate days for control samples.

The microsphere samples were washed with distilled water three times and dried in fumehood and then in the desiccator at room temperature. The molecular weights of the degraded PLG microspheres were determined by GPC. The surface morphologies of the degraded PLG microspheres were observed and recorded using SEM. The glass transition temperature ($T_g$) of each sample was also determined using DSC.

3.3.6. Co-60 irradiation of PLG microspheres and paclitaxel

Pure paclitaxel and the microspheres with or without paclitaxel loading were accurately weighed (100 ± 0.02 mg) and transferred into individual 5.0 mL vials with Teflon® caps. The vials were labeled and shipped to Chiron (US) for Co-60 irradiation. All samples were irradiated with a dose of 2.5 Mrad over 350 minutes. Samples were surrounded with blue ice (2°C) to avoid heat generation during irradiation. The irradiated samples were characterized using DSC, GPC and HPLC.

3.3.7. Statistical treatment of data

Data collected by measurement of several samples from different batches of material were presented as “average ± standard deviation”. For ANOVA tests and t-tests, the level of significance was a $p$-value of less than 0.05 and the hypothesized difference between populations was zero.

For particles size distributions of the microspheres, two 60-seconds scans were performed on each sample and data were summarized as the mean ± standard deviation of the particle size distribution.
For *in vitro* release studies, measurements were made on four samples. Values of the average and standard deviation were calculated from cumulative amounts of paclitaxel released from each of the four samples. In figures representing these data, the average values are plotted against time and the error bars represent the standard deviations.

For all other data collected by repeated measurement of samples from a single batch material, the average was calculated. The relative standard deviation (RSD) of this type of average was used to calculate the precision in the measurements.
4. RESULTS

4.1. Paclitaxel and 7-epi-taxol

Paclitaxel was used as received from Hauser and stored at -4°C until required. Paclitaxel has two epi-isomers. Paclitaxel has an alpha hydroxy group (α-OH) at C-7 while 7-epitaxol has a beta hydroxy group (β-OH) at C-7. Scanning electron micrographs of paclitaxel and 7-epitaxol revealed that the crystals exhibited a needle shaped crystal habit for both crystals (Figure 9).

4.2. Physical characterization of PLG polymers

4.2.1. The molar compositions of PLG polymers

Polymers were synthesized by BPI in compliance with the FDA guidelines for current good manufacturing practices (cGMP). PLG polymer with a targeted inherent viscosity (I.V.) of greater than 0.50 were within ±10% of its lactide:glycolide composition. Physical data obtained from BPI indicated that PLG either had different inherent viscosities (I.V. = 0.58-1.06) for the same lactide:glycolide (50:50) composition group of polymers or had different lactide:glycolide compositions for the similar inherent viscosity (I.V. = 0.55-0.60) group of polymers. PLG polymers contained < 100 ppm of residual Sn+2 and < 1% of residual monomer.

The molar compositions of the above polymers were determined by H1-NMR spectroscopy. Representative results are shown in Figure 10. The molar compositions of the polymers (expressed as a percentage) were calculated from the peak areas of methylene, methyl and methine proton signals (Amecke et al., 1995). Table 1 compares the LA:GA molar compositions of the PLG polymers provided by the manufacturer and determined by H1-NMR. The results of NMR analysis are in good agreement with the
targeted compositions provided by the manufacturer with the exception of two samples of PLG50:50.

4.2.2. *Standard GPC curve and molecular weights of polymers*

The elution profiles for polystyrene standards are shown in Figure 11. The calibration curve was obtained by plotting the logarithm of the molecular weights of the polystyrene standards against the peak retention time (Figure 12). Results indicate that the PLgel 10$^4$ column can be used to measure the polymers with a molecular weight between 4,000 and 170,600 g/mole.
<table>
<thead>
<tr>
<th>PLG Polymers</th>
<th>LA:GA ratio$^b$</th>
<th>I.V.</th>
<th>Mole$^a$%</th>
<th>Z-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lactide</td>
<td>Glycolide</td>
</tr>
<tr>
<td>100:0</td>
<td>0.60</td>
<td>100±0.0</td>
<td>0±0.0</td>
<td>N/A</td>
</tr>
<tr>
<td>85:15</td>
<td>0.56</td>
<td>85.2±0.4</td>
<td>14.8±0.4</td>
<td>0.29$^d$</td>
</tr>
<tr>
<td>75:25</td>
<td>0.55</td>
<td>75.6±0.5</td>
<td>24.4±0.4</td>
<td>0.69$^d$</td>
</tr>
<tr>
<td>65:35</td>
<td>0.55</td>
<td>65.7±0.6</td>
<td>34.3±0.3</td>
<td>1.35$^d$</td>
</tr>
<tr>
<td>50:50</td>
<td>1.06</td>
<td>53.7±0.7</td>
<td>46.3±0.5</td>
<td>3.05$^c$</td>
</tr>
<tr>
<td>50:50</td>
<td>0.78</td>
<td>51.0±0.4</td>
<td>49.0±0.5</td>
<td>1.44$^d$</td>
</tr>
<tr>
<td>50:50</td>
<td>0.74</td>
<td>50.9±0.4</td>
<td>49.1±0.5</td>
<td>1.30$^d$</td>
</tr>
<tr>
<td>50:50</td>
<td>0.58</td>
<td>52.8±0.4</td>
<td>47.2±0.5</td>
<td>4.04$^c$</td>
</tr>
</tbody>
</table>

$^a$ Molar percents of lactide and glycolide were calculated from the peak area of methine (-CH), methylene (-CH$_2$) and methyl (-CH$_3$) protons (Amecke et al., 1995).

$^b$ Molar percents of lactide and glycolide provided by manufacturer.

$^c$ Z-values are greater than 1.96 (two-tailed) indicating LA and GA values are significantly different from the theoretical values.

$^d$ Z-values are lower than 1.96 (two-tailed) indicating LA and GA values are not significantly different from the theoretical values.
Figure 9 SEM micrographs of A: paclitaxel crystal (magnification: 5.0k× at 10kV, 25mA) and B: 7-epitaxol crystal (magnification: 5.0k× at 10kV, 25mA).
Figure 10  Representative H¹-NMR (200MHz) spectra of PLG polymers [A: a PLG polymer with a LA:GA composition of 100:0 and I.V. = 0.60, B: a PLG polymer with a LA:GA composition of 75:25 and I.V. = 0.55, and C: a PLG polymer with a LA:GA composition of 50:50 and I.V. = 0.78. Solvent used: Chloroform-D (99.8%+)].
Figure 11  GPC elution profiles of polystyrene standards with molecular weights ranging from 2,000 to 100,000 g/mole at 40°C (Solvent used: chloroform. Solvent flow rate: 1.0 mL/min. Injection volume: 20μL).
Figure 12  GPC standard curve for polystyrene standards with molecular weights of 4, 9, 17.5, 30, 50, 100 and 170 kg/mole on a PL-gel column with a nominal pore size of $10^4$ Å at 40°C (Solvent used: chloroform. Solvent flow rate: 1.0 mL/min. Injection volume: 20 μL).
4.3. **Size distribution of the microspheres**

The size distributions of dried microspheres were determined by laser scattering. The small size microspheres showed 98% (by volume) of microspheres falling into the range of 1-20\(\mu\)m as shown in Figure 13A. The large size microspheres had a size range between 20-100\(\mu\)m, as shown in Figure 13B, with over 95% (by volume) of the microspheres falling into this range.

4.4. **Surface morphologies of microspheres**

The surface morphologies of microspheres were revealed with SEM. An extensive pore network (pinholes) was found on the surface of 20% paclitaxel-loaded PLG100:0 (I.V. = 0.60) microspheres (Figure 14A). This surface morphology was not seen on the 10% paclitaxel-loaded (Figure 15A) or control PLG100:0 (I.V. = 0.60) microspheres (Figure 16A). The control, 10% and 20% paclitaxel-loaded PLG85:15 (I.V. = 0.56), PLG75:25 (I.V. = 0.55) and PLG65:35 (I.V. = 0.55) microspheres had very smooth surface morphologies as shown in Figures 14B, C, D and 15B, C, D as well as in 16B, C, D.

The SEM micrographs of control, 10% and 20% paclitaxel-loaded PLG50:50 (I.V. = 1.06), PLG50:50 (I.V. = 0.78), PLG50:50 (I.V. = 0.74), and PLG50:50 (I.V. = 0.58) microspheres are shown in Figures 17-19. No differences in the surface morphologies were observed among these microspheres. The SEM micrographs of PLG50:50 (I.V. = 1.06) microspheres with 1 to 5% paclitaxel loading also revealed that their surface morphologies were smooth and there were no differences due to different paclitaxel loading (Figure 20).
Figure 13  Representative size distributions of A: small (1-20μm) microspheres (mean = 3.4 ± 1.5μm) and B: large (20-100μm) microspheres (mean = 65 ± 21μm). The size distributions of the microspheres were the averages of the two 60-seconds scans. Microspheres were resuspended in polysorbate 80 solution.
Figure 14  SEM micrographs of 20% paclitaxel-loaded PLG microspheres  [A: PLG100:0, 20-100μm (1k× at 5kV, 25mA); B: PLG85:15, 1-20μm (10k× at 6kV, 25mA); C: PLG75:25, 1-20μm (4k× at 6kV, 25mA); and D: PLG65:35, 1-20μm (1k× at 5kV, 25mA)].
Figure 15  SEM micrographs of 10% paclitaxel-loaded PLG microspheres [A: PLG100:0, 20-100µm (2k× at 5kV, 25mA); B: PLG85:15, 1-20µm (10k× at 6kV, 25mA); C: PLG75:25, 1-20µm (600× at 5kV, 25mA); and D: PLG65:35, 1-20µm (10k× at 6kV, 25mA)].
Figure 16 SEM micrographs of control PLG microspheres [A: PLG100:0, 20-100μm (1.5k× at 5kV, 25mA); B: PLG85:15, 1-20μm (6k× at 5kV, 25mA); C: PLG75:25, 1-20μm (3k× at 5kV, 25mA); and D: PLG65:35, 1-20μm (2k× at 7kV, 25mA)].
Figure 17  SEM micrographs of 20% paclitaxel-loaded PLG50:50 microspheres [A: I.V. = 0.58, 1-20μm (1k× at 5kV, 25mA); B: I.V. = 0.74, 1-20μm (10k× at 5kV, 25mA); C: I.V. = 0.78, 1-20μm (2k× at 5kV, 25mA); and D: I.V. = 1.06, 1-20μm (3k× at 5kV, 25mA)].
Figure 18  SEM micrographs of 10% paclitaxel-loaded PLG50:50 microspheres [A: I.V. = 0.58, 1-20μm (1k× at 5kV, 25mA); B: I.V. = 0.74, 1-20μm (5k× at 5kV, 25mA); C: I.V. = 0.78, 1-20μm (5k× at 5kV, 25mA); and D: I.V. = 1.06, 1-20μm (4k× at 5kV, 25mA)].
Figure 19  SEM micrographs of control PLG50:50 microspheres [A: I.V. = 0.58, 1-20μm (400× at 5kV, 25mA); B: I.V. = 0.74, 1-20μm (2k× at 8kV, 25mA); C: I.V. = 0.78, 1-20μm (2k× at 5kV, 25mA); and D: I.V. = 1.06, 1-20μm (5k× at 5kV, 25mA)].
Figure 20  SEM micrographs of paclitaxel-loaded PLG50:50 (I.V.= 1.06, 1-20 µm) microspheres [A: 1% paclitaxel (4k× at 5kV, 25mA); B: 5% paclitaxel, (1k× at 5kV, 25mA); C: 10% paclitaxel, (4k× at 5kV, 25mA); and D: 20% paclitaxel, (3k× at 5kV, 25mA)].
4.5. Characterization of microspheres

4.5.1. X-ray diffraction patterns of the microspheres

Figure 21 shows the X-ray diffraction pattern of paclitaxel as received from Hauser. Paclitaxel gave three strong diffraction peaks between 5-15° (2θ). Figure 22 shows the X-ray diffraction patterns of 20% paclitaxel-loaded and control PLG100:0 (I.V. = 0.60, 20-100μm) microspheres. A typical amorphous halo pattern was observed for all control and paclitaxel-loaded microspheres. X-ray data for all microspheres are given in Table 2.

Figure 21 X-ray powder diffraction pattern of paclitaxel. Paclitaxel sample as received was scanned at 2°/min at 20mA and 40kV at room temperature (25°C).
Figure 22  X-ray diffraction patterns of A: control and B: 20% paclitaxel-loaded PLG100:0 microspheres. Microsphere samples were scanned at 2°/min at 20mA and 40kV at room temperature (25°C).
Table 2  X-ray diffraction results for control and paclitaxel-loaded PLG microspheres. Microsphere samples were scanned at 2°/min at 20mA and 40kV at room temperature (25°C)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>I.V.</th>
<th>Paclitaxel Loading</th>
<th>X-ray Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLG100:0</td>
<td>0.60</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG85:15</td>
<td>0.56</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG75:25</td>
<td>0.55</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG65:35</td>
<td>0.55</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.74</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.58</td>
<td>0%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG100:0</td>
<td>0.60</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG85:15</td>
<td>0.56</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG75:25</td>
<td>0.55</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG65:35</td>
<td>0.55</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.74</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.58</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>20%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>10%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>5%</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>1%</td>
<td>Amorphous</td>
</tr>
</tbody>
</table>

a  Microsphere size = 20-100μm.
b  Microsphere size = 1-20μm.
c  A typical halo pattern as shown in Figure 22 was observed.
4.5.2. Glass transition temperatures of control and paclitaxel-loaded microspheres

The DSC thermograms of paclitaxel as well as control and 20% paclitaxel-loaded PLG microspheres are given in Figures 23 and 24. The paclitaxel sample had a melting peak at 224°C (Figure 23A). Exothermic peaks at 230-240°C were due to the decomposition of paclitaxel at high temperatures. However, no paclitaxel melting peaks were found in any of the paclitaxel-loaded PLG microspheres (Figure 24A). The exothermic peaks at about 250-260°C were observed in the paclitaxel-loaded PLG microsphere samples, similar to the paclitaxel sample. The DSC thermogram of the control PLG microspheres did not show any exothermic peak after 230°C (Figure 24B), but the decomposition of the PLG polymer was found after 296°C.

The glass transition temperatures of the microsphere samples are shown as endothermic peaks at about 51°C in Figures 23 and 24 and are summarized in Table 3. The glass transition temperatures increased with an increase in the paclitaxel loading in the microspheres with either different LA:GA compositions or the same 50:50 LA:GA composition but different molecular weights. The \textit{p-value} from two-tailed tests indicated that increases in the glass transition temperatures were significant for all 20% paclitaxel-loaded microspheres regardless of the sizes and LA:GA compositions of the PLG microspheres.

4.5.3. Fourier transform infra-red spectroscopy (FTIR)

Figure 25 shows the FTIR spectrum of paclitaxel and the FTIR spectrum of control PLG50:50 (I.V. = 0.74, 1-20μm) microspheres. Figure 26 shows the FTIR spectra of a physical mixture of paclitaxel/control PLG50:50 (I.V. = 0.74, 1-20μm) microspheres and 20% paclitaxel-loaded PLG50:50 (I.V. = 0.74, 1-20μm) microspheres. The
wavenumber of paclitaxel C=O in-plane (δC=O) and out-of-plane (γC=O) deformation was 710 cm⁻¹ while the wavenumbers of C=O in-plane (δC=O) and out-of-plane (γC=O) deformation from paclitaxel-loaded microspheres increased significantly (about 721-730 cm⁻¹). The p-values for the two-tailed student t-tests revealed that the wavenumbers of C=O in-plane (δC=O) and out-of-plane (γC=O) deformation for paclitaxel-loaded PLG microspheres were increased significantly compared to control PLG microspheres (Table 4).
Table 3  Glass transition temperatures ($T_g$) of control and paclitaxel-loaded PLG microspheres
(heating rate: 10 °C/min; N₂ purging rate: 40 mL/min.)

<table>
<thead>
<tr>
<th>Polymers</th>
<th>L.V.</th>
<th>Size ranges (μm)</th>
<th>Glass transition temperatures ($T_g$) (°C)</th>
<th>p-value</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>1% paclitaxel</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>1 – 20</td>
<td>51.5 ± 0.9</td>
<td>52.6 ± 1.1</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>1 – 20</td>
<td>48.3 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>20 – 100</td>
<td>48.2 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.74</td>
<td>1 – 20</td>
<td>49.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>PLG100:0</td>
<td>0.60</td>
<td>20 – 100</td>
<td>57.0 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>PLG85:15</td>
<td>0.56</td>
<td>1 – 20</td>
<td>54.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>PLG75:25</td>
<td>0.55</td>
<td>1 – 20</td>
<td>53.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>PLG65:35</td>
<td>0.55</td>
<td>1 – 20</td>
<td>51.2 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Values obtained by two-tailed student t-tests with equal variances between the control and 20% paclitaxel-loaded microspheres. Glass transition temperatures of paclitaxel-loaded microspheres are significantly increased compared with control microspheres when p ≤ 0.05.

<sup>b</sup> All data are the average of N = 3, ± standard deviation.
Figure 23  DSC thermograms of A: paclitaxel and B: a physical mixture of paclitaxel/PLG50:50 (I.V.= 1.06, 1-20μm) microspheres (1:4). Samples were scanned at a heating rate of 10 °C/min with a N₂ purging rate of 40 mL/min.
Figure 24  DSC thermograms of A: 20% paclitaxel-loaded PLG85:15 (I.V. = 0.56, 1-20μm) and B: control PLG65:35 (I.V. = 0.55, 1-20μm) microspheres. Samples were scanned at a heating rate of 10 °C/min with a N₂ purging rate of 40 mL/min.
Table 4  Wavenumbers of C=O double bond in-plane (δC=O) / out-of-plane (γC=O) deformation absorption of the PLG microspheres (± paclitaxel). Samples were run at a resolution of 2 cm⁻¹.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>I.V.</th>
<th>Wavenumber of control PLG microspheres (cm⁻¹)</th>
<th>Wavenumber of 20% paclitaxel-loaded PLG microspheres (cm⁻¹)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLG100:0</td>
<td>0.60</td>
<td>707 ± 4</td>
<td>730 ± 8</td>
<td>0.009</td>
</tr>
<tr>
<td>PLG85:15</td>
<td>0.56</td>
<td>708 ± 2</td>
<td>727 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PLG75:25</td>
<td>0.55</td>
<td>709 ± 2</td>
<td>727 ± 4</td>
<td>0.002</td>
</tr>
<tr>
<td>PLG65:35</td>
<td>0.55</td>
<td>708 ± 2</td>
<td>726 ± 3</td>
<td>0.001</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>708 ± 1</td>
<td>724 ± 2</td>
<td>0.002</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>710 ± 2</td>
<td>726 ± 7</td>
<td>0.02</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.74</td>
<td>709 ± 3</td>
<td>721 ± 4</td>
<td>0.01</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.58</td>
<td>708 ± 2</td>
<td>730 ± 6</td>
<td>0.003</td>
</tr>
</tbody>
</table>

a Value obtained by two-tailed student t-test assuming equal variances. Wavenumbers of paclitaxel-loaded microspheres are significantly higher than control microspheres when p ≤ 0.05.

b Data are the average of three samples, ± standard deviation.
A. FTIR spectrum of paclitaxel as received

B. FTIR spectrum of control PLG50:50 (I.V. = 0.74, 1-20μm) microspheres

Figure 25 Representative FTIR spectra of A: paclitaxel and B: control PLG50:50 (I.V. = 0.74, 1-20μm) microspheres. Samples were run at a resolution of 2 cm⁻¹.
Figure 26  Representative FTIR spectra of A: a physical mixture of control PLG50:50 (I.V. = 0.74, 1-20μm) microspheres/paclitaxel and B: 20% paclitaxel-loaded PLG50:50 (I.V. = 0.74, 1-20μm) microspheres. Samples were run at a resolution of 2 cm⁻¹.
4.5.4. Total content and encapsulation efficiency of paclitaxel in microspheres

Paclitaxel recoveries during extraction, expressed as a percentage, are shown in Table 5. A high percentage of paclitaxel (≥ 99%) can be recovered from this process.

Table 6 summarizes the encapsulation efficiencies of different paclitaxel-loaded PLG microspheres. The microsphere fabrication process had high encapsulation efficiency for paclitaxel. Most microspheres had paclitaxel encapsulation efficiency of greater than 90% except for 20% paclitaxel-loaded PLG100:0 (I.V. = 0.60, 20-100μm) microspheres. The actual loading of paclitaxel in the microspheres is listed in Table 7.

4.5.5. Stability of paclitaxel in acetonitrile, PBS-albumin, distilled water and methanol

Paclitaxel was very stable in ACN with less than 0.5% of paclitaxel degrading over one month (at 37°C) or over six months (at 4°C). Paclitaxel in methanol and distilled water were relatively stable within 72 hours, with less than 1% of paclitaxel degrading (at 37°C). Paclitaxel was less stable in PBS-albumin. About 2% of paclitaxel degraded in 24 hours (37°C). However, no 7-epitaxol peak was found in the HPLC chromatogram during release studies.
Table 5  Percentages of paclitaxel recovery during extraction from control PLG microspheres/paclitaxel mixtures

<table>
<thead>
<tr>
<th>Polymers</th>
<th>I.V.</th>
<th>% of paclitaxel recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLG100:0</td>
<td>0.60</td>
<td>98.8 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG85:15</td>
<td>0.56</td>
<td>99.5 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG75:25</td>
<td>0.55</td>
<td>98.3 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG65:35</td>
<td>0.55</td>
<td>97.5 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>99.0 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.74</td>
<td>99.0 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.58</td>
<td>99.8 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>98.7 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are the averages of three samples (N = 3), ± standard deviation.

<sup>b</sup> Average of paclitaxel recovery from all PLG polymer samples during extraction, ± standard deviation.
Table 6  Paclitaxel encapsulation efficiency in PLG microspheres with different loading, sizes and LA:GA compositions

<table>
<thead>
<tr>
<th>Polymers</th>
<th>I.V.</th>
<th>Microspheres sizes (µm)</th>
<th>Encapsulation efficiency (%) of paclitaxel in PLG microspheres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1% paclitaxel&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>1 - 20</td>
<td>100 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>20 - 100</td>
<td></td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>1 - 20</td>
<td></td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.74</td>
<td>1 - 20</td>
<td></td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.58</td>
<td>1 - 20</td>
<td></td>
</tr>
<tr>
<td>PLG100:0</td>
<td>0.60</td>
<td>20 - 100</td>
<td></td>
</tr>
<tr>
<td>PLG85:15</td>
<td>0.56</td>
<td>1 - 20</td>
<td></td>
</tr>
<tr>
<td>PLG75:25</td>
<td>0.55</td>
<td>1 - 20</td>
<td></td>
</tr>
<tr>
<td>PLG65:35</td>
<td>0.55</td>
<td>1 - 20</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are the averages of three samples, ± standard deviation. Encapsulation efficiency (%) = 100 × [(paclitaxel content) / (paclitaxel + polymer content)] × [(1/ (initial paclitaxel loading))]

<sup>b</sup> Numbers are the theoretical calculation based on the weights of PLG polymers and paclitaxel.
Table 7  Actual loading of paclitaxel in PLG microspheres with different loading, sizes and LA:GA compositions

<table>
<thead>
<tr>
<th>Polymers</th>
<th>I.V.</th>
<th>Microspheres sizes (μm)</th>
<th>1% Paclitaxel&lt;sup&gt;a&lt;/sup&gt;</th>
<th>5% Paclitaxel&lt;sup&gt;b&lt;/sup&gt;</th>
<th>10% Paclitaxel&lt;sup&gt;b&lt;/sup&gt;</th>
<th>20% Paclitaxel&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLG50:50</td>
<td>1.06</td>
<td>1 - 20</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.78</td>
<td>20 - 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.74</td>
<td>1 - 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLG50:50</td>
<td>0.58</td>
<td>1 - 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLG100:0</td>
<td>0.60</td>
<td>20 - 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLG85:15</td>
<td>0.56</td>
<td>1 - 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLG75:25</td>
<td>0.55</td>
<td>1 - 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLG65:35</td>
<td>0.55</td>
<td>1 - 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Actual loading of paclitaxel in the microspheres (N = 3).

<sup>b</sup> Numbers are the theoretical loading calculated based on the weights of PLG polymers and paclitaxel.
4.5.6. Release studies of paclitaxel-loaded microspheres

4.5.6.1. Statistical treatment of release study data

The two-tailed t-tests were used to compare the cumulative amounts of paclitaxel released on each pair of release curves involved for comparison on day 1, 2, 5, 10, 15, 20, 25 and 30 if applicable. When the p-value was ≤ 0.05, the difference between the amounts of paclitaxel released would be considered significant. The slopes (rate of paclitaxel released) of linear regressions on each of three segments (segment 1: day 1 to day 2; segment 2: day 5 to day 10 and segment 3: day 15 to 25) from the same release curves, or on the same segments of different cumulative amounts of paclitaxel released curves were also compared.

4.5.6.2. Paclitaxel release from PLG microspheres

Figure 27 shows the paclitaxel release profiles from PLG50:50 (I.V. = 1.06, 1-20μm) microspheres with paclitaxel loading from 1 to 20%. A burst phase of paclitaxel release was observed from the microspheres during the first 2 days; then followed by a relatively steady release of paclitaxel from microspheres from day 4 to day 28. The 20% paclitaxel-loaded microspheres showed the fastest release rate (amounts of paclitaxel release per day) than any other microspheres. Two-tailed t-tests on the cumulative amounts of paclitaxel released at about day 5, 10, 15, 20, 25 and 30 indicated that the differences among the amounts of paclitaxel released for the 1, 5, 10 and 20% paclitaxel-loaded PLG50:50 (I.V. = 1.06, 1-20μm) microspheres were significant (p-values ≤ 0.05).

Figure 28 shows the release profiles of paclitaxel from PLG100:0 (I.V. =0.60, 20-100μm) microspheres with 10 and 20% paclitaxel loading. There was a very large burst
phase for the 20% paclitaxel-loaded microspheres, which was followed by a period of slow release to approximately day 6, then another phase of fast release.

Figures 29 and 30 show the release profiles of paclitaxel from PLG85:15, PLG75:25 and PLG65:35 microspheres with the same size ranges (1-20 μm), similar inherent viscosities (I.V. = 0.55 – 0.56) and 10 and 20% paclitaxel loading. A burst phase of paclitaxel release was observed during the first 2 days; this was then followed by relatively steady release. The cumulative amounts of paclitaxel-released at day 23 for the PLG75:25 and PLG65:35 microspheres were similar and were significantly higher than the cumulative amounts of paclitaxel-released for the PLG85:15 microspheres (t-tests).

Figures 31 and 32 show the release profiles for 10 and 20% paclitaxel-loaded PLG50:50 microspheres with the same size ranges (1-20 μm) and different inherent viscosities (I.V. = 0.74 – 1.06) or molecular weights. Burst phases were observed up to day 2. Paclitaxel release was significantly lower for 10% paclitaxel-loaded PLG50:50 microspheres with I.V. = 1.06 compared to microspheres either with I.V. = 0.78 or with I.V. = 0.74. For 20% paclitaxel-loaded microspheres, the cumulative amounts of paclitaxel released were significantly different for all microspheres with different molecular weights.

Figure 33 compares the release profiles of two different sizes (1-20 μm and 20-100 μm) of 10% paclitaxel-loaded PLG50:50 (I.V. = 0.78) microspheres. The burst phases of these two sizes of microspheres were observed during the first two days; this was followed by a period of relatively steady release. The microspheres with smaller size range produced slightly higher burst phase release than microspheres with larger size range.
range. The rates of paclitaxel release from both size ranges of the microspheres were not significantly different (t-tests).
Figure 27 Cumulative amounts of paclitaxel released from PLG50:50 (I.V. = 1.06, 1-20μm) microspheres in PBS-albumin at 37°C (N = 4).
Figure 28 Cumulative amounts of paclitaxel released from PLG100:0 (I.V. = 0.60, 20-100μm) microspheres in PBS-albumin at 37°C (N = 4).
Figure 29  Cumulative amounts of paclitaxel released from 10\% paclitaxel-loaded PLG microspheres (1-20\mu m) with different LA:GA compositions in PBS-albumin at 37\textdegree C (N = 4).
Figure 30  Cumulative amounts of paclitaxel released from 20% paclitaxel-loaded PLG microspheres (1-20μm) with different LA:GA compositions in PBS-albumin at 37°C (N = 4).
Figure 31  Cumulative amounts of paclitaxel released from 10% paclitaxel-loaded PLG microspheres (1-20μm) with a 50:50 LA:GA composition in PBS-albumin at 37°C (N = 4).
Figure 32  Cumulative amounts of paclitaxel released from 20% paclitaxel-loaded PLG microspheres (1-20\,\mu m) with a 50:50 LA:GA composition in PBS-albumin at 37°C (N = 4).
Figure 33  Cumulative amounts of paclitaxel released from 10% paclitaxel-loaded PLG50:50 (I.V. = 0.78, 1-20\,\mu m \& 20-100\,\mu m) microspheres in PBS-albumin at 37°C (N = 4).
4.6. *In vitro* degradation of microspheres in PBS-albumin buffer

4.6.1. Molecular weights of degraded PLG microspheres determined by GPC

Figure 34 shows the GPC elution profiles for PLG polymers used in the studies. Table 8 summarizes the molecular weights of control PLG50:50 microspheres of differing inherent viscosities degraded in PBS-albumin at 37°C. Table 9 provides the molecular weights of control PLG100:0, PLG85:15, PLG75:25 and PLG65:35 microspheres of similar inherent viscosities (I.V. = 0.55 – 0.56) after incubation in PBS-albumin at 37°C. Table 10 shows the molecular weights of 10% paclitaxel-loaded PLG85:15, PLG75:25, PLG65:35 and PLG50:50 microspheres which were incubated in PBS-albumin at 37°C.

Degradation profiles for control PLG50:50 microspheres with differing inherent viscosities (I.V. = 0.58 – 1.06) were obtained by plotting their molecular weights (M<sub>GPC</sub>) against degradation time in the PBS-albumin (Figure 35). The molecular weights of the PLG50:50 microspheres decreased over a period of 4-6 weeks. The higher the initial molecular weights, the faster the degradation rate of the polymers. Figure 36 shows the degradation profiles of control PLG100:0, PLG85:15, PLG75:25 and PLG65:35 microspheres with similar inherent viscosities. The molecular weights of these PLG polymers remained quite stable over 4-6 weeks with little or no decrease in molecular weight, compared with PLG polymers with a LA:GA ratio of 50:50.

Figures 37 and 38 show the degradation profiles of control and 10% paclitaxel-loaded PLG75:25 and PLG65:35 (Figure 37) and PLG85:15 and PLG50:50 (Figure 38) microspheres. There was no significant difference between the degradation profiles of the control and 10% paclitaxel-loaded microspheres (*p* > 0.05).
Figure 39 compares the degradation profiles of two different sizes (1-20μm and 20-100μm) of control PLG50:50 (I.V. = 0.78) microspheres. The smaller microspheres appeared to show faster degradation over the first 2 weeks compared to larger microspheres.
A: GPC elution profiles of PLG polymers with the same LA:GA composition (50:50) but different inherent viscosities.

B: GPC elution profiles of PLG polymers with different LA:GA compositions but similar inherent viscosities (I.V. $\approx 0.55$).

Figure 34  GPC elution profiles of A: PLG polymers with the same LA:GA composition (50:50) but different inherent viscosities and B: PLG polymers with different LA:GA compositions but similar inherent viscosities (I.V. $\approx 0.55$) at 40°C (Solvent: chloroform. Solvent flow rate: 1.0 mL/min).
Table 8  Molecular weights of PLG50:50 microspheres with different inherent viscosities degraded over time in PBS-albumin [molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min)]

<table>
<thead>
<tr>
<th>LV</th>
<th>Size range</th>
<th>LA:GA</th>
<th>0 day</th>
<th>1 day</th>
<th>2 days</th>
<th>3 days</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
<th>35 days</th>
<th>42 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.78</td>
<td>1-20</td>
<td>50:50</td>
<td>70.8</td>
<td>67.5</td>
<td>70.0</td>
<td>47.5</td>
<td>50.0</td>
<td>39.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>± 1.1</td>
<td>± 1.2</td>
<td>± 1.0</td>
<td>± 0.7</td>
<td>± 0.6</td>
<td>± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.78</td>
<td>20-100</td>
<td>50:50</td>
<td>72.1</td>
<td>70.1</td>
<td>69.9</td>
<td>69.5</td>
<td>60.9</td>
<td>43.3</td>
<td>27.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.74</td>
<td>1-20</td>
<td>50:50</td>
<td>63.0</td>
<td>60.3</td>
<td>64.8</td>
<td>60.4</td>
<td>55.7</td>
<td>46.2</td>
<td>34.8</td>
<td>26.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>± 0.9</td>
<td>± 1.0</td>
<td>± 0.7</td>
<td>± 0.4</td>
<td>± 0.5</td>
<td>± 0.3</td>
<td>± 0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.58</td>
<td>1-20</td>
<td>50:50</td>
<td>46.2</td>
<td>42.0</td>
<td>39.9</td>
<td>39.8</td>
<td>36.0</td>
<td>31.4</td>
<td>25.8</td>
<td>20.0</td>
<td>13.8</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>± 0.5</td>
<td>± 0.7</td>
<td>± 0.5</td>
<td>± 0.5</td>
<td>± 0.6</td>
<td>± 0.8</td>
<td>± 0.2</td>
<td>± 0.3</td>
<td>± 0.3</td>
<td>± 0.1</td>
</tr>
</tbody>
</table>

a Molecular weights are the average of three GPC sample runs, ± standard deviation.
Table 9  Molecular weights of PLG microspheres with different LA:GA compositions and similar inherent viscosities degraded over time in PBS-albumin [molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min)]

<table>
<thead>
<tr>
<th>I.V.</th>
<th>Size range</th>
<th>LA:GA mole (%)</th>
<th>M&lt;sub&gt;GPC&lt;/sub&gt; at different time intervals (kg/mole)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μm)</td>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>0.60</td>
<td>20-100</td>
<td>100:0</td>
<td>96.1</td>
</tr>
<tr>
<td>0.56</td>
<td>1-20</td>
<td>85:15</td>
<td>91.6</td>
</tr>
<tr>
<td>0.55</td>
<td>1-20</td>
<td>75:25</td>
<td>81.9</td>
</tr>
<tr>
<td>0.55</td>
<td>1-20</td>
<td>65:35</td>
<td>46.2</td>
</tr>
</tbody>
</table>

a Molecular weights are the average of three GPC sample runs, ± standard deviation.
Table 10  \( M_{GPC} \) of PLG microspheres with different LA:GA compositions and 10% paclitaxel loading degraded over time in PBS-albumin [molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min)]

<table>
<thead>
<tr>
<th>I.V.</th>
<th>Size range</th>
<th>LA:GA</th>
<th>( M_{GPC} ) at different time intervals (kg/mole)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μm)</td>
<td>mole (%)</td>
<td>1 day</td>
</tr>
<tr>
<td>0.74</td>
<td>1-20</td>
<td>50:50</td>
<td>63.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.9</td>
<td>± 0.9</td>
</tr>
<tr>
<td>0.56</td>
<td>1-20</td>
<td>85:15</td>
<td>82.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1.4</td>
<td>± 1.1</td>
</tr>
<tr>
<td>0.55</td>
<td>1-20</td>
<td>75:25</td>
<td>87.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1.2</td>
<td>± 1.3</td>
</tr>
<tr>
<td>0.55</td>
<td>1-20</td>
<td>65:35</td>
<td>43.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.7</td>
<td>± 0.7</td>
</tr>
</tbody>
</table>

* Molecular weights are the average of three GPC sample runs, ± standard deviation.
Figure 35  Degradation profiles of control PLG50:50 microspheres with differing inherent viscosities (1-20μm). Molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min).
Figure 36  Degradation profiles of control PLG microspheres (1-20µm) with different LA:GA compositions and similar inherent viscosities. Molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min).
Figure 37  Degradation profiles of control and 10% paclitaxel-loaded PLG microspheres (1-20μm) with different LA:GA compositions. Molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min).
Figure 38  Degradation profiles of control and 10% paclitaxel-loaded PLG microspheres (1-20μm) with different LA:GA compositions. Molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min).
Figure 39 Degradation profiles of control PLG50:50 (I.V. = 0.78, 1-20μm & 20-100μm) microspheres. Molecular weights were measured by GPC at 40°C using polystyrenes as standards (Solvent: chloroform. Solvent flow rate: 1.0 mL/min)
4.6.2. Surface morphology by SEM

Figure 40A-H shows the SEM morphologies of control PLG100:0 microspheres after incubation in PBS-albumin at 37°C over 6 weeks. Surface erosion was evident at day 3 and became more pronounced at day 28. At day 35, the outer layer of the microspheres started to peel off on some of the microspheres.

Figure 41A-H shows surface morphologies of control PLG65:35 microspheres after incubation in PBS-albumin at 37°C. At day 28, pinhole structures were found in some of the microspheres and there was evidence of bulk degradation in some of the microspheres at day 42. The surface morphologies of control PLG85:15 and PLG75:25 microspheres with similar inherent viscosities were similar to those of control PLG65:35 microspheres.

Figure 42A-H shows micrographs of control PLG50:50 microspheres with the lowest inherent viscosity of 0.58 degrading in PBS-albumin at 37°C over 6 weeks. Microspheres showed relatively smooth surface morphologies until day 21, at which time pore structures appeared on some of the microspheres. The number of pores increased and at day 42, due to the extensive bulk degradation inside the microsphere matrix, the microspheres collapsed and an extensive pore network was observed.

Figures 43A-F are micrographs of 10% paclitaxel-loaded PLG50:50 (I.V. = 0.74, 1-20 µm) microspheres degraded in PBS-albumin at 37°C over 4 weeks. There was evidence of significant degradation of the microsphere matrix by day 28.
Figure 40  SEM micrographs of control PLG100:0 (I.V. = 0.60, 20-100μm) microspheres degrading in PBS-albumin at 37°C.
Figure 41  SEM micrographs of PLG65:35 (I.V. = 0.55, 1-20µm) control microspheres degrading in PBS-albumin at 37°C.
Figure 42  SEM micrographs of PLG50:50 (I.V.= 0.58, 1-20μm) control microspheres degrading in PBS-albumin at 37°C.
Figure 43 SEM micrographs of PLG50:50 (I.V.= 0.74, 1-20μm) 10% paclitaxel-loaded microspheres degrading in PBS-albumin at 37°C.
4.6.3. Glass transition temperatures of the degraded PLG microspheres

Figure 44 shows a change in peak position of the glass transition temperatures of the control PLG50:50 (I.V.= 1.06) microspheres during degradation over 4 weeks in PBS-albumin buffer. Table 11 summarizes the change in glass transition temperatures of all control microspheres during 28 or 42 days of degradation in PBS-albumin. There was a consistent decrease in the glass transition temperatures for all PLG microspheres with a LA:GA composition of 50:50. In contrast, the PLG polymer microspheres with different LA:GA compositions did not show any significant changes in their glass transition temperatures.

![Graph showing glass transition temperatures](image)

Figure 44   Glass transition temperatures of control PLG microspheres (I.V.1.06, 1-20μm) with a LA:GA ratio of 50:50 after 28 days of degradation in PBS-albumin at 37°C (A: day 1, B: day 3, C: day 7, D: 14 and E: day 28).
Table 11  Glass transition temperatures of control PLG microspheres with LA:GA ratios of 50:50, 65:35, 75:25, 85:15 and 100:0 and different molecular weights after 42-days of degradation in PBS-albumin at 37°C

<table>
<thead>
<tr>
<th>I.V.</th>
<th>Size range</th>
<th>LA:GA</th>
<th>p-value</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.06</td>
<td>1-20</td>
<td>50:50</td>
<td>0.003b</td>
<td>52.4 ± 0.8</td>
<td>51.7 ± 0.9</td>
<td>51.6 ± 1.2</td>
<td>51.6 ± 0.9</td>
<td>50.9 ± 0.9</td>
<td>47.5 ± 1.1</td>
<td>48.0 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.78</td>
<td>20-100</td>
<td>50:50</td>
<td>0.05c</td>
<td>51.7 ± 0.9</td>
<td>51.6 ± 0.6</td>
<td>50.2 ± 0.5</td>
<td>49.4 ± 0.4</td>
<td>49.4 ± 0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.74</td>
<td>1-20</td>
<td>50:50</td>
<td>0.01g</td>
<td>51.2 ± 1.0</td>
<td>50.4 ± 0.7</td>
<td>51.2 ± 0.5</td>
<td>50.9 ± 0.8</td>
<td>50.6 ± 0.7</td>
<td>48.9 ± 1.2</td>
<td>47.9 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.58</td>
<td>1-20</td>
<td>50:50</td>
<td>0.05d</td>
<td>47.3 ± 0.7</td>
<td>47.8 ± 0.8</td>
<td>48.7 ± 0.7</td>
<td>49.7 ± 0.7</td>
<td>49.4 ± 1.1</td>
<td>49.2 ± 1.3</td>
<td>47.8 ± 0.8</td>
<td>46.3 ± 1.7</td>
<td>45.6 ± 0.9</td>
</tr>
<tr>
<td>0.60</td>
<td>20-100</td>
<td>100:0</td>
<td>0.31f</td>
<td>55.5 ± 1.2</td>
<td>55.6 ± 0.8</td>
<td>55.2 ± 0.9</td>
<td>57.0 ± 1.0</td>
<td>56.6 ± 0.6</td>
<td>55.8 ± 0.7</td>
<td>56.0 ± 0.7</td>
<td>54.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>0.56</td>
<td>1-20</td>
<td>85:15</td>
<td>0.15h</td>
<td>55.2 ± 0.8</td>
<td>55.0 ± 0.8</td>
<td>54.3 ± 0.7</td>
<td>55.0 ± 1.0</td>
<td>54.4 ± 0.7</td>
<td>53.3 ± 0.5</td>
<td>54.1 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.55</td>
<td>1-20</td>
<td>75:25</td>
<td>0.51i</td>
<td>53.9 ± 0.9</td>
<td>52.7 ± 0.9</td>
<td>53.3 ± 0.7</td>
<td>53.2 ± 0.8</td>
<td>53.7 ± 0.9</td>
<td>51.6 ± 1.3</td>
<td>53.4 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.55</td>
<td>1-20</td>
<td>65:35</td>
<td>0.2gj</td>
<td>51.0 ± 1.2</td>
<td>50.4 ± 1.2</td>
<td>50.7 ± 1.0</td>
<td>52.5 ± 0.8</td>
<td>52.5 ± 0.8</td>
<td>52.5 ± 0.9</td>
<td>52.1 ± 1.0</td>
<td>51.3 ± 1.3</td>
<td>49.8 ± 1.2</td>
</tr>
</tbody>
</table>

a. Tg's are the average of three determinations, ± standard deviation  
b. Two-tailed t-tests on the glass transition temperatures between day 1 and day 28.  
c. Two-tailed t-tests on the glass transition temperatures between day 3 and day 28.  
d. Two-tailed t-tests on the glass transition temperatures between day 1 and day 42.
4.7. The effect of γ-irradiation on paclitaxel, control and paclitaxel-loaded microspheres

The impact of γ-irradiation at a dose of 2.5 Mrad on paclitaxel recovery and paclitaxel loading in the microspheres was determined. There are no significant differences for either paclitaxel recovery or loading of paclitaxel in microspheres before and after gamma irradiation.

The effect of gamma irradiation on the molecular weights of PLG85:15 polymer in 20% paclitaxel-loaded microspheres showed that gamma irradiation caused a significant decrease in molecular weight of the polymer.

Table 12 summarizes the molecular weights of non-irradiated control PLG85:15 microspheres and non-irradiated and gamma irradiated 20% paclitaxel-loaded PLG85:15 microspheres after incubation in PBS-albumin at 37°C. The degradation profiles are shown in Figure 45. Slopes of the linear regressions of these degradation curves revealed that the in vitro degradation rates of control and 20% paclitaxel-loaded microspheres (non-irradiated) were not significantly different, but that they were significantly different from the degradation rate of the gamma irradiated (2.5 Mrad) 20% paclitaxel-loaded microspheres.
Table 12  Summaries of the $M_{GPC}$ of non-irradiated control PLG85:15 microspheres and non-irradiated and gamma irradiated (2.5 Mrad) 20% paclitaxel-loaded PLG85:15 microspheres after incubation in PBS-albumin at 37°C

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>$M_{GPC}$ of control and 20% paclitaxel-loaded PLG85:15 microspheres $^a$ (×1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control $^b$</td>
</tr>
<tr>
<td>0</td>
<td>88.4 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>83.4 ± 1.1</td>
</tr>
<tr>
<td>7</td>
<td>87.1 ± 1.4</td>
</tr>
<tr>
<td>10</td>
<td>83.4 ± 1.3</td>
</tr>
<tr>
<td>14</td>
<td>85.9 ± 1.3</td>
</tr>
<tr>
<td>17</td>
<td>88.2 ± 1.2</td>
</tr>
<tr>
<td>24</td>
<td>92.0 ± 1.3</td>
</tr>
<tr>
<td>28</td>
<td>87.9 ± 1.3</td>
</tr>
<tr>
<td>35</td>
<td>83.4 ± 1.3</td>
</tr>
<tr>
<td>42</td>
<td>82.0 ± 1.3</td>
</tr>
<tr>
<td>51</td>
<td>83.6 ± 1.3</td>
</tr>
<tr>
<td>58</td>
<td>76.9 ± 1.4</td>
</tr>
<tr>
<td>65</td>
<td>74.0 ± 1.2</td>
</tr>
<tr>
<td>72</td>
<td>78.1 ± 1.2</td>
</tr>
<tr>
<td>79</td>
<td>76.1 ± 1.3</td>
</tr>
</tbody>
</table>

$^a$ Molecular weights (g/mole) of control, paclitaxel-loaded PLG85:15 microspheres (before and after irradiation) are the averages of three sample runs, ± standard deviation.
Figure 45 The degradation profiles in PBS-albumin at 37°C of non-irradiated control and 20% paclitaxel-loaded PLG85:15 (I.V.= 0.56, 1-20μm) microspheres as well as 20% paclitaxel-loaded PLG85:15 (I.V.= 0.56, 1-20μm) microspheres after irradiation at a dose of 2.5 Mrad. The slopes of linear regression of the degradation profiles were -0.16 (g/mole/day) for the control microspheres, -0.18 (g/mole/day) and -0.38 (g/mole/day) for the paclitaxel-loaded PLG85:15 microspheres (before and after irradiation respectively).
5. DISCUSSION

5.1. Composition of the PLG polymers

The area under each NMR signal in the spectrum was proportional to the numbers of hydrogen atoms in that group. The average molar compositions of the polymers could be determined by the ratio of the corresponding $H^1$-NMR integrated peak area (Amecke et al., 1995; Deasy et al., 1989). The chemical shift ($\delta$), which was the difference in absorption frequency between a given proton and the proton from the internal standard (TMS), was used to identify the existence of certain protons. A peak at chemical shift ($\delta$) = 7.2 ppm was due to the impurity from the solvent CDCl$_3$. The lactide unit had two different protons: methine and methyl. These proton peaks appeared at 5.2 ppm and 1.5 ppm respectively (Asano et al., 1989). The methyl proton appeared as a doublet peak and the methine proton was a quartet peak, due to spin coupling interactions between neighboring protons, and was related to the number of possible spin orientations that these neighbors could possess, or so-called spin-spin splitting or spin coupling. The methylene proton in the glycolide unit (Figure 7) had a multiple peak at 4.8 ppm, which may be due to vicinal coupling (Schakenraad, et al., 1989; Amecke et al., 1995). The additional small peaks at 1.3 ppm could be from residual catalyst in the polymer (Amecke et al., 1995). Our data confirmed the molar ratios of LA:GA in the polymers received from BPI.

5.2. Microsphere fabrication

5.2.1. Size and surface morphology of the microspheres

The solvent evaporation method has been commonly used in manufacturing many controlled release formulations for hydrophobic drugs with high encapsulation efficiency.
Acceptable amounts of residual solvent ranging from 8.8 ± 1.2 to 463.1 ± 20.8 ppm have been reported using this method (Bitz et al., 1996).

Factors affecting the size ranges of microspheres manufactured have been widely investigated. These factors include polymer concentration, drug concentration, ratio of organic/aqueous phase volume, concentration of PVA solution and rate of agitation (Jeffery et al., 1991; Conti et al, 1995 and Nihant et al., 1995). The concentration of the PVA solution and the rate of agitation during emulsification have been shown to have a profound influence on the sizes of the microspheres (Jeffery et al., 1991). Increasing the PVA concentration or increasing the agitation rate decreases the sizes of the microspheres by causing an increased shear stress on the organic phase droplets and decreasing the size of the dispersed phase. Two size ranges of microspheres were produced in these studies. Microspheres with sizes of less than 20 μm were prepared using 5% (w/v) PVA solution and a stirring rate of 900 ± 50 rpm at room temperature. Microspheres with size ranges between 20-100 μm were prepared by changing the PVA concentration and stirring rate to 2.5% (w/v) and 550 ± 50 rpm, respectively.

Microspheres produced were spherical, with sizes controlled within the targeted ranges. The results indicated that polymers with either different molecular weights but the same LA:GA composition or different LA:GA compositions did not produce microspheres with any significant differences in their size distributions. All small microsphere batches were less than 20 μm, whereas the large microsphere batches were within 20-100 μm.
Scanning electron micrographs showed that all 3 batches of the 20% paclitaxel-loaded PLG100:0 (I.V. = 0.60, 20-100 μm) microspheres (Figure 14A) possessed an extensive pinhole-like network on the microsphere surfaces. However, this effect was not observed in the scanning electron micrographs of the 10% paclitaxel-loaded (Figure 15A) and control PLG100:0 (Figure 16A) microspheres. Bodmeier et al. (1987) reported that the surfaces of quinidine-loaded PLG100:0 microspheres changed from virtually smooth at a low drug loading of 7% (w/w) to a pinhole-like structures at a loading of 24% (w/w). The pinholes became more evident as the loading of quinidine was increased from 34 to 62%. A porous substructure in the microsphere matrix was found under this pinhole-like skin structure on the microspheres (Bodmeier et al., 1987). Similar results were also reported in a hormone-loaded PLG75:25 microsphere system by Takada et al. (1994).

These pinhole structures were not seen on other 10 and 20% paclitaxel-loaded microspheres in which glycolide was present in the polymer chain (Figure 14, 15 and 16). Several factors, which include the rate of solvent removal, the compositions of the polymer wall material and the loading of paclitaxel, may influence the surface morphology of the microspheres. A lower solubility of paclitaxel in the PLG100:0 polymer microspheres may have led to precipitation of the drug in the superficial layers of the microsphere surface. The pinhole network might then form following the washing and drying steps of microsphere manufacture.

5.2.2. X-ray diffraction patterns of control and paclitaxel loaded microspheres

X-ray diffraction data for all microsphere samples showed only an amorphous matrix. There was no evidence of a crystalline form of paclitaxel present in the
microspheres. Paclitaxel may have been present in the polymer matrix either as a solid solution and/or in an amorphous form. Scanning electron micrographs of sectioned microspheres did not provide any evidence of crystalline paclitaxel present in the bulk microsphere matrix. In addition, DSC analysis of microsphere samples showed an absence of any melting peaks due to crystalline paclitaxel.

5.2.3. **Paclitaxel total content and encapsulation efficiency**

The encapsulation efficiencies of paclitaxel in PLG microspheres were found to be independent of the paclitaxel loading or polymers. Except for the 20% paclitaxel-loaded PLG100:0 microspheres, all PLG microspheres showed over 90% encapsulation efficiency. This is comparable with many other PLG microsphere systems, which have reported encapsulation efficiencies of about 90-95% for hydrophobic drugs (Okada *et al.*, 1995). The reason for PLG100:0 20% paclitaxel-loaded microspheres having the lowest encapsulation efficiency (86.5%) among the microspheres could be related to the pinhole structure seen in the scanning electron micrographs (Figure 14A). Paclitaxel may have been lost from the outer superficial layers either during the fabrication process and/or during the final washing step.

5.2.4. **Glass transition temperatures of control and paclitaxel-loaded microspheres**

DSC data for control and paclitaxel-loaded microspheres revealed that the glass transition temperatures increased with an increase in the paclitaxel loading, regardless of the sizes of the microspheres, molecular weights and compositions of the polymers. However, there was no increase in the $T_g$ of the control PLG microspheres/paclitaxel physical mixture (Figure 23B), suggesting that the increase in the glass transition temperatures were related to the presence of paclitaxel in the microsphere matrix. Similar
increases in glass transition temperatures were also reported by Yamakawa et al. (1992) for neurotensin-loaded PLG microspheres. The increase in $T_g$ could be due to either a reduction of the free volume in the polymer matrix after the incorporation of paclitaxel, or an interaction between the paclitaxel and the polymers. This interaction would likely make the polymer chains more rigid and require more energy to achieve segmental motion. Okada et al. (1995) speculated that drug molecules in the microspheres would be dispersed throughout the PLG matrix and that the motion of PLG segments would be hindered by the interaction between the polymer and drug. Maulding (1987) and Prinos et al. (1995) have suggested that drug-polymer interactions might be due to intermolecular hydrogen bonds in the polymer-drug matrix. Menikh et al. (1993) and Lin et al. (1993 & 1995) used Fourier transform infrared spectroscopy (FTIR) to characterize drug-polymer solid-state interactions and confirmed the existence of intermolecular hydrogen bonds.

5.2.5. Investigation of polymer-paclitaxel interaction by Fourier transform infra-red spectroscopy (FTIR)

The C=O stretching vibration is the most characteristic band of the C=O group, which absorbs very strongly in the region $1750 \pm 80 \text{ cm}^{-1}$. However, the peak position of the absorbance band is greatly affected by the adjacent group and the surrounding environment such as CO$_2$, making it unsuitable for detecting any subtle changes due to hydrogen bonds (Ishida, 1987).

The deformation of the C=O bond as part of the ester group can be detected in the region $710 \pm 80 \text{ cm}^{-1}$, which has been observed for many organic compounds and has been attributed to in-plane and out-of-plane deformation of the C=O bond (Roeges, 1995). The $\delta$C=O (in-plane deformation) and $\gamma$C=O (out-of-plane deformation) would
appear as one peak if there was no influence from outside molecules (Roeges, 1995). Figure 25A showed that only one absorbance band existed at 710 cm\(^{-1}\) for both the \(\delta \text{C}=\text{O}\) (in-plane deformation) and \(\gamma \text{C}=\text{O}\) (out-of-plane deformation) for paclitaxel. Two absorbance bands were observed for control microspheres and control microspheres/paclitaxel physical mixture as well as paclitaxel-loaded microspheres. The peak with lower wavenumber was identified as C=O deformation since it was intensified in the FTIR spectra of paclitaxel-loaded microspheres and physical mixture of control microspheres/paclitaxel. Therefore, the C=O deformation peak for control microspheres was 708 cm\(^{-1}\) (Figure 25B). The peak with higher wavenumber was relatively stable regardless of polymer compositions.

A significant shift of the C=O deformation band was observed for the 20% paclitaxel-loaded microsphere sample (722 cm\(^{-1}\)) (Figure 26B). This change in the band position implies that the required energy for C=O deformation was increased due to the presence of paclitaxel in the polymer matrix. It is possible that hydrogen bonds between the hydroxyl group of paclitaxel and the ester group of the polymer may be the major cause of the shift of the C=O deformation band.

Intermolecular hydrogen bonds between paclitaxel and PLG polymer chains could result in the loss of some degree of flexibility, which would increase the energy required for the glass transition. This may explain the increase in glass transition temperatures with an increase in paclitaxel loading in the microspheres.
5.2.6. In vitro degradation of microspheres

5.2.6.1. Molecular weights and morphological changes

The PLG polymers with different lactide:glycolide compositions possessed very similar inherent viscosities. Except for PLG65:35 which had a molecular weight of about 46,000 g/mole, this group of polymers had molecular weights between 82,000 and 96,000 g/mole. After 28 days of incubation in the PBS-albumin at 37°C, slight decreases (between 3-13%) in the molecular weights were observed in this group of polymers, either with or without paclitaxel loading. Ramchandani, et al. (1997) also reported a slow degradation of a PLG85:15 implant with a M$_w$ of 90,801 g/mole in phosphate buffer (pH = 7.4) in the first 63 days. A period of slow degradation in phosphate buffer (pH = 7.4) has been reported for most PLG microspheres with higher lactide content (> 50 mole%) by Park (1995). Microspheres loaded with or without paclitaxel had almost identical degradation profiles (Figures 37, 38 and 45), indicating that the presence of paclitaxel in the polymer matrix had little effect during the first four to six weeks on the degradation of this group of PLG polymers. SEM micrographs showed very little or no changes in the surface morphologies of PLG85:15, 75:25 and 65:35 microspheres over 4-6 weeks, providing additional evidence of slow degradation over this time period.

The period of slow degradation is always accompanied by little mass loss as reported by Fukuzaki et al. (1991). Amorphous poly(l-lactide-co-glycolide) polymers with 70 mole% of l-lactide and molecular weights of 16,900, 24,000 and 41,300 g/mole showed little change in the masses following implantation in rats for about 5 weeks. Poly(l-lactide-co-glycolide) containing 85 mole% of l-lactide (M$_w$ = 114,000 g/mole) showed minimal mass loss in the first 10 weeks in phosphate buffer (Vert et al., 1991).
The *in vitro* degradation profiles of PLG polymers with 80 mole% ($M_w = 54,000$ g/mole) and 90 mole% ($M_w = 19,200$ g/mole) of $d,l$-lactide content were alike, but were significantly different from a PLG polymer containing 50 mole% ($M_w = 48,500$ g/mole) of $d,l$-lactide content (Park, 1995).

The group of PLG polymers with the same composition of glycolide and lactide (50:50) possessed very different inherent viscosities. The molecular weights were about 140,000 g/mole for PLG50:50 (I.V. = 1.06), 71,000 g/mole for PLG50:50 (I.V. = 0.78), 63,000 g/mole for PLG50:50 (I.V. = 0.74), and 46,000 g/mole for PLG50:50 (I.V. = 0.58). During 28 or 42 days of degradation in the PBS-albumin at 37°C, the molecular weights of this group of PLG polymers decreased significantly, regardless of paclitaxel loading. Degradation of the polymers was generally slow in the first three days and then occurred more rapidly. By day 28, the molecular weights had decreased to about 18 to 43% of their initial molecular weights. Polymers with higher initial molecular weights had a greater reduction than the polymers with lower initial molecular weights in the same period of time. This was because polymers with longer chains possess a greater number of sites for hydrolysis, leading to a faster decrease in the molecular weight. Similar results were reported by Shah *et al.* (1992), who also employed 50:50 PLG polymers in the studies.

There appeared to be no evidence of changes in the surface morphology of PLG50:50 microspheres until about 21 days of incubation in PBS-albumin (Figure 42). By day 28, SEM micrographs showed evidence of significant mass loss. Reed and Gilding (1981) also showed that mass loss did not occur until after 21 days of *in vitro* degradation of PLG50:50 polymers.
The higher degradation rates for PLG50:50 polymers are due to the higher glycolide content which is more hydrophilic than the lactide component (Park, 1995). The degradation half-life of a PLG50:50 implant ($M_w \approx 46,000$ g/mole) in Sprague-Dawley rats was about one week, while the degradation half-life of a PLG100:0 implant was about 6 months (Miller et al., 1977). Complete degradation of PLG50:50 microspheres ($M_w \approx 15,500$ g/mole) was seen after 63 days implantation in male Sprague-Dawley rats (Visscher et al., 1985).

Figure 29 compared the degradation profiles of two different sizes of PLG50:50 (I.V. = 0.78) control microspheres. The rate of degradation was slow for the larger microspheres for the first 10 days, followed by more rapid degradation. The smaller microspheres showed a steady drop in molecular weight similar to the PLG50:50 (I.V. = 0.74) microsphere degradation profile (Figure 28). The slopes of the two degradation curves indicated that there was no significant difference between the overall degradation rates. Visscher et al. (1988) also reported that the degradation profiles of PLG50:50 microspheres (MW $\approx 43,000$ g/mole) with three size ranges (45-75 μm, 75-106 μm and 106-177 μm) were similar.

The degradation of PLG microspheres involves the uptake of water into the polymer matrix, random chain scission of the linkage of ester bonds in the polymer backbone, decrease in molecular weight of the polymer and ultimately mass loss from the microspheres. It has also been suggested that degradation of PLG microspheres occurs more rapidly in the centre than at the surface due to the autocatalytic action of the carboxylic acid end groups of degrading PLG polymers (Li et al., 1990A and 1990B; Therin et al., 1992). Similar results were also seen in our studies (Figure 42).
5.2.6.2. The effect of degradation on glass transition temperatures

There was a small but consistent drop in the glass transition temperatures for all PLG microspheres with the same 50:50 LA:GA composition after incubation in PBS-albumin. PLG microspheres with different LA:GA compositions, in contrast, did not show any significant changes in their glass transition temperatures. Shah et al. (1992) reported a similar decrease in glass transition temperature associated with a decrease of molecular weight of a PLG50:50 polymer. According to the Flory-Fox equation, a decrease in the molecular weight of a polymer would lead to a decrease in its glass transition temperature. The drop in glass transition temperatures of PLG polymers with the same 50:50 LA:GA composition was likely related to the change in molecular weights. For the PLG polymers with differing LA:GA compositions, no significant changes in glass transition temperatures were observed up to 28 or 42 days of degradation in PBS-albumin at 37°C since the changes in the molecular weights in this group of microspheres were small.

5.2.7. In vitro release of paclitaxel from paclitaxel-loaded microspheres

The solubility of paclitaxel in the PBS-albumin was about 3 μg/mL (Winternitz, 1997 and Zhang 1997). Sampling intervals were selected in order to prevent the concentration of paclitaxel in the release buffer from exceeding 15% of its aqueous solubility. This was to ensure that sink conditions were maintained during the entire course of the in vitro release studies (Carstensen, 1977). Nevertheless, due to the rapid release of paclitaxel during the burst phase, sink conditions may not have been maintained in the first 3 days, particularly for the 20% paclitaxel-loaded microspheres.
However, this would not be expected to alter the cumulative amount of paclitaxel released in the course of the study.

The release profiles of paclitaxel from PLG50:50 microspheres with paclitaxel loading from 1 to 20% (Figure 27) showed that a significant amount of paclitaxel, which accounted for about 10-20% of total paclitaxel loading, was released during the first two or five days. The initial burst phase is believed to be due to the large amount of drug released from the superficial surface layers of the microspheres (Alonso et al., 1993). Paclitaxel was released at a relatively steady rate between day 5 and day 30 until the secondary burst phase appeared at day 31 for the 20% paclitaxel-loaded microspheres (Figure 27). The microspheres with higher paclitaxel loading provided faster paclitaxel release due to the higher concentration of paclitaxel in the microsphere matrix. According to the kinetic theory for diffusion controlled release from a sphere, the amount of drug released at a given time is directly proportional to the drug loading (Baker, 1987).

Paclitaxel release from the microsphere matrices was probably due to the combination of diffusion and degradation process. Diffusional control is usually the major mechanism of drug release in the early phase of release (Heya et al., 1991). Degradation controlled release of drug would play a more important role at the later phase of drug release (Shah et al., 1992). There is evidence of a triphasic pattern of drug release for the PLG50:50, 20% paclitaxel-loaded microspheres (Figure 27) due to the appearance of a secondary burst phase at about day 31. This is likely due to the contribution of significant degradation to the drug release rates. Sturesson et al. (1993) reported a similar triphasic release pattern in PLG50:50 (I.V.= 0.2) microspheres loaded with timolol maleate. Alonso et al. (1993) and Shah et al. (1993) also showed this release
pattern by using different model compounds (tetanus vaccine and red dye) encapsulated in PLG50:50 microspheres ($M_w = 100,000$ g/mole and I.V. = 0.4-0.5).

The 20% paclitaxel-loaded PLG100:0 microspheres showed a much higher initial burst phase of release compared to 10% paclitaxel-loaded PLG100:0 microspheres (Figure 28). The extensive pinhole-like structures on the microsphere surfaces might be related to the high burst phase from the 20% paclitaxel-loaded PLG100:0 microspheres. Bodmeier and McGinity (1987) showed that the burst phase of quinidine release from PLG100:0 microspheres increased from zero to almost 100% when the loading of quinidine in the microspheres increased from 7 to 53% (w/w). Extensive pores were found on the microsphere surfaces when the loading of quinidine was over 24% (w/w). Similar results were also reported by Takada et al. (1994) on thyrotropin-releasing-hormone (TRH) loaded PLG75:25 microspheres ($M_w = 14,000$). Extensive pores formed at high TRH loading, contributed to the high initial burst phase. The cause of the apparent phase of faster release at about day 6 for the 20% paclitaxel-loaded PLG100:0 microspheres is not clear. It may be related to the pinhole surface morphology of these microspheres. Fong et al., (1986) reported a similar pattern of faster release after the burst phase on a 44% thioridazine-loaded PLG100:0 microsphere system.

The amounts of paclitaxel released in the first 15 days from PLG85:15, 75:25 and 65:35 microspheres were almost identical (Figures 29 and 30). Different lactide:glycolide compositions did not result in any differences in the paclitaxel release rates in the early phase of release. However, the release rates of PLG85:15 microspheres with 10% and 20% paclitaxel loading started to decrease after day 15 compared with the PLG75:25 and PLG65:35 microspheres. Spenlehauer et al. (1988) also showed that the presence of
different amounts of glycolic units in the polymer backbones for PLG75:25 and PLG90:10 microspheres loaded with 30% cisplatin had no influence on the drug release.

No differences in the paclitaxel release rates from 10% paclitaxel-loaded PLG50:50 (I.V. = 0.78) and PLG50:50 (I.V. = 0.74) microspheres were found in the first 24 days of release (Figure 31). However, the paclitaxel release rates from 20% paclitaxel-loaded PLG50:50 (I.V. = 0.78) and PLG50:50 (I.V. = 0.74) microspheres were significantly different after day 10. The paclitaxel release rates from 10 and 20% paclitaxel-loaded PLG50:50 (I.V. = 1.06) microspheres were significantly lower than the release rates from the PLG50:50 microspheres with lower inherent viscosities of 0.74 and 0.78. The slow release rate for paclitaxel from the higher molecular weight PLG50:50 (I.V. = 1.06) microspheres was likely due to decreased diffusion of paclitaxel through the polymer and increased hydrophobicity of the matrix due to fewer carboxyl end groups in the higher molecular weight polymers. Fong et al. (1986) reported that thioridazine released from PLG100:0 microspheres with different molecular weights but similar loading and size ranges were almost identical in the first 6 days. The release rate of thioridazine from microspheres with the lower molecular weight increased after day 6. Heya et al. (1991) also showed that higher molecular weight PLG microspheres gave lower release rate of TRH.

The release profiles of two different sizes (1-20 μm and 20-100 μm) of 10% paclitaxel-loaded PLG50:50 (I.V. = 0.78) microspheres (Figure 33) showed that the larger size microspheres had a burst phase which released about 5% of paclitaxel from the total loading, while the burst phase of smaller microspheres was equivalent to about 10-12% of paclitaxel total loading. The slightly higher cumulative amounts of paclitaxel released
from the smaller microspheres may have been due to a higher burst phase of release, which was related to the increased surface area of the smaller microspheres. The slopes of the two release-curves after the burst phases were not significantly different. Visscher et al. (1988) also found that drug release rates from 9% ergot-alkaloid loaded PLG50:50 microspheres (45-75 µm, 75-106 µm and 106-177 µm) were similar. Cha and Pitt (1988) also found that drug (L-methadone) release rate was independent of the sizes of PLG microspheres.

5.2.8. Effects of gamma irradiation on paclitaxel and PLG polymers

No paclitaxel degradation occurred in paclitaxel-loaded microspheres after gamma irradiation. The molecular weight of 20% paclitaxel-loaded PLG85:15 microspheres dropped significantly after gamma irradiation. The rate of degradation of the gamma irradiated 20% paclitaxel-loaded PLG85:15 microspheres in PBS-albumin was increased compared with non-irradiated control PLG85:15 microspheres and 20% paclitaxel-loaded microspheres. Volland et al. (1994) reported that gamma irradiation of polyester could result in simultaneous chain scission and cross-linking. Gamma irradiation caused the formation of free radicals in the PLG polymer chains, which either underwent a chain scission to form smaller molecular weight polymer chains, or two radicals combined to form a cross-linked PLG polymer. However, an irradiation dose-dependent decrease in molecular weight was usually reported (Volland et al., 1994; Hausberger et al., 1995). Spenlehauer et al. (1988) showed that gamma irradiation had minimal influence on drug release profiles and the release of cisplatin from PLG75:25 microspheres was found to be independent of the doses of gamma irradiation.
5.3. Intra-articular microsphere formulation and future work

The overall objective of the project was to develop a paclitaxel loaded microsphere formulation suitable for prolonged intra-articular drug delivery. The ideal properties of the formulation include biocompatibility with joint tissue, injectability and controlled release of drug over a period of about 3 months.

We believe that of the PLG polymers studied in this work, the PLG polymer with 50:50 mole % LA:GA provides the most suitable matrix for the formulation since the degradation lifetime of the PLG50:50 polymer is the most appropriate. Drug release and degradation of the polymer matrix would likely be complete in approximately 3 months.

Future studies would involve the determination of the tolerability and efficacy of control and paclitaxel loaded microsphere formulations in animal models. For example, the total amount or dose of microspheres that could be safely administered into joints is not known. Therefore, a series of in vivo tolerability studies in animal models would be required. Increasing doses of control microspheres would be injected into animal joints to determine an appropriate dose range for the microspheres, which could be tolerated in animal joints. Once the maximum dose of microspheres is established, efficacy studies with paclitaxel loaded PLG50:50 microspheres would be undertaken in animal models of rheumatoid arthritis.
6. SUMMARY AND CONCLUSIONS

6.1. Control and paclitaxel-loaded microspheres with two different size ranges of 1-20μm and 20-100μm were prepared using the solvent evaporation method. The microspheres thus obtained had smooth and spherical shapes except for the 20% paclitaxel-loaded PLG100:0 microspheres which showed a pinhole network.

6.2. DSC results indicated that there was an increase in the glass transition temperatures for the paclitaxel-loaded microspheres compared to control PLG microspheres.

6.3. FTIR results suggested that the glass transition temperature increase in the paclitaxel-loaded microspheres might be due to the formation of hydrogen bonds between paclitaxel and PLG polymers.

6.4. Degradation studies of control and paclitaxel-loaded microspheres indicated that the molecular weights of PLG with 50:50 lactide:glycolide deceased rapidly following incubation in PBS-albumin, while the molecular weights of higher lactide content (> 50 mole% of lactide) PLG polymers did not decrease significantly until after 3 weeks of incubation. This was due to the greater hydrophilicity of the PLG50:50 polymers.

6.5. In general, the release profiles of paclitaxel from all PLG microsphere formulations showed a burst phase of release, followed by a phase of slower release. The burst phase was caused by rapid release of paclitaxel from the superficial surface layers of the microspheres.
6.6. The release rates of paclitaxel from PLG50:50 microspheres were influenced by paclitaxel loading and molecular weights of the PLG50:50 polymers. Increased loading and decreased molecular weight led to faster paclitaxel release rates.

6.7. PLG microspheres prepared from polymers with LA:GA ratios of 85:15, 75:25 and 65:35 showed minimal effect on paclitaxel release rates.

6.8. The two size ranges of microspheres showed minimal effects on the rates of paclitaxel releases from the microspheres.

6.9. Gamma irradiation had no influence on the paclitaxel encapsulated in PLG microspheres. The molecular weight of PLG polymers decreased significantly after gamma irradiation. The degradation rate of the PLG microspheres was also affected significantly after gamma irradiation.
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