POLY(ε-CAPROLACTONE) AND METHOXYPOLYETHYLENE GLYCOL BLENDS AS A SURGICAL PASTE DELIVERY SYSTEM FOR PACLITAXEL

by

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ABSTRACT

A biodegradable, polymeric surgical paste formulation was developed in which the anticancer drug paclitaxel was incorporated by mixing into a blend of poly(ε-caprolactone) (PCL) and methoxypolyethylene glycol, MW 350, (MePEG). The potential application of this paste formulation is to a surgical cavity following tumour resection surgery. The paste would be applied in the molten state and would solidify to form a solid depot for the slow release of paclitaxel to prevent regrowth of cancerous tissue which may not have been surgically removed. The MePEG was used to modify the thermal and mechanical properties of PCL, specifically to reduce the melting point and melt viscosity, and to increase the time taken to crystallization from the melt.

The effect of the incorporation of MePEG into PCL on the physical and chemical properties of polymer matrix was investigated. The melt viscosity at 60° C of PCL:MePEG blends was decreased from 120 to 11 poise as the MePEG concentration was increased from 0 to 40%. X-ray analysis revealed that PCL was a semicrystalline polymer, and the incorporation of MePEG did not alter the crystalline form of the PCL crystallites. Differential scanning calorimetry showed that the melting points of PCL and MePEG were 59° C and -1° C respectively, so at room temperature, PCL crystallites could form but MePEG would still be above its melting point. On cooling paste blends from the melt to -100° C, recrystallization of both PCL and MePEG occurred. At all blend compositions tested, only one glass transition (Tg) was observed and it was intermediate between the Tg’s of the individual components indicating that the components were miscible. The observed Tg’s deviated from those predicted by the Fox equation and this was thought to be due to the blend not being completely amorphous. Blend composition did not affect the degree of crystallinity of either component. Blend composition had no effect on either the crystallization or melting temperature of MePEG but melting point
depression was observed with the PCL crystallites. Analysis of the melting point depression indicated that the stability parameters of PCL crystallites in the presence of between 0 and 30\% MePEG did not change, but the equilibrium melting point of the PCL crystallites was decreased from 59.4\° C to 55.2\° C. A Flory interaction parameter value of -0.16 was calculated for the PCL:MePEG blend and the melting point depression of PCL was shown to be due, at least in part, to an interaction between the melting PCL chains and the liquid MePEG present in the matrix. Microscopic analysis of PCL spherulites crystallizing in blends with MePEG at 37\° C showed that the spherulites impinged on each other, suggesting that the MePEG was incorporated intraspherulitically. Measurement of spherulite growth across divisions in a micrometer etched in the microscope eyepiece as a function of time, revealed that the presence of MePEG did not affect the growth rate of PCL spherulites.

The effect of MePEG blending on the tensile strength of PCL tablets was measured using a CT-40 tablet hardness tester. MePEG was found to decrease the tensile strength of PCL from 179.4 to 26.7 N/cm².

Incubation of PCL and PCL:MePEG 80:20 at 4\°, 25\° and 37\° C and in phosphate buffered saline at 37\° C over 13 weeks showed that no degradation of PCL occurred in the samples stored dry, but that in buffer, the PCL molecular weight was decreased from 20k to 14k (100\% PCL) and 17k (PCL:MePEG 80:20). Storage for 13 weeks also resulted in an increase in both the melting point and degree of crystallinity of PCL, both alone or in a blend with MePEG.

In vitro release studies of 20\% paclitaxel loaded PCL and PCL:MePEG 80:20 in phosphate buffered saline with albumin showed a biphasic pattern of paclitaxel release consisting of a burst phase of about 1 day, followed by a period of slow sustained release lasting at least 3 months. Paclitaxel release followed a diffusion model and sterilization by gamma irradiation did not affect the release profile of paclitaxel. The presence of MePEG in the surgical paste
formulation decreased the rate and extent of paclitaxel release by about 50% although the percentage of MePEG in the polymer matrix, between 1 and 30% MePEG, did not change the paclitaxel release. When paste samples were incubated in an aqueous solution, it was found that the MePEG diffused out of the paste leaving a PCL matrix containing channels into which water could diffuse. It was suggested that the formation of water channels in the PCL:MePEG formulations contributed to the precipitation of a more stable form of paclitaxel crystals, paclitaxel dihydrate, within the polymer matrix resulting in altered release profiles.

Formulations of paclitaxel in PCL and PCL:MePEG 80:20 were shown to result in inhibition of angiogenesis using a chick chorioallantoic membrane (CAM) model. Molten surgical paste, which subsequently solidified as pellets, containing $^3$H-paclitaxel was injected subcutaneously in mice at a dose of 25 mg paclitaxel. The distribution of $^3$H-paclitaxel in the mice over 1 month was measured using a radioassay. Analysis of the surgical paste pellet remaining in the mice showed that 35% of the $^3$H-paclitaxel was released from the pellet in vivo over 15 days. Treated mice did not show any obvious signs of gross toxicity due to treatment, although inhibition of wound healing at the injection site was observed. The $^3$H-paclitaxel was found to distribute to the liver and to the muscle tissue adjacent to the injected pellet, which was analyzed to represent the site of action for the drug. The level of $^3$H-paclitaxel detected on the muscle tissue remained above the minimum level required for activity for at least 30 days.
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\[
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Figure 34: Ageing of surgical paste, under different storage conditions as shown by the melting point of the PCL component (Tm) and the degree of crystallinity of PCL (Xc) measured using DSC. A) Tm of PCL; B) Tm of PCL:MePEG 80:20; C) Tm of 20% paclitaxel loaded PCL:MePEG 80:20; D) Xc of PCL; E) Xc of PCL:MePEG 80:20 and F) Xc of 20% paclitaxel loaded PCL:MePEG 80:20. Data represent the mean of 4 determinations ± 1 S.D.

Figure 35A: Effect of paclitaxel loadings on the cumulative release profile of paclitaxel from PCL surgical paste pellets which were tumbled end over end at 37° C in phosphate buffered saline with albumin, normalized to 2.5 mg pellets. The inset shows the burst phase release data over the first day of the study on an expanded scale. Data represent the mean of 4 samples with error bars shown only in the positive direction (1 S.D.) for clarity.
Figure 35B: Effect of paclitaxel loadings on the cumulative release profile of paclitaxel from PCL:MePEG 80:20 surgical paste pellets which were tumbled end over end at 37° C in phosphate buffered saline with albumin, normalized to 2.5 mg pellets. The inset shows the burst phase release data over the first day of the study on an expanded scale. Data represent the mean of 4 samples with error bars shown only in the positive direction (1 S.D.) for clarity.

Figure 36: Cumulative release of paclitaxel, normalized to 2.5 mg pellet weight, from 20% paclitaxel loaded PCL and PCL:MePEG 80:20 as a function of the square root of time. Data represent the mean of 4 samples. Error bars have been omitted for clarity.

Figure 37: Effect of paclitaxel loading on the percent of paclitaxel released from 2.5 mg PCL, and PCL:MePEG 80:20 pellets, which were tumbled end over end in phosphate buffered saline with albumin at 37° C for 14 days. Data represent the mean of 4 determinations with error bars of 1 S.D.

Figure 38: Time course of paclitaxel released from 20% paclitaxel loaded PCL:MePEG blends at varying blend ratios. Release was measured from pellets which were tumbled end over end at 37° C in phosphate buffered saline with albumin, and is reported per mg of paste. Data represent the mean of 4 determinations (n = 5 for PCL:MePEG 100:0) with error bars (1 S.D.) shown in the positive direction only for clarity.

Figure 39: Time course of cumulative paclitaxel release from 20% paclitaxel loaded PCL and PCL:MePEG 80:20 pellets. Release was measured from pellets which were tumbled end over end at 37° C in phosphate buffered saline with albumin and was normalized to release from 2.5 mg pellets. Data represent the mean of 4 determinations ± 1 S.D.

Figure 40: Time course of cumulative paclitaxel release from 20% paclitaxel loaded A) PCL and B) PCL:MePEG 80:20 surgical paste samples. Release was measured from pellets which were tumbled end over end at 37° C in phosphate buffered saline and was normalized to release from 2.5 mg pellets. For each surgical paste formulation the effect of sterilization by 60Co gamma irradiation on the paclitaxel release profiles was compared. Data represent the mean of 4 determinations ± 1 S.D.

Figure 41: Representative optical micrographs showing the effect of 3 mg surgical paste disks on chick chorioallantoic membrane (CAM). The disks were placed on the CAM after 6 days of shell-less culturing and the images were taken 2 days later. A) Control surgical paste (PCL without paclitaxel) showing normal blood vessel architecture of CAM located about the surgical paste disk. B) 5% paclitaxel loaded PCL showing induced avascular zone measuring approximately 6 mm in diameter characterized by blood stasis and vessel disruption. Magnification = 8X.
Figure 42: Representative HPLC chromatograms for paclitaxel in mouse tissue. A) Paclitaxel in kidney spiked with 150 μg paclitaxel / g kidney tissue with a mobile phase flow rate of 1.5 mL/min; B) paclitaxel in mouse whole blood spiked with 150 μg paclitaxel / g whole blood using a mobile phase flow rate of 1 mL/min and C) sample of blank whole mouse blood using a mobile phase flow rate of 1 mL/min.

Figure 43: GPC chromatograms of PCL:MePEG 80:20 paste pellets removed from mice following 2 days implantation (A) and 17 days implantation (B and C). The PCL peak can be seen at 5.6 minutes and no MePEG peak was observed. Chromatographic conditions: 10^3 Å column with mobile phase of chloroform flowing at 1 mL per minute with refractive index detection.

Figure 44: DSC thermograms of PCL:MePEG 80:20 (± 20% paclitaxel) pellets removed from mice after 2 and 17 days. Both cooling and heating runs were done using a scan rate of 40° per minute.

Figure 45: Change in weight observed in mice treated with ^3^H-paclitaxel labelled surgical paste pellets. The mice were divided into 3 groups. Sham group, (n = 4), underwent procedure but no paste injected; control group, (n = 6) paste matrix injected, no drug; and treatment group, (n = 10) ^3^H-paclitaxel labelled PCL:MePEG 80:20 injected (20% paclitaxel loaded). Injections were made with 125 mg surgical paste, subcutaneously in the back of the neck.

Figure 46: Concentration of ^3^H-paclitaxel, expressed as the percent of ^3^H-paclitaxel remaining, measured in surgical paste pellets which were surgically removed following implantation in mice. ^3^H-paclitaxel was measured using a radioassay and points reflect the mean of 10 samples ± 1 S.D.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Omega )</td>
<td>Bond angle</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>Constant cooling rate</td>
</tr>
<tr>
<td>( \theta )</td>
<td>Fraction of polymer left uncrystallized</td>
</tr>
<tr>
<td>( \pi )</td>
<td>Osmotic pressure</td>
</tr>
<tr>
<td>( \varepsilon )</td>
<td>Porosity</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>Shear rate</td>
</tr>
<tr>
<td>( \eta' )</td>
<td>Shear rate dependent viscosity</td>
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<tr>
<td>( \psi )</td>
<td>Shear stress</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>Solubility parameter</td>
</tr>
<tr>
<td>( \nu )</td>
<td>Specific volume</td>
</tr>
<tr>
<td>( \varphi )</td>
<td>Stability parameter</td>
</tr>
<tr>
<td>( \tau )</td>
<td>Tortuosity</td>
</tr>
<tr>
<td>( \eta )</td>
<td>Viscosity</td>
</tr>
<tr>
<td>( \phi(T) )</td>
<td>Volume fraction of transformed polymer at temperature ( T )</td>
</tr>
<tr>
<td>( \chi_{12} )</td>
<td>Flory interaction parameter</td>
</tr>
<tr>
<td>( \Delta E )</td>
<td>Energy required to transport polymer segments across a solid-liquid interface</td>
</tr>
<tr>
<td>( \sigma_c )</td>
<td>Surface free energy</td>
</tr>
<tr>
<td>( \nu_f )</td>
<td>Free volume of a polymer</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
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</tr>
<tr>
<td>$\Delta F^*$</td>
<td>Work required to form a critical nucleus on crystal surface</td>
</tr>
<tr>
<td>$\Delta F^* m$</td>
<td>Work required to form a critical nucleus on crystal surface modified by amorphous component</td>
</tr>
<tr>
<td>$\Delta G_m$</td>
<td>Change in free energy on fusion</td>
</tr>
<tr>
<td>$\Delta G_{mix}$</td>
<td>Change in free energy on mixing</td>
</tr>
<tr>
<td>$\Delta h$</td>
<td>Enthalpy of fusion per repeat unit of polymer</td>
</tr>
<tr>
<td>$\Delta H_{2u}$</td>
<td>Enthalpy of fusion per mole of repeat unit of semicrystalline polymer</td>
</tr>
<tr>
<td>$\Delta H_m$</td>
<td>Change in enthalpy on fusion</td>
</tr>
<tr>
<td>$\Delta H_{mix}$</td>
<td>Change in enthalpy on mixing</td>
</tr>
<tr>
<td>$\phi_i$</td>
<td>Volume fraction of polymer “i”</td>
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<tr>
<td>$\omega_i$</td>
<td>Weight fraction of phase “i”</td>
</tr>
<tr>
<td>$\nu_s$</td>
<td>Volume of solidly packed molecules</td>
</tr>
<tr>
<td>$\Delta S_m$</td>
<td>Change in entropy on fusion</td>
</tr>
<tr>
<td>$\Delta S_{mix}$</td>
<td>Change in entropy on mixing</td>
</tr>
<tr>
<td>$\Delta T$</td>
<td>Degree of supercooling</td>
</tr>
<tr>
<td>$[\eta]$</td>
<td>Intrinsic viscosity</td>
</tr>
<tr>
<td>A</td>
<td>Total amount of drug present in matrix per unit volume</td>
</tr>
<tr>
<td>c</td>
<td>Concentration of polymer in solution</td>
</tr>
<tr>
<td>CAM</td>
<td>Chick chorioallantoic membrane</td>
</tr>
</tbody>
</table>
Cs  Solubility of drug in matrix

c_x  Concentration of polymer in chloroform

D  Diffusivity of drug in homogeneous matrix

DSC  Differential scanning calorimetry

Ev  Activation energy

G  Growth rate of a crystal

GPC  Gel permeation chromatography

i.p.  Intraperitoneal

i.v.  Intravenous

K(T)  Cooling function

k_1  Rate of transport of crystalline segments

k_2  Rate at which amorphous component can be removed from growth front

k_B  Boltzmann constant

L  Load

I  Thickness of lamella

M_{14}  Cumulative paclitaxel released from surgical paste in 14 days

M_{90}  Cumulative paclitaxel released from surgical paste in 90 days

MePEG  Methoxypolyethylene glycol

M_{GPC}  Molecular weight as determined by GPC

M_i  Molecular weight of species "i"
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>MEC</td>
<td>Minimum effective concentration</td>
</tr>
<tr>
<td>Mn</td>
<td>Number average molecular weight</td>
</tr>
<tr>
<td>Mv</td>
<td>Viscosity average molecular weight</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>Mw</td>
<td>Weight average molecular weight</td>
</tr>
<tr>
<td>n</td>
<td>Avrami exponent</td>
</tr>
<tr>
<td>Ni</td>
<td>Number of molecules of species “i”</td>
</tr>
<tr>
<td>p.o.</td>
<td>Orally</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly(ε-caprolactone)</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>Q</td>
<td>Amount of drug released after time t</td>
</tr>
<tr>
<td>R</td>
<td>Universal gas constant</td>
</tr>
<tr>
<td>Rₜ</td>
<td>Hydrodynamic radius</td>
</tr>
<tr>
<td>s.c.</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>T</td>
<td>Absolute temperature (Kelvin)</td>
</tr>
<tr>
<td>Tc</td>
<td>Isothermal holding temperature of a sample</td>
</tr>
<tr>
<td>tc</td>
<td>Time under constant load</td>
</tr>
<tr>
<td>Tg</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>Tg°</td>
<td>Glass transition temperature of polymer with infinite chain length</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Melting point of a polymer</td>
</tr>
<tr>
<td>$T_m^*$</td>
<td>Equilibrium melting point of blended polymer</td>
</tr>
<tr>
<td>$T_m^\circ$</td>
<td>Equilibrium melting point of polymer</td>
</tr>
<tr>
<td>$T_o$</td>
<td>Temperature at which motions needed for transport cease</td>
</tr>
<tr>
<td>$t_o$</td>
<td>Time required for chloroform to flow through Canon-Fenske viscometer</td>
</tr>
<tr>
<td>$T_r$</td>
<td>Retention time</td>
</tr>
<tr>
<td>$t_r$</td>
<td>Time under constant strain</td>
</tr>
<tr>
<td>$t_x$</td>
<td>Time required for polymer solution in chloroform to flow through Canon-Fenske viscometer.</td>
</tr>
<tr>
<td>$v$</td>
<td>Velocity</td>
</tr>
<tr>
<td>$V_0$</td>
<td>Initial release rate defined as slope of in vitro release curve of paclitaxel from surgical paste over first 12 hours of study.</td>
</tr>
<tr>
<td>$V_{10}$</td>
<td>Sustained release rate defined as slope of in vitro release curve of paclitaxel from surgical paste over last 10 days of study</td>
</tr>
<tr>
<td>$V_{60}$</td>
<td>Sustained release rate defined as the slope of the in vitro release curve of paclitaxel from surgical paste over the last 60 days of the study</td>
</tr>
<tr>
<td>$V_i$</td>
<td>Molar volume of polymer “i”</td>
</tr>
<tr>
<td>$V_u$</td>
<td>Molar volume of repeat units</td>
</tr>
<tr>
<td>$W_i$</td>
<td>Weight contribution of species “i”</td>
</tr>
<tr>
<td>$X(T)$</td>
<td>Volume fraction of transformed polymer at temperature T</td>
</tr>
<tr>
<td>$X_c$</td>
<td>Degree of crystallinity</td>
</tr>
</tbody>
</table>
$\delta$  Strain

$\Delta H_r$  Heat of fusion
ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Helen Burt, for her guidance, support, wisdom, patience, and spelling ability. Her leadership, and commitment to her profession and family served as an inspiration to me throughout my entire stay with this faculty. She is a good person, and it is a blessing to be associated with good people.

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1. INTRODUCTION

Biomaterials are materials which are compatible with biological tissue and fluids and can therefore be safely implanted into biological systems. Their function can be one of physical support or they may provide some other therapeutic benefit such as sutures, protective dressings, or drug delivery devices (Piskin 1994).

The development of new polymeric biomaterials for use in drug delivery is a major goal in drug delivery research, with formulations for oral, topical and parenteral drug delivery being developed (Langer and Peppas 1981; Hadgraft and Guy 1987; and Holland and Tighe 1986). Many drugs, including most proteins and peptides are orally inactive and have short half-lives in the blood. These drugs require continuous parenteral infusion to be effective, or alternatively, they may be delivered by slow release from a drug/polymer depot within the body (Amsden and Cheng 1995). Other drugs, such as anticancer agents, have serious systemic toxicities. These drugs can be administered locally at the site of action, for example using polymeric implants placed adjacent to inoperable brain tumours, to minimize systemic exposure to the drug and maximize the drug concentration at the site of action (Walter et al. 1994; Kubo et al. 1994; Brem et al. 1995). Drugs which must be taken for extended periods of time are also candidates for polymeric drug delivery since they can be incorporated into polymers and administered via various routes to give controlled release over a long time period.

Paclitaxel has shown promise as an effective anticancer agent (Spencer and Faulds 1994). Its current formulation, a 50:50 mix of Cremophor EL®, and dehydrated alcohol USP, has been associated with a high incidence of hypersensitivity in patients, in addition to the other adverse
effects of paclitaxel including neutropenia, peripheral neuropathy, arthralgia, myalgia, mucositis, nausea and vomiting, and alopecia (Spencer and Faulds 1994; Onetto et al. 1993).

In addition to its cytotoxic effects, paclitaxel has also been shown to be a potent inhibitor of angiogenesis (Oktaba et al. 1995). Cancer may be considered to be an angiogenesis-dependent disease because cancerous tumours, through the release of angiogenic factors, induce new blood vessel growth, or neovascularization, in order to sustain their continued growth (Folkman and Klagsburg 1987). Paclitaxel has been shown to inhibit many of the steps involved in angiogenesis, such as cell proliferation, cell migration, and collagenase secretion (Stearns and Wang 1992). The combination of both cytotoxic and anti-angiogenic properties may result in a more effective agent to treat cancer.

We are developing novel drug loaded polymer formulations which we term “surgical pastes”. The application for these surgical pastes would be at a tumour resection site where drug would be released locally over a period of weeks to months to prevent local recurrence of the disease. The polymer base of this surgical paste is poly(ε-caprolactone) (PCL).

PCL is a biocompatible, biodegradable polymer which either alone, or with other biocompatible polymers, has been investigated for various uses including drug delivery devices (Pitt 1990, Pitt et al. 1979a), nerve guides (Perego et al. 1994), and artificial skin (Bruin et al. 1990). PCL has a long biodegradation lifetime of the order of 1 year (Pitt 1981a; Woodward et al. 1985) and is therefore potentially suitable for the surgical paste formulation in terms of providing a slow release of drugs from the matrix. The physical and chemical properties of PCL were optimized for use as a surgical paste by blending it with methoxypolyethylene glycol (MePEG). The MePEG served to decrease the melting point and viscosity and modify the mechanical properties of PCL.
Paclitaxel has been incorporated into blends of PCL and MePEG which have a low melting point (onset of melting is 45° - 50° C). Following gentle warming, the molten paclitaxel-polymer material may be delivered from a syringe directly to the tumour resection site where it solidifies at 37° C to form a waxy solid.

In this project it was hypothesized that physicochemical characteristics of PCL, including thermal, mechanical and viscoelastic properties, could be altered by blending a low molecular weight oligomer with PCL. The resulting blend could then be used as a polymer matrix for the sustained delivery of paclitaxel in a surgical paste formulation \textit{in vitro}.

The objective of this work was to characterize PCL and blends of PCL with MePEG to be used as the basis of a surgical paste formulation. The \textit{in vitro} release rates of paclitaxel from these pastes were investigated and the antiangiogenic activity of paclitaxel released from surgical paste was assessed using a chick chorioallantoic membrane (CAM) bioassay. A preclinical biodistribution study was performed in mice to determine the distribution of drug released from a subcutaneously implanted pellet of paclitaxel loaded PCL:MePEG surgical paste.

2. BACKGROUND

2.1 Site directed delivery of anticancer agents

Polymeric implants have been used by several groups to administer anticancer drugs. Wu \textit{et al.} (Wu \textit{et al.} 1994) investigated the possibility of incorporating carmustine into wafers of the bioerodible copolymer poly \textit{[bis(p-carboxyphenoxy)propane-sebacic acid]} for intracranial implantation in rats. They found that the degradation of the implant occurred more slowly \textit{in vivo} than \textit{in vitro} but that the \textit{in vivo} rate was not affected by the presence of carmustine, tumour
(9-L-gliosarcoma), or an absorbable hemostatic material. Walter et al. (Walter et al. 1994) used paclitaxel at 20-40% loading in the above copolymer and incorporated discs of the polymer into 9-L-gliosarcoma tumours which had been implanted intracranially in rats. The paclitaxel loaded polymer was found to double or triple the mean survival time of treated rats over that of control rats (no paclitaxel). After 30 days post-implantation, paclitaxel could be measured 8 mm from the disc (the size limit of the rat brain) at a tissue concentration of 5 μM. In a multicenter, randomized, placebo controlled, prospective study, carmustine was incorporated in the same copolymer and administered as discs to treat patients with recurrent malignant brain tumours who required a repeat operation. Patients who received drug loaded polymer had a longer mean survival time than those receiving polymer alone. There were no clinically important adverse reactions related to the carmustine loaded polymeric drug delivery system, either in the brain or systemically (Brem et al. 1995).

Kubo et al. (Kubo et al. 1994) developed a delivery system for anticancer agents using a non-biodegradable polymer containing 10% polymetacrylic methyl acid. This polymer was loaded with mitomycin, adriamycin, 5-fluorouracil (5-Fu) or nimustine hydrochloride. The drug loaded polymers were administered to patients, either intratumourally to treat malignant brain tumours or were applied into the tumour cavity following resection surgery. Following resection and implantation of 5-Fu loaded polymer, the tumour mass was markedly reduced and tumour tissue around the polymer had high concentrations of 5-Fu (Kubo et al. 1994).

Cisplatin formulations in poly(methyl methacrylate) have been investigated for use in osteosarcoma. Studies in rabbits have found that drug concentrations in bone marrow were higher than in bone tissue following implantation of the formulation into bone (Mestiri et al. 1995).
About 64% of all cancer patients present with localized disease. Following initial
treatment of patients with localized disease, it has been estimated that 32% will have a recurrence
of the disease and of these, 66% will relapse due to local recurrence of the disease compared to
34% who will relapse due to distant metastases of the disease (Devita et al. 1989). Local
recurrence of tumours generally occurs near the previous surgical excision site of the primary
tumour. In studies of recurrence patterns of glioblastoma multiforme and anaplastic astrocytoma,
it was found that 90% of all recurrences are located within a 2 cm margin of the primary tumour
(Hochberg et al. 1980, Wallner et al. 1988). The application of a surgical paste formulation
directly to the tumour resection site would prevent regrowth of local tumours by providing a high
dose of anticancer drug to the tumour resection site, without the toxicities associated with
systemic delivery of drug.

2.2 Paclitaxel

2.2.1 Historical development

Paclitaxel was collected in 1962 from the bark of the Western Yew tree, Taxus brevifolia,
as part of a screening program by the National Cancer Institute (Suffness, 1995). By 1971 the
drug was isolated and its structure had been determined (Wani et al. 1971). Development of the
drug was delayed because it did not show superior anticancer activity, relative to other agents
being studied at the time, and because large scale isolation and extraction of the drug was very
difficult. Interest in paclitaxel was renewed however, when it was discovered that it had a unique
mechanism of action and the drug entered preclinical and Phase I trials. Paclitaxel is very
hydrophobic and it required dissolution in a vehicle containing Cremophor EL®
(polyoxyethylated castor oil) prior to infusion. This vehicle caused a high incidence of severe
hypersensitivity reactions which again delayed the development of the drug (Koeller and Dorr 1994). The incidence and severity of these hypersensitivity reactions could be reduced by the administration of antihistamines and steroids prior to each paclitaxel dose, and the development of paclitaxel moved ahead.

Paclitaxel has been extracted from the harvested needles of a different species of yew tree, *Taxus x media Hicksii* (Witherup et al. 1990). A semisynthetic method for producing paclitaxel was developed by Potier from the starting material 10-deacetyl baccatin III (10-DAB) which is relatively abundant and can be extracted from the needles of the English yew, *Taxus baccata* (Suffness 1995). This method is advantageous because the needles are renewable, the yield is 6 to 10 fold greater than for paclitaxel, and the isolation of 10-DAB is simpler and more economical than for paclitaxel (Suffness 1995).

The total synthesis of paclitaxel has also been achieved (Nicholaou et al. 1994; Holton et al. 1994).

### 2.2.2 Physical properties

#### 2.2.2.1 Structure

The structure of paclitaxel is shown in Figure 1. It is characterized by the tricyclic ring system in the structure and is part of a group of compounds called taxanes (Suffness 1995). The chemical structure of paclitaxel is composed of the 20 carbon taxane skeleton, a complex side chain at C-13, two acetoxy groups at C-4 and C-10, and a phenoxy group at C-2. The numbering scheme conforms to the IUPAC nomenclature.

The structure of paclitaxel crystals grown from a mix of dioxane, water and xylene has been reported to be monoclinic with unit cell dimensions $a = 9.661$, $b = 28.275$, and
Figure 1: Chemical structure of paclitaxel
c = 19.839 Å, and a β angle of 99.730° (Mastropaolo et al. 1995). The d-spacings for paclitaxel and its hydrate form determined using X-ray powder diffraction have also been reported (Liggins et al. 1997).

2.2.2.2 Physicochemical properties

Paclitaxel is a hydrophobic drug with a very low water solubility. Values for the aqueous solubility vary in the literature, and include values of 0.7 μg/mL (Mathew et al. 1992), 1 μg/mL (Liggins et al. 1997), 6 μg/mL (Swindell et al. 1991), and about 30 μg/mL (Tarr and Yalkowsky 1987). The discrepancies in the reported solubilities of paclitaxel in water have been suggested to be due to the anhydrous form of paclitaxel achieving an initially higher apparent solubility, but then precipitating as a more stable dihydrate form with a lower equilibrium solubility of 1 μg/mL at 37° C (Liggins et al. 1997). The solubility of paclitaxel in soybean oil is reported to be 30 μg/mL, which is too low for the drug to be formulated into simple oil-water emulsions (Adams et al. 1993). Paclitaxel is more soluble in organic solvents however, with values of greater than 19 and 22 mg/mL being reported for methylene chloride and acetonitrile respectively (Adams et al. 1993), and with an octanol-water partition coefficient of 311 (Lundberg 1997).

2.2.2.3 Assays for paclitaxel

A variety of analytical methods have been used for the measurement of paclitaxel including enzyme immunoassay (Grothaus et al. 1995), time of flight mass spectrometry (Gimon et al. 1994), and LC/MS/MS (Kerns et al. 1994). High-performance liquid chromatography (HPLC) has been used to measure paclitaxel in human urine, plasma and serum as well as mouse plasma and tissues (Richheimer et al. 1992; Andreeva et al. 1997; Willey et al. 1993; Sparreboom et al. 1995; Huizing et al. 1995). In addition, a radio assay for tritium labelled
paclitaxel has also been described for detection of paclitaxel in rat plasma and tissues including liver, spleen, heart, lung, and muscle (Lesser et al. 1995).

### 2.2.3 Pharmacology and indications

Paclitaxel is administered by i.v. infusion to patients in a dose range of between 135 to 175 mg/m\(^2\) over 3 hours or at a dose of between 200 and 250 mg/m\(^2\) over 24 hours. These treatment regimens are repeated every 3 weeks. Paclitaxel has been demonstrated to be effective against a variety of cancers but is only approved for ovarian and breast cancers (Rowinsky 1994; Spencer and Faulds 1994). Phase II trials of paclitaxel used as a single agent against ovarian cancer showed that paclitaxel produced total response rates in the range of 20 to 37%, with complete responses in 3 to 12% of cases (McGuire et al. 1989; Enzig et al. 1992; Thigpen et al. 1994). In Phase II trials of paclitaxel against breast cancer, response rates of about 60% were found (Seidman et al. 1992; Holmes et al. 1991). These patients had advanced breast cancer and had received no prior treatment. In patients who had received prior therapy, the response rates were reported to be between 22 and 29% depending on the dose (Gelmon et al. 1994). Phase II studies assessing the effectiveness of paclitaxel as a single agent against non-small-cell lung cancer in patients who had received no prior chemotherapy reported response rates of between 10 and 38% (Millward et al. 1996; Sekine et al. 1996).

Animal work with paclitaxel has included a study by Riondel et al. (Riondel et al. 1986) in which the ability of paclitaxel to inhibit growth of tumour tissue transplanted from humans to 6-8 week old Swiss nude female mice was assessed. Primary breast, endometrium, ovary, brain and lung tumours as well as a recurrent tongue tumour were transplanted and treated using s.c. injections of paclitaxel at a dose of 12.5 mg/kg per injection for 5 consecutive days out of 7 over
a three week period (15 injections total). The animals were followed for three months before being sacrificed. It was found that for all tumours, paclitaxel treatment resulted in a significant delay in tumour growth. Total tumour regression was found in 4 of the 5 mice implanted with the breast cancer tumour tissue while 1 of 11, 1 of 7 and 1 of 6 mice with brain, lung and tongue cancer tissue respectively, showed regression of the tumours.

Paclitaxel also possesses antiangiogenic activity (Belotti et al. 1996; Oktaba et al. 1995; Burt et al. 1995). Angiogenesis is the growth of new vascular tissue, which is a process mediated by angiogenic factors such as fibroblast growth factor and endothelial cell growth factor (Folkman and Klagsburg 1987). Tumours secrete angiogenic factors, which stimulate additional blood vessel growth into the tumour which is necessary for continued tumour growth (Folkman and Klagsburg 1987). Paclitaxel inhibits several steps involved in angiogenesis such as cell division, migration and collagenase secretion (Stearns and Wang 1992). A chick chorioallantoic membrane (CAM) assay may be used to determine angiogenic or antiangiogenic activity of compounds placed on the membrane (Dugan et al. 1991). This assay can be used to confirm the antiangiogenic activity of paclitaxel released from the surgical paste formulations.

The mechanism of action of paclitaxel is through the promotion of microtubule formation from α and β tubulin subunits (Spencer and Faulds 1994; Schiff et al. 1979). Within a cell, microtubules are critical in the processes of spindle formation, movement, nutrient ingestion, and controlling the shape of cells (Panchagnula 1998). Microtubules are polymers of tubulin, and their normal operation requires the periodic polymerization and depolymerization of tubulin. That is, the microtubules are continually forming and breaking down in a cell (Eisenhauer and Vermorken 1998). Paclitaxel binds to tubulin and prevents its depolymerization, resulting in microtubules which do not break down. Without functional microtubules, the cell is unable to
divide and cell mitosis is prevented. Paclitaxel also promotes abnormal, branched microtubule formation in the cytoplasm of cells, decreasing cell motility (Keller and Zimmerman 1986).

Chemical modifications to the paclitaxel molecule can affect the activity of the drug. The C-13 ester is necessary for antimitotic activity (Guéritte-Voegelein et al. 1991) and the loss of this side chain produces baccatin III. Loss of this ester can occur by mild basic hydrolysis, however, this process is slowed when paclitaxel is bound to proteins or microtubules. Other modifications are less critical but in vitro activity can be lost with acetylation at C-2' and C-7 (Kingston 1991). Epimerization of the hydroxyl on carbon 7 occurs in solution but 7-epipaclitaxel maintains over 90% of paclitaxel’s activity (Ringel et al. 1994).

2.2.4 Pharmacokinetics

Eiseman et al. (Eiseman et al. 1994) studied the pharmacokinetics of paclitaxel in CD₂F₁ mice by administering paclitaxel at a dose of 22.5 mg/kg via the intravenous (i.v.), interperitoneal (i.p.), oral (p.o.), and subcutaneous (s.c.) routes. Paclitaxel was not detected in the plasma of the mice following p.o. and s.c. administration and a 10% bioavailability was was measured after i.p. administration. Following i.v. administration, it was found that paclitaxel distributed extensively to all tissues except the brain and testes. In male mice, the clearance of paclitaxel was found to be 3.25 mL/min/kg with a half-life of 69 minutes. The clearance in female mice was 4.54 mL/min/kg with a half-life of 43 minutes. Innocenti et al. (Innocenti et al. 1995) administered paclitaxel to female Swiss mice with a single dose of 18 or 36 mg/kg via the i.p. route and found maximum plasma concentration (Cₘₐₓ) values of 13.0 and 25.7 μg/mL respectively. The paclitaxel clearance and half-life values were 0.06 mL/min and 3.0 hours for the 18 mg/kg dose and 0.10 mL/min and 3.7 hours for the 36 mg/kg dose. Intra-peritoneal
administration resulted in high levels of paclitaxel in peritoneal organs including the liver, pancreas and ovary.

Five Phase I studies of paclitaxel in humans were reviewed by Kuhn (Kuhn 1994), and paclitaxel pharmacokinetic data were summarized. Paclitaxel was administered as a single agent in 1-, 6-, and 24-hour continuous intravenous infusions. The paclitaxel dosages ranged from 15 to 275 mg/m². Serum concentration versus time curves showed biphasic elimination profiles from plasma with the half-lives of elimination ranging from 1.4 to 8.3 hours. Peak plasma levels were proportional to dose but increased throughout the infusion period. The steady state volumes of distribution ranged from 49 to 119 L/m² with a mean of 110 L/m², while clearances ranged from 8 to 53 L/h/m². Renal elimination accounts for between 4 and 8% of the dose (Wiernik et al. 1987; Longnecker et al. 1987) while biliary excretion accounts for approximately 20% of the dose (Monsarrat et al. 1990). The major route of paclitaxel elimination is via metabolism in the liver and excretion in the feces. After 7 days, 75% of a radiolabelled dose of paclitaxel was recovered from human feces (Monsarrat et al. 1990).

2.2.5 Toxicities

A high incidence of anaphylactic reactions has been reported following paclitaxel administration which has been attributed to the Cremophor vehicle. As a result, premedication regimens using H₁ and H₂ antihistamines and steroids are required (Rowinsky et al. 1990). Even with this premedication however, anaphylaxis type reactions were still observed in under 5% of patients (Weiss et al. 1990; Arbuck et al. 1993).

Paclitaxel has also been implicated in causing systemic toxicities, common with other antineoplastic agents. These include neutropenia, peripheral neuropathy, arthralgia, myalgia,
mucositis, nausea and vomiting and alopecia (Spencer and Faulds 1994; Onetto et al. 1993). Administration of paclitaxel at a dose of 250 mg/m\(^2\) over 24 hours seems to result in a significantly greater incidence of neutropenia compared to a 175 mg/m\(^2\) paclitaxel dose infused over 3 hours (Eisenhauer and Vermorken 1998).

2.2.6 Paclitaxel formulations

Because of its poor water solubility, paclitaxel is currently formulated in a vehicle of 50% polyoxyethylated castor oil (Cremophor EL\textsuperscript{®}) and 50% ethanol (Dorr 1994) at a concentration of 6 mg/mL.

Controlled release formulations for paclitaxel have also been reported in the literature. Paclitaxel has been formulated in liposomal carriers (Sharma and Straubinger 1994) and as complexes with cyclodextrins (Sharma et al. 1995). Paclitaxel loaded polymeric microspheres have been developed using a variety of different polymers including polyanhydride (Jampel et al. 1993), polyvinylpyrrolidone (Sharma et al. 1996), poly(lactic-co-glycolic acid) (Wang et al. 1996), blends of ethylene-vinyl acetate and poly(d,l-lactic acid) (Burt et al. 1995), and PCL (Dordunoo et al. 1995). Poly \textit{[bis(p-carboxyphenoxy)propane-sebacic acid]} was used to make paclitaxel loaded polymeric discs (Walter et al. 1994), and diblock copolymers of poly(d,l-lactide) and MePEG formed micellar carriers for paclitaxel (Zhang et al. 1996). The \textit{in vitro} release of paclitaxel was also evaluated from biodegradable polyanhydride polymer for sustained release (Park et al. 1998).

2.3 Polymer chemistry

A polymer can be defined as a large molecule constructed from many smaller structural units called monomers, covalently bonded together in any conceivable pattern (Cowie 1973).
The properties that a polymeric material possesses as a whole will depend on the chemical structure of the monomer, the chemical arrangement of monomers such as straight chain, branched chain or 3-D matrix, length of chains, and the orientation of the chains.

2.3.1 Constitution and conformation

Polymer constitution refers to the atomic structure of the monomers which make up the polymer. This also takes into account elements of chirality, isomerism, the presence of side groups and rigidity. For a polymer to change its constitution, it would need to break and reform interatomic covalent bonds. Polymer conformation refers to the three dimensional shape that a polymer chain possesses. A polymer chain with a given constitution can adopt a variety of conformations by rotating bonds in the polymer backbone (Miller 1962; Billmeyer 1984).

2.3.2 Morphology and models of crystallinity

Polymer morphology describes the three dimensional arrangement of polymer chains in the solid state with respect to long range order (Wunderlich 1973; Rosen 1993). In the solid state, polymer chains do not achieve an equilibrium state but rather remain disordered, or only partially ordered, with respect to adjacent chains, which gives rise to amorphous or semicrystalline materials, respectively.

There are two models used to describe the observed properties of semicrystalline polymers. The “fringed micelle” model (Figure 2A) describes crystalline regions known as fringed micelles or crystallites interspersed in an amorphous matrix. The crystallites consist of portions of different chains which are aligned parallel to each other, tightly packed in a crystal lattice (Rosen 1993). The individual chains are many times longer than the crystallite and so they pass from one crystallite to another through amorphous areas.
This model explains the coexistence of crystalline and amorphous regions within a polymer and shows why perfect crystallinity cannot be achieved. The model also represents the crystallites as crosslinks which tie the individual chains together and can reduce the mobility of individual chains resulting in a reduction of the material's ability to flow. Unlike crosslinks however, crystallites generally melt before the polymer degrades, and can be dissolved by solvents that form strong secondary bonds with the chains (Rosen 1993).

The second model of polymer crystallinity is that of chain folded crystals or lamellar crystals. This model came about when it was observed that single polymer crystals could be grown from dilute solutions as flat discs approximately 0.01 μm thick, which was about one tenth the length of the polymer chains. X-ray measurements showed that the polymer chains were aligned perpendicularly to the flat faces of the crystals and hence, the chains must fold back on themselves as shown in Figure2B (Rosen 1993).
Figure 2: A) Fringed-micelle and B) Lamellar (chain folding) model of polymer crystallinity. Grey areas represent crystalline regions.
2.3.3 Degree of crystallinity

Polymer samples are, at best, only partially crystalline. The proportion of a solid polymer incorporated into crystallites, commonly on a weight basis, is known as the degree of crystallinity (Xc) (Blackadder 1975). A semicrystalline polymer is a two phase system consisting of crystallites and amorphous phases. To measure the degree of crystallinity of a polymer sample, an overall property of the sample is measured such as density, specific heat or heat of fusion. The property measured is chosen on the basis that the crystallites and amorphous phase have different values and that the theoretical values for pure crystalline and amorphous polymer can be determined. The value measured in a semicrystalline polymer will depend on the relative contributions of the crystallites and the amorphous phase and can be used to calculate the degree of crystallinity using the equation:

\[ P = XcP_1 + (1-Xc)P_2 \]  

Equation 1

where \( P \) is the overall property per unit mass, \( Xc \) is the degree of crystallinity expressed as the weight fraction of the crystalline phase, and \( P_1 \) and \( P_2 \) correspond to the values of the property per unit mass of pure crystalline and amorphous phases respectively (Blackadder 1975). The validity of this equation depends on how accurately \( P \), \( P_1 \) and \( P_2 \) can be measured (or calculated) and on the assumption that a specific phase property, particularly the amorphous phase, will be the same whether it is measured as a pure sample or as part of a semicrystalline sample.

The degree of crystallinity is an important parameter in the characterization of a polymeric drug delivery system since it may influence both the mechanical properties and the drug release kinetics (Langer and Peppas 1981; Piskin 1994). In practice, the measurement of
the heat of fusion of a semicrystalline sample provides a convenient measure of \( X_c \) provided that a value for the heat of fusion of a 100% crystalline sample can be obtained from the literature.

2.3.4 Molecular Weight

2.3.4.1 Definitions

The molecular weights of polymeric samples are described in terms of a distribution of weights and of average molecular weights. The two most commonly used averages are the number average molecular weight (\( M_n \)) and the weight average molecular weight (\( M_w \)). \( M_n \) is the average weight of the polymer chains calculated by dividing the total weight of the polymer sample by the total number of chains present:

\[
M_n = \frac{\sum N_i M_i}{\sum N_i}
\]

Equation 2

where \( N_i \) are the number of molecules of species \( i \) of molecular weight \( M_i \). \( M_n \) values are sensitive to the presence of small chains in the polymer sample since all molecules contribute equally to the average value (Rosen 1993). That is, a small fraction of molecules by weight may exist as low molecular weight species, yet this fraction may represent a large proportion of the total number of molecules present in the sample.

To characterize the molecular weight distribution by taking into account the contribution of each chain to the total mass of the sample, a weight average molecular weight can be used (\( M_w \)), which can be calculated according to the formula:

\[
M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum w_i M_i}{\sum w_i}
\]

Equation 3
where \( w_i \) is equal to the weight contribution of species \( i \). As a result, \( M_w \) is less sensitive to the presence of short chains in a polymer sample. The lower sensitivity of \( M_w \) to low molecular weight chains than \( M_n \), means that \( M_w \) values will always be greater than \( M_n \) values for a given polymer sample. The ratio of \( M_w \) to \( M_n \) for a given polymer sample is called the polydispersity index (PDI). This value gives information about the molecular weight distribution of the sample, since a wider distribution will give a greater PDI value. For many “typical” polymerization reactions the PDI is about 2 but there is a lot of variability encountered among samples (Cowie 1973).

The average degree of polymerization of a polymer is obtained by dividing the molecular weight of the polymer by the molecular weight of each monomer unit. It represents the number of monomer residues which make up each chain. The value obtained will depend on which molecular weight is used in the calculation (\( M_n \) or \( M_w \)).

2.3.4.2 Measurement of molecular weight

Measurement of the average molecular weight of a polymer sample can be either absolute, where measured quantities are theoretically related to the average molecular weight, or relative, where the relationship between the measured quantity and the molecular weight of the sample must first be established by calibration with one of the absolute methods (Rosen 1993).

2.3.4.2.1 Absolute methods

An absolute method is end group analysis, which involves determining the number of chains present using a standard analytical technique to determine the concentration of end groups present in the sample. Given the mass of polymer, the \( M_n \) of the sample may be obtained. As the molecular weight of the sample increases, the concentration of end groups per unit volume
decreases and the sensitivity drops off rapidly. For this reason, end group analysis is usually limited to polymers of Mn < 10 - 15 000 (Rosen 1993; Cowie 1973).

Another technique for counting the number of polymer molecules present in a sample is through the use of colligative property measurements. A colligative property is one which is a function only of the number, and not of the nature, of the solute molecules contained in a unit volume of solution (Cowie 1973). Examples are freezing point depression, boiling point elevation, and osmotic pressure. In the case of osmotic pressure the equation would be:

\[
\lim_{c \to 0} \frac{\pi}{c} = \frac{RT}{Mn}
\]

where \(\pi\) is the osmotic pressure, \(c\) is the concentration of polymer in solution (mass/volume), \(R\) is the universal gas constant, and \(T\) is absolute temperature. The Mn of a polymer sample can now be measured by measuring \(\pi\) at various concentration and plotting \((\pi/c)\) as a function of \(c\) and extrapolating to zero concentration (Rosen 1993). A requirement of this technique is that the solution is sufficiently dilute such that it behaves as an ideal solution and obeys Raoult’s Law. As the Mn of the polymer is increased, fewer polymer chains can be included while still maintaining a dilute solution. This decreases the sensitivity of the method and limits the applicability of the method to 50 000 < Mn < 1 000 000 (Rosen 1993).

If a measurement is used to give information on the mass or size of each chain then this method could be used to measure the Mw of the polymer sample. One of the most common of these methods is that of light scattering (Rosen 1993). This method is based on the fact that the intensity of light scattered by a polymer molecule is proportional to the square of its mass. Provided the dependence of the refractive index to polymer concentration is known, then by measuring the intensity of scattered light as a function of the scattering angle at different polymer
concentrations, and extrapolating both the angle and concentration to zero (Zimm plot), Mw can be obtained. This technique is useful for 10 000 < Mw < 10 000 000. Ultracentrifugation can also be used to measure the Mw of polymers since the molecules will be distributed according to their mass in a centrifugal force field (Rosen 1993).

2.3.4.2.2 Relative methods

Relative methods of molecular weight determinations measure quantities which are related to the molecular weight of the sample but must be calibrated using one of the absolute methods (Rosen 1993). One example is solution viscosity, since small amounts of dissolved polymer can cause large increases in viscosity which are proportional to the molecular weight of the polymer. The solution viscosity will depend also on the solvent viscosity, temperature, solute concentration, the particular solvent and polymer.

In particular, solution viscosity measurements can be used to determine the intrinsic viscosity ([η]) of the polymer sample (Martin 1993). If the molecular size of the polymer (described by the hydrodynamic radius (R_\eta)) can also be measured, then the molecular weight of the polymer can be determined using Einstein's equation of intrinsic viscosity:

\[
[\eta] = \frac{CR^3}{\eta MW}
\]  

Equation 5

where C is a proportionality constant, and MW is the molecular weight of the polymer. Gel permeation chromatography (GPC) is a technique which measures the molecular size of polymer chains. A polymer solution is passed through a column containing beads which have specified pore sizes. The presence of these pores will result in a fractionation of the polymer according to molecular size, since smaller polymer chains will tend to be entrapped in the pores to a greater
extent than larger molecules and as a result will elute at longer retention times. The shape of the resulting GPC peak will therefore represent the distribution of molecular weights in the polymer sample. A standard curve for a given polymer solution at a specified temperature under specified column conditions can be constructed, relating retention time and molecular weight. If no standards are available, then the intrinsic viscosity of the polymer must be determined and can be used in conjunction with the molecular size data to determine the molecular weight. A universal standard curve can be constructed by plotting \( \log ([\eta]M_{GPC}) \) against retention time (Cowie 1973).

Alternatively, intrinsic viscosity can be used to determine molecular weight by using the Mark-Houwink equation:

\[
[\eta] = K(M_v)^a
\]  
Equation 6

where \( K \) and \( a \) are constants and \( M_v \) is the viscosity average molecular weight of the polymer.

The constants \( K \) and \( a \) are specific for a given polymer solvent system at a particular temperature.

If these values can be obtained from the literature, then a universal standard curve for GPC can be plotted as \( \log [K (M_v)^{a+1}] \) against retention time.

2.3.5 Thermal properties of polymers

2.3.5.1 Glass transition

In the amorphous state, polymer chains are oriented in a completely random manner. The mobility of polymer chains in an amorphous material are not restricted by extensive interchain bonding and the material is therefore provided with a measure of flexibility and elasticity. The physical state of an amorphous polymer is governed by the chain flexibility and the temperature of the system (Rosen 1993), and the polymer may exist in the glassy, leathery, viscoelastic, rubbery flow, or viscous states.
At very low temperatures, a polymer is in what is called the glassy region. Here, virtually all molecular motion is frozen, and the material behaves like a brittle elastic material. In this state, the polymer chains are locked in a tangled ball and thermal energy is not sufficient to allow slippage of the chains past each other, which would be necessary for any kind of flow to occur. Without chain slippage, the only strain which can occur is elastic chain stretching. In this region, the typical modulus is $10^9$ to $10^{10}$ N/m$^2$, and is referred to as the “glassy” modulus (Rosen 1993; Cowie 1973).

At higher temperatures, an amorphous polymer is in the viscoelastic state. In this region, the polymer chains have enough mobility to assume a variety of conformations, without significant chain untangling taking place. Application of a stress to a viscoelastic material causes rotation about the chain bonds resulting in an elongation of the molecule in the direction of the applied stress. The polymer chains adopt less stable conformations in response to the stress and if this stress is removed quickly, the viscoelastic polymer will return to its original shape as the chains return to their preferred conformation. However, if the stress is applied for a greater length of time, the chains begin to unravel and slip past each other into new positions where, when the stress is removed, the chains can relax into a stable coil which is different from its original conformation. That is, the polymer possesses both elastic and viscous properties and is termed “viscoelastic”.

Between the glassy and viscoelastic states, over a temperature range of about 30 K, is a transition region or leathery region. Within this region lies the glass transition temperature (Tg) of the polymer.

At temperatures above the viscoelastic state the polymer moves through the rubbery flow region which is characterized by a decrease in the modulus (down to about $10^{4.5}$ N/m$^2$), and into
the region where the material is in a viscous state. In response to a stress, a polymer in the viscous state will flow and there will be no evidence of elastic recovery. As the temperature is increased, the modulus of the material will decrease.

In some polymers, bonds exist between amorphous chains which restrict the flexibility of the chains and the movement of these chains past one another. These bonds serve to stiffen the polymeric material and give the material as a whole, greater elasticity. If the bonds are weak, for example hydrogen bonds or Van der Waal's interaction, then energy would have to be applied to break these bonds before the chains would be able to flow past each other. In some cases the amorphous chains are tied together or crosslinked by molecules which are covalently bonded to the chains. As a result, the crosslinks cannot be broken by melting or dissolution without degradation of the material as a whole. The effect of crosslinking is to stiffen the material causing it to behave more like a brittle glass (Hearle 1982) at all temperatures.

The glass transition temperature (Tg) is the temperature which separates the glassy region from the viscoelastic region of the amorphous phase of a polymer. Polymer chains can exhibit four types of molecular motion. These are, vibration of atoms about equilibrium positions, motions in small chain segments and side chains, cooperative “wriggling” and “jumping” of large chain segments of about 40 to 50 carbons in length, and translational motion of entire polymer chains. The latter two types of motion are necessary for the flexing, uncoiling and flow of polymer chains. In terms of molecular motion, below Tg, these types of motion are “frozen out” and the material behaves as a brittle glass (Rosen 1993). Above Tg, enough energy is present to permit the cooperative and translational motions and the material can exhibit viscoelastic properties.
Thermal analysis using differential scanning calorimetry (DSC) provides a convenient way of measuring the Tg of a polymer (Wunderlich 1990). The method monitors the heat capacity of a sample as a function of temperature. At the Tg of the sample, the heat capacity increases and is observed as a change in the measured baseline.

There are at least five factors which influence the Tg of a given polymer (Rosen 1993):

i) Free volume of the polymer ($v_f$).

This is defined as the volume of the polymer mass not actually occupied by the molecules themselves, and can be calculated by the equation:

$$v_f = v - v_s$$  

Equation 7

where $v_s$ is the volume of the solidly packed molecules. At higher values of $v_f$ there is more space available for the polymer chains to move around and the Tg will be decreased.

ii) Attractive forces between molecules.

The more strongly the molecules are attracted to each other, the more energy will be required to break these connections to allow the chains to slip past each other and Tg will be increased.

iii) Internal mobility, or rotational freedom of the chains.

Each bond in the polymer chain has a minimum energy configuration. As the bond is rotated, energy is required to push the substituent groups past each other and the maximum energy is needed to get the two chain substituents past each other. Chain mobility requires the complete rotation of bonds, for which energy is needed. As the resistance to rotation is increased by the interactions of the substituent groups, the Tg will increase.
iv) Chain stiffness.

Chains which resist coiling and folding will have a higher Tg. This effect is very similar to internal mobility.

v) Chain length.

$T_g$ is related to $M_n$ according to the equation:

$$T_g = T_g^\infty - \frac{k}{M_n}$$

Equation 8

where $T_g^\infty$ is the glass transition temperature of the polymer at infinite chain length, and $k$ is a constant for the particular polymer. This reflects the increased ease of motion of shorter chains.

2.3.5.2 Melting transition

Crystallites in a semicrystalline polymer vary in both size and degree of crystalline perfection depending on the thermal history of the sample (Cowie 1973). The result is that the overall melting transition of the polymer sample takes place over a broad temperature range and is not a discreet value. In practice, the melting of polymers is not observed at the equilibrium melting temperature ($T_m^\circ$) because near-perfect crystals cannot be obtained, except under very specific conditions, for only certain polymers such as polyethylene (Wunderlich 1973). Instead, the observed melting point ($T_m$) is found, which deviates from $T_m^\circ$ as a function of the lamellar thickness and the surface energy of the crystallites (Holdsworth and Turner-Jones 1971) according to the equation:

$$T_m = T_m^\circ \left(1 - \frac{2\sigma}{\Delta h \cdot l}\right)$$

Equation 9
where $\Delta h$ is the enthalpy of fusion per repeat unit of the polymer chain and $\sigma_e$ and $l$ are the surface free energy and lamellar thickness, respectively, which are both related to the chain folding frequency. In some polymers, the chains can crystallize in different polymorphic forms which will exhibit different $T_m^\circ$'s (Flanagan and Rijke 1972). The broad temperature range over which polymer samples of a given molecular weight are seen to melt, reflects the range in the degree of crystalline perfection of the crystallites present and the possible existence of polymorphic forms within the crystals.

The melting of polymers is sometimes observed as multiple endotherms in thermal analysis. The cause of this has been discussed in several publications (Sweet and Bell 1972; Todoki and Kawagughi 1977; Nichols and Robertson 1992). The presence of the multiple endotherms are a result of metastable crystals within the polymer sample melting at a $T_m$ below the $T_m^\circ$ of the polymer crystal. The melt can subsequently recrystallize at temperatures which are still below $T_m^\circ$ to form more stable crystals which have a melting point which is closer to the equilibrium value. These crystals will subsequently melt, giving rise to the second endotherm.

2.4 Poly(e-caprolactone)

2.4.1 Physical properties

Poly(e-caprolactone) (PCL) is widely used in biomaterial applications (Pitt 1990). The repeating unit of PCL consists of five ethylene groups linked by ester bonds (Figure 3) and the polymer is relatively hydrophobic. PCL is between 40 and 80% crystalline with the degree of crystallinity increasing as molecular weight decreases (Pitt et al. 1981b). X-ray diffraction studies of PCL show that it has two major X-ray diffraction peaks (Ong 1978a). The melting
point of PCL is between 59-64° C and it has a glass transition temperature (Tg) of approximately -60° C (Pitt 1990).

2.4.2 Biodegradation

Some polymers such as ethylene vinyl acetate (EVA) can be used as implants for the sustained release of drugs. However, the polymer is not broken down \textit{in vivo} and the polymer is considered to be non-biodegradable. When the drug supply is exhausted, the polymer must be removed surgically. An example is the Norplant\textsuperscript{®} drug delivery system for birth control, which contains polydimethyl siloxane (PDMS).

If an implanted polymer undergoes hydrolytic, enzymatic or bacteriologic degradation to biocompatible products it is considered to be biodegradable (Holland and Tighe 1986). Two classes of biodegradable polymers include the polyanhydrides and polyesters, which consist of monomers linked by anhydride and ester bonds respectively (Holland and Tighe 1992). These bonds are susceptible to hydrolytic cleavage, which is dependent on the ability of water to react with the susceptible functional groups in the polymer backbone (Holland 1992; Woodward \textit{et al}. 1985; Pitt \textit{et al}. 1981a). Factors affecting the hydrolysis rate of the polymer are, the hydrophobicity of the polymer (Holland and Tighe 1992), the presence of substituents which sterically impede access to the susceptible functional group (Pitt \textit{et al}. 1984), the crystallinity of the polymer (Pistner \textit{et al}. 1993; Pitt \textit{et al}. 1981a), and environmental factors such as temperature (Aso \textit{et al}. 1994), pH, buffer concentration and ionic strength (Makino \textit{et al}. 1986; Chu 1981).

As a polymer degrades, its molecular weight decreases and eventually the degradation products become soluble and separate from the matrix, resulting in a loss of polymer implant mass (Woodward \textit{et al}. 1985; Pitt \textit{et al}. 1981a). This loss of mass is called bioerosion. The
effects of biodegradation and subsequent bioerosion are seen in changes in polymer mass and molecular weight, as well as in mechanical properties such as tensile strength (Migliaresi et al. 1994) and drug release kinetics (El-Arini and Leuenberger 1995; Zhang et al. 1994). Biodegradable polymers have been used as biomaterials for drug delivery implants, surgical sutures, and a variety of other medical uses (Engelberg and Kohn 1991).

The hydrolytic biodegradation of PCL is dependent on the ability of water to insert itself into the ester groups in the PCL matrix which will result in cleavage of the bond (Pitt et al. 1981b; Woodward et al. 1985; Pitt 1990). The biodegradation of semicrystalline polymers such as PCL proceeds first in the amorphous regions of the polymer because water can penetrate the amorphous regions more easily than the crystallites. This results in an initial increase in the degree of crystallinity of the PCL sample (Holland and Tighe 1992). The relatively high hydrophobicity and degree of crystallinity of PCL give it a long biodegradation time of over 9 months (Woodward et al. 1985). Initially, hydrolysis of the ester bonds results in a decrease in the molecular weight of the PCL but not a decrease in the mass of the polymer sample, since the PCL fragments are too large to diffuse from the bulk of the polymer (Holland and Tighe 1986; Pitt 1990; Woodward et al. 1985). Eventually, when the fragments fall below about molecular weight 3000 they begin to diffuse away and the polymer mass is reduced (Woodward et al. 1985). The effects of biodegradation and subsequent bioerosion of PCL samples would be reflected in changes in the mass of the implant, inherent viscosity and molecular weight of the PCL and in mechanical properties such as tensile strength and drug release kinetics (Pitt 1990).

In addition to hydrolytic cleavage of PCL chains, chemical hydrolysis mechanisms, in which a molecule other than water catalyzes the cleavage reaction, have been studied including free radical catalyzed hydrolysis (Ali et al. 1993), accelerated degradation by organic amines
(Lin *et al.* 1994) and enzyme catalyzed hydrolysis *in vivo* (Pitt *et al.* 1984; Holland and Tighe 1986). Since the initial *in vivo* rate of PCL degradation was similar to the *in vitro* rate, it was concluded that enzymatic degradation of PCL is insignificant compared to hydrolysis. This was thought to be due to the PCL having a high degree of crystallinity which inhibited the mobility of the PCL chains and prevented them from achieving correct chain conformation for enzymatic attack (Pitt 1990; Holland and Tighe 1986). Small molecular weight fragments of PCL which eventually diffuse from the PCL implant are ultimately degraded *in vivo* by phagosomes in macrophages, giant cells and fibroblasts (Woodward *et al.* 1985).

### 2.5 Blending of PCL and MePEG

#### 2.5.1 Rational

Polymer blends are mixtures of two or more different polymers. Blends can be prepared by physical mixing, melt fusion, or casting from a common solvent. Different polymers can be blended together to give a new material with its own unique set of properties (Bates 1991).

PCL has an “exceptional” ability to form blends with other polymers (Pitt 1990) and PCL blending has been studied extensively (Li and Prud’Homme 1993; Pitt 1990; Cha and Pitt 1990; Domb 1993; Embleton and Tighe 1993). By mixing other biocompatible polymers with PCL, a polymer blend may be produced which will have a lower melting point than PCL alone, and which will have a decreased tensile strength for greater ease of spreading. If the added polymer is more soluble in an incubation medium than PCL, it may act as a porosigen (Baker 1987). In this case the added polymer may diffuse out of the PCL matrix into the surrounding medium leaving spaces in the PCL where the added polymer used to be. This will facilitate the formation of channels within the PCL and may lead to increased wetting of the interior of the PCL pellet.
PCL's melting point and hard, brittle, mechanical properties are not optimal for a surgical paste formulation, because the paste must be administered in a molten state to the exposed tissue of the tumour resection site and the solid paste which subsequently forms, must not damage the surrounding tissue. Since the administration of paste at temperatures greater than 50° C would likely cause tissue necrosis (Nelson et al. 1986) the surgical paste must melt at less than 50° C. Once deposited as a solid paste, the final formulation must be either soft enough or fail under a low enough stress that it does not injure surrounding tissues during normal activities of the patient following administration.

In this work, methoxypolyethylene glycol (MePEG) has been blended with PCL in order to optimize the physicochemical properties of the material to meet the requirements of a surgical paste formulation. This represents the first report of methoxypolyethylene glycol (MePEG) as a blend with PCL to optimize the formulation characteristics.

2.5.2 Methoxypolyethylene glycol

2.5.2.1 Physical properties

Methoxypolyethylene glycols (MePEG’s) are polyethylene oxide polymer chains with a hydroxyl group at one end and a methoxy group at the other. The structure of MePEG is shown in Figure 4. Literature from the suppliers, Union Carbide, Danbury, CT states that MePEG is completely soluble in water, has a density of about 1.1 g/cm³, and a heat of fusion of 184 J/g.

The melting point of MePEG is dependent on MW, with the MW 350 samples melting between -5 and 10° C and the MW 750 samples melting between 27 and 32° C.

MePEGs are produced by Union Carbide to meet all of the National Formulary (NF, Volume 18) specifications for use in drug applications.
2.5.2.2 Methoxypolyethylene glycol formulations

MePEG chains have been grafted on the external surfaces of existing formulations to give the product some of MePEG's characteristics. Increased water solubility and emulsification properties were achieved by using MePEG to make micelles (Geetha et al. 1993), and bonding MePEG to poly(bis(trifluoroethoxy) phosphazene) films gave them better compatibility with biological tissues, while allowing the films to retain their mechanical properties (Lora et al. 1993). When poly(D,L-lactic acid) (PLA) was copolymerized with MePEG before nanoparticle manufacture, the resulting nanoparticles were found to have a longer plasma half-life than PLA nanoparticles alone, due to their enhanced ability to avoid uptake by the mononuclear phagocytic system (Bazile et al. 1995). Covalent attachment of MePEG to various proteins has been reported in the literature as a means of decreasing the immunogenicity of the proteins when administered to animals (King et al. 1979; Abuchowski et al. 1977; Lang et al. 1992; Savoca et al. 1979).

MePEG was copolymerized with PCL and investigated for potential use as a drug carrier (Shin et al. 1998; Kim et al. 1998). The copolymer was formulated as micelles in which indomethacin, an anti-inflammatory drug, was encapsulated. The formulation provided sustained release of indomethacin in vitro for more than 2 weeks. A diblock copolymer of MePEG with poly(d,l-lactic acid) was developed as a micellar delivery system for paclitaxel (Zhang et al. 1996).

Polyethylene glycol (PEG) has been used to enhance the release rate of drugs from formulations, due to its ability to dissolve many drugs and its own complete solubility in water. For example, the addition of PEG to Avicel PH101 pellets enhanced the in vitro release rate of hydrochlorothiazide in a concentration dependent manner (Vervaet et al. 1994).
Figure 3: Chemical structure of poly(ε-caprolactone) (PCL).

Figure 4: Chemical structure of methoxypolyethylene glycol (MePEG).
2.5.3 Thermal properties of blends

The Gibbs Helmholtz Equation can be used to predict whether polymer miscibility in the blend is thermodynamically favorable. The Gibbs free energy of mixing ($\Delta G_{\text{mix}}$) is given by the equation:

$$\Delta G_{\text{mix}} = \Delta H_{\text{mix}} - T\Delta S_{\text{mix}}$$  \hspace{1cm} \text{Equation 10}

where $\Delta H_{\text{mix}}$ and $\Delta S_{\text{mix}}$ are the enthalpy and entropy of mixing at temperature (Liu and Donovan 1995). The entropy of mixing is given by the equation:

$$\Delta S_{\text{mix}} = -R \left[ \frac{\phi_1}{V_1} \ln \phi_1 + \frac{\phi_2}{V_2} \ln \phi_2 \right]$$  \hspace{1cm} \text{Equation 11}

where $R$ is the gas constant, $\phi_1$, $\phi_2$, $V_1$ and $V_2$ are the volume fraction and molar volume of polymer 1 or 2, respectively (Flory et al. 1953b). Since the molar volume is usually quite large for polymers, $\Delta S_{\text{mix}}$ is negligible (Song et al. 1997; Liu and Donovan 1995). As a result, the only way that polymers can be miscible is if $\Delta H_{\text{mix}}$ is very close to zero, if not negative, so that the free energy of mixing can be negative. This occurs if there are specific interactions between the two polymer components, such as hydrogen bonding (Coleman et al. 1996), acid-base attractions (Zhou and Eisenberg 1983), ion-ion interaction (Kokufuta et al. 1981), amide exchange reaction (Shibayama et al. 1995), and n-π complex formation (Cruz et al. 1979). These interactions reduce the free energy of mixing and contribute to a negative interaction parameter, $\chi$ (Liu and Donovan 1995; Coleman et al. 1996). In addition, $\chi$ can be affected by other factors such as crystallization, differences in polymer stiffness and spatial geometry, by affecting the free energy of mixing (Liu and Donovan 1995). Miscibility between polymers has been shown to be
enhanced by increasing the intramolecular repulsion in one of the polymer components (Ahn et al. 1997).

The Tg of polymers and their blends can be used to determine the miscibility of blended polymers. Polymer blends have been categorized as being a) compatible, exhibiting a single Tg, b) mechanically compatible, exhibiting the Tg values of each component but with superior mechanical properties, and c) incompatible, exhibiting the unenhanced properties of phase-separated materials (Pitt 1990). Miscible blends form clear, homogeneous one phase systems and exhibit a single Tg intermediate between the Tg’s of the individual blend components (Mark et al. 1969). For a pair of miscible polymers, the Tg of the blend can be approximated by the Fox equation:

\[
\frac{1}{T_g} = \frac{\omega_1}{T_{g1}} + \frac{\omega_2}{T_{g2}}
\]

Equation 12

where \(\omega_1, \omega_2, T_{g1}, \text{and } T_{g2}\) are the weight fractions and Tg’s of components 1 and 2 of the blends respectively.

For semicrystalline polymers, blending can affect the Tm of the polymers, often resulting in a depression of the polymer melting point. Melting point depression in polymer blends can be due to either morphological effects such as size and perfection of the crystalline regions (Flory 1953a) or to thermodynamic effects. Blending can also affect the degree of crystallinity of the polymer because the temperature over which crystallization may occur has been changed due to changes in Tg and Tm (Runt and Martynowicz 1986).

Nishi and Wang (Nishi and Wang 1975) reported that the melting point depression observed in miscible blends of polymers in which one of the polymers was semicrystalline, was due to thermodynamic effects of the polymers. Miscibility of blended polymers arises from
specific interactions between the two constituent polymers (Lee et al. 1997). In a miscible blend containing one semicrystalline polymer, this interaction will lead to a lowering of the polymer melting point, due to a lowering of the ΔG of the melting event by a negative enthalpy of mixing term which occurs as the melting polymer chains interact with the amorphous diluent (Nishi and Wang 1975). This effect was measured by several authors for blends of poly(vinylidene fluoride) and poly(methyl methacrylate) (Nishi and Wang 1975), poly(ethylene terephthalate) and poly(butylene terephthalate) (Avramova 1995), poly(3-hydroxybutyrate) and poly(vinylidene chloride-co-acrylonitrile) (Lee et al. 1997), and polyethylene with a compatible paraffin oligomer (Martinez-Salazar et al. 1996). The melting point depression which resulted from thermodynamic effects could be differentiated from the morphology effects by measuring the depression in the Tm° of the solute polymer, since Tm° corrects for differences in Tm resulting from differences in crystal morphology (Nishi and Wang 1975).

Determination of equilibrium melting point depression has been most often measured using Hoffman-Weeks plots (Nishi and Wang 1975; Lee et al. 1997). Polymers were crystallized isothermally from the melt at different holding temperatures (Tc) and the melting points (Tm) of the crystals were measured. The Hoffman-Weeks equation was then used:

\[ T_m = \phi T_c + (1-\phi)T_m^o \]  

Equation 13

in which \( \phi \) is a stability parameter (Nishi and Wang 1975). A \( \phi \) value of 1 would reflect an inherently unstable crystal (\( T_m = T_c \)), while for \( \phi = 0 \) the crystals would be perfectly stable (\( T_m = T_m^o \)). From a plot of \( T_m \) against \( T_c \), the \( \phi \) and \( T_m^o \) can be calculated from the slope and y-intercept.

The depression in equilibrium melting point of a semicrystalline polymer, when blended with another non-crystallizing polymer, is represented by the equation:
\[
\frac{1}{T_{m^*}} - \frac{1}{T_{m^o}} = -\frac{RV_{2u}}{\Delta H_{2u} V_1 m_2} \left[ \ln \phi_2 \left( \frac{1}{m_2} - \frac{1}{m_1} \right) + (1 - \phi_2) + \chi_{12} (1 - \phi_2)^2 \right] \]

Equation 14

where \(T_{m^*}\) and \(T_{m^o}\) are the equilibrium melting points of blended polymer and the pure polymer respectively, the subscripts 1 and 2 refer to the amorphous (solvent) and semicrystalline (solute) polymers respectively, \(\Delta H_{2u}\) is the enthalpy of fusion per mole of repeat unit of crystalline polymer, \(V_u\) is the molar volume of the repeat units, \(m\) is the degree of polymerization, \(\phi\) is the volume fraction, \(\chi_{12}\) is the polymer-polymer interaction parameter, \(R\) is the gas constant and \(T\) is the absolute temperature. Depression of the equilibrium melting point of polymers in a blend indicates miscibility, and this miscibility is due to specific interactions between the two constituent polymers (Lee et al. 1997). This interaction is reflected in the \(\chi_{12}\) value for the two polymers. For two polymers to be miscible, \(\chi_{12}\) must be nearly zero or negative (Nishi and Wang 1975). Measurement of equilibrium melting point depression by blending has been used as a means of estimating \(\chi_{12}\) for polymers (Nishi and Wang 1975; Lee et al. 1997).

The Flory interaction parameter (\(\chi_{12}\)) is a quantity that gives some information about the interaction between materials. If two materials are characterized by a high \(\chi_{12}\) then energy would be required for interaction between them and the two materials will not be miscible. Conversely if \(\chi_{12}\) is low or negative, the materials will interact together and they will be miscible. It has been reported that for a material to be soluble in another, \(\chi_{12}\) should be less than 0.5 (Nishi and Wang 1975).

Equilibrium melting point depression data can be used to calculate \(\chi_{12}\) (Nishi and Wang 1975). Equation 14 can be rearranged under the assumption that \(m_1\) and \(m_2 \gg 1\) to give:

\[
\frac{1}{T_{m^*}} - \frac{1}{T_{m^o}} = -\frac{BV_{2u}}{\Delta H_{2u} T_{m^*}} (\phi_2)^2
\]

Equation 15
where B is the interaction energy density characteristic of a given polymer pair. It can be calculated from the slope of a plot of the difference in the reciprocal melting points against \((\phi_i)^2/Tm^*\), provided that \(V_2u\) and \(\Delta H_{2u}\) are known. The Flory interaction parameter can then be calculated using the equation:

\[ \chi_{12} = \frac{BV_{1u}}{RT} \]

at a temperature T.

Alternatively, individual chemical groups within a repeat unit of a polymer can be assigned a molar attraction constant. The sum of these constants divided by the molar volume of the polymer repeat unit is an estimate of the solubility parameter (\(\sigma\)) of the polymer (Martin 1993). The Flory interaction parameter can be calculated from \(\sigma\) by the equation:

\[ \chi_{12} = \frac{V}{RT} (\sigma_1 - \sigma_2)^2 \]

Equation 17

Calculation of \(\chi_{12}\) in this way does not account for specific interactions between the two different polymers and therefore cannot give a negative value for \(\chi_{12}\).

2.6 Crystallization of polymers and polyblends

2.6.1 General mechanisms of polymer crystallization

Sections of polymer chains can come together to form a stable crystal lattice. Factors which contribute to polymer crystallization have been identified and include: a lack of side groups or branching, rigidity of the backbone, regular monomer configuration and the ability to form hydrogen bonds or dipole interactions (Rosen 1993). The crystallization of polymer chains can occur from a polymer melt or from precipitation in a dilute solution, where the chain can take up all possible conformations in space (Hearle 1982).
The crystallization of polymers is different from crystallization of small molecules because of the constraints imposed by polymer atoms being joined in a chain. Events occurring on one portion of a chain have long range implications along the length of the chain. As a chain crystallizes, the portion of the chain which still lies in the amorphous region is affected because it is pulled and extended. This strain causes an increase in free energy which affects the overall thermodynamics of the crystallization process. In addition, thermal and stress gradients, nucleation and growth rate phenomena, memory in melt, impurities and short chains, as well as the influence of chain ends can all affect the crystallization process (Hearle 1982).

Crystallinity in a polymer sample can have a significant effect on the density, optical clarity, modulus and general mechanical response of the sample. A polymeric sample is rarely completely crystalline and the overall properties of the polymer will depend on the amount of crystalline order present in the sample (Cowie 1973).

In this work the crystallization of PCL from the melt is a critical parameter contributing to our understanding of the properties of the surgical paste formulation. The following description of polymer crystallization will therefore concentrate on crystallization from the melt.

2.6.1.1 Nucleation

Crystallization of polymer chains from the melt requires that a stable site, called a stable nucleus, exists onto which the chains can crystallize. The formation of these stable nuclei in a polymer melt is referred to as nucleation and is the first step in the crystallization of polymers. This process has been discussed in several publications (Cowie 1973; Hearle 1982; Wunderlich 1973; Wunderlich 1990).
Nucleation can be either homogeneous or heterogeneous. Homogeneous nucleation occurs as polymer chains from the melt become ordered in a parallel array and are held together by intermolecular forces. Polymer chains can then crystallize onto this nucleus and crystal growth can occur. The formation of homogeneous nuclei requires time, since several chains must come together in order to form a nucleus large enough to support crystal growth. At temperatures just below the melting point of the polymer (Tm), small crystals are not stable due to the high surface free energy of small crystals. As a result, stable, homogeneous nuclei do not form just below Tm but rather at lower temperatures where the small nuclei will be stable. That is, the degree of supercooling (ΔT) must be sufficient to allow homogeneous nucleation to take place. The number of homogeneous nuclei which develop over time is a function of the temperature at which crystallization takes place (Tc) and the viscosity of the melt (Wunderlich 1976).

Polymer chains in the melt can also crystallize onto sites such as impurities or remnants of crystalline polymer which had not been completely melted. This process is referred to as heterogeneous nucleation. Since heterogeneous nuclei are present in the melt and do not need to form, the number of stable nuclei present at a given Tc is not a function of time. Rather, the Tc under which the samples are held will define a minimum size of nucleus which is large enough to support crystal growth (Wunderlich 1990; Hearle 1982).

At a Tc just below Tm, a number of nuclei will be present in the sample which are large enough to initiate crystal growth. This number will not change over time as long as the temperature remains constant and is termed athermal nucleation. At a lower Tc, smaller nuclei will also be effective in initiating crystal growth, and so the number of crystals will be greater, but will not increase over time, so crystallization would still be athermal. At even lower
crystallization temperatures however, homogeneous nucleation becomes possible. In homogeneous nucleation, time is required for the stable nuclei to form, and so the number of effective nuclei that are present will increase as a function of time over which the polymer is held at Tc. This is called thermal nucleation. At very low Tc’s, the degree of supercooling is so great that homogeneous nucleation occurs simultaneously throughout the sample in an athermal process (Wunderlich 1990; Hearle 1982).

2.6.1.2 Crystal growth

Polymer crystals which grow from the melt grow in a completely different environment than those which grow from dilute solution. A crystallizing polymer molecule in a dilute solution will have time to fully incorporate itself into a crystal before another molecule arrives and nucleates. A polymer crystallizing from the melt however, will have in its immediate surroundings, other polymer molecules and not solvent. This will result in mutual interference of polymer chains in growing crystals, which in turn leads to the formation of complex three dimensional crystalline structures (Hearle 1982; Wunderlich 1973).

2.6.1.2.1 Lamellar structures

Chain folding occurs because crystallizing polymer chains are under the influence of competing driving forces. A stable crystal requires that interactions between and within polymer chains are maximized. For a long chain molecule to maximize these interactions as it attaches to a crystal face, whose length is several orders of magnitude smaller than the chain length, the chain must fold back on itself to attach to the growing face with as many segments as possible. After a chain attaches to the crystal in these lamellar structures the chain folds are not available for crystal growth. Further crystallization occurs on a single crystalline face and therefore the
polymer crystal grows as a needle shaped fibril. The end of a growing fibril represents the area on which crystallization can occur. It is not necessary that a crystal lamella will travel the entire width of the fibril. That is, there may be more than one distinct lamellae on the end of a growing fibril. A distinction can be made then between the interlamellar regions, which are between lamellae on the same fibril, and the interfibrillar regions which separate groups of lamellae. The relationship between interlamellar and interfibrillar regions is depicted in Figure 5A. Figure 5B shows a polymer chain crystallizing on the end of a growing fibril with a chain folding mechanism. Although each fold results in a greater number of interactions for the chain, it also necessarily results in a greater number of chain folds. These folds represent strain in the polymer chains, resulting in an increased surface energy, which serves to decrease crystal stability (Hearle 1982).

At a given crystallization temperature, an optimal lamellar thickness exists. Higher crystallization temperatures result in thicker crystal lamellae because the degree of supercooling is lower and the chains have more time to achieve more stable conformations (Hearle 1982). If the polymer is subsequently stored at a temperature above the crystallization temperature, the lamellae will refold to the thickness characteristic of the new temperature. This process whereby the crystallinity of the polymer is increased on storage is called annealing. Since the most stable conformation is one where the chains are completely extended and aligned with no folds present, the annealing process continues indefinitely, but at a steadily decreasing rate. For this reason the lamellar thickness of a given polymer is characteristic of the annealing temperature.
Figure 5: Polymer crystallization diagrams. A) Relationship between interlamellar and interfibrillar regions of crystallizing polymer. Crystal growth is depicted coming out of the page. B) Polymer chain crystallizing on the end of a growing fibril by a chain folding mechanism; C) Diagram of fibril branching leading to spherulite formation; D) Photographs of growing spherulites viewed between crossed polarizing lenses showing the Maltese Cross pattern. (Diagrams 5B to 5D adapted from: Hearle 1982)
2.6.1.2.2 Spherulites

Spherulites are characteristic of polymeric crystal growth and consist of a network of crystal fibrils radiating outwards from a central nucleus. They appear when the initial crystal fibril undergoes repeated branching which results in three dimensional growth, proceeding equally in all directions, such that the chain axis and fold direction are both perpendicular to the radius of the spherulite. Figure 5C shows how branching of the initial crystal fibril leads to spherulite formation. This branching must not show a bias for certain directions relative to the initial fibril, otherwise preferred growth would occur in certain directions and dendrite formation would result (Hearle 1982; Keith and Padden 1964a). Rather, a spherulite is characterized by uniform growth in all directions. It is usually found that a particular crystal axis lies parallel to the radius of the spherulite, which means that the crystal orientation at the growing faces is unrelated to the orientation of the original crystal. Therefore, a spherulite is not a single crystal but a polycrystalline aggregate which was formed from a single nucleus (Keith and Padden 1964a). The development of the three dimensional fibril structure is referred to as primary crystallization. However, amorphous polymer exists between the lamellae, in what is called the interlamellar region. Over time, crystallization of this amorphous polymer can occur and would result in the widening of the fibrils. This process is termed secondary crystallization (Keith and Padden 1963).

The reason for the branching is discussed in several publications (Hearle 1982; Keith and Padden 1963; Keith and Padden 1964a; Keith and Padden 1964b; Wunderlich 1973). The crystallization environment for polymer chains from the melt is usually relatively viscous. The process of crystallization involves the diffusion of polymer chains to, and nucleation on, the
growing crystal face. At the same time, impurities, which include any substance not able to crystallize on the growing crystal face, must diffuse away from the crystal front. If the viscosity of the melt is such that the impurities cannot diffuse away fast enough, they may be "overtaken" by the crystal front. This will cause a divergence of the crystal front and one crystal face will be split into two faces (Keith and Padden 1963). In addition to this direct effect, impurities can cause branching another way. During crystallization there is a balance which exists between the growth rate of the crystals and the diffusion of impurities or polymer away from, or to the crystal face. As fibril growth occurs, polymer chains leave the melt, which consists of a mix of polymer and impurity, and crystallize onto the fibril. As a result, the immediate environment surrounding the fibril becomes relatively rich in impurities and deficient in polymer. Regions of differing concentrations of polymer and impurities can be quite localized and can be smaller than the width of the fibril. When this occurs, crystallization on one portion of the crystal fibril may be preferred and the crystal growth rates may be unequal on two sides of the same crystal leading to branching.

In general, if fewer spherulites are formed they will be larger because they will be able to grow for a longer period of time before impinging upon a neighboring spherulite. Likewise, the presence of many nucleation sites will result in many smaller spherulites. If the number of initial nuclei is so large that impingement occurs right away, such that only the central portion of the spherulites can form, the resulting polymer will consist of small crystallites separated by disordered regions. This is a description of the fringed micelle model of polymer crystallinity (Hearle 1982).
2.6.1.2.3 Optical properties

The birefringence of the crystalline substructure of spherulites, allows for the study of crystallizing polymers between crossed polarizers using an optical microscope (Wunderlich 1973). Crystals which are birefringent will refract polarized light so that some of the refracted beam will pass through the second polarizer and will be visible. If the polymeric material does not refract light then complete extinction of light passing through the sample would occur when the sample is between crossed polarizers. This occurs in amorphous material or crystals with a cubic lattice. Complete extinction of the light would also occur if the crystals are aligned in such a way that the polarized light rays travel parallel to their optical axis and no birefringence occurs. The amount of rotation of light passing through a sample is proportional to the thickness of the sample. Therefore, certain thicknesses of sample exist which would rotate light at multiples of 180°, resulting in total extinction of light.

The crystallographic axes of growing fibrils in a spherulite are not fixed in space but are aligned relative to the spherulite radius and therefore the axes rotate about the center of the spherulite. This will result in four positions where complete extinction of light will occur, because the crystallites show no birefringence in these directions. On viewing these spherulites under the microscope, the resultant image will be that of a Maltese cross (Figure 5D). In some cases the growing fibrils twist as they grow outwardly from the center of the spherulite. This will cause the crystallographic axes to twist as well, which can sometimes lead to periodic zones of extinction which are viewed as a banding pattern with the microscope (Wunderlich 1973).
2.6.1.3 Crystallization kinetics

The crystallization rate of a polymeric material depends largely on the temperature of crystallization (Hearle 1982). As the Tc is decreased, the driving force for crystallization (ΔT) increases, but the mobility of the polymer chains decreases. As a result, a plot of crystallization rate against Tc would be a bell shaped curve with a maximum between Tg and Tm.

The kinetics of polymer crystallization are often described by using the Avrami equation to calculate the Avrami exponent (Cowie 1973):

$$\theta = e^{-kt'}$$

Equation 18

where θ is the fraction of the polymer still molten, k is a rate constant, and t is time. The Avrami exponent is designated as n and is an integer which provides information on the geometric form of polymer crystal growth. Polymer crystals may grow in one, two, or three dimensions as rods, discs or spherulites, respectively (Hearle 1982; Cowie 1973). The Avrami exponent represents the sum of the order of the rate process and the number of dimensions that the polymer crystal possesses. This equation is used for isothermal crystallization and is valid during the early part of the crystallization process before the growing crystals impinge on each other. The exponent is determined by plotting log(-ln θ) vs log t and calculating the slope (n) (Blackadder 1975).

In the case of non-isothermal crystallization, the Avrami exponent has been calculated by some authors using the Ozawa equation (Cazé et al. 1997; Gopakumar et al. 1997):

$$\ln\{-\ln[1-X(T)]\} = K(T) - n (\ln \alpha)$$

Equation 19

where X(T) is the volume fraction of the crystalline polymer at temperature T, K(T) is the cooling function, and α is the constant cooling rate.
2.6.2 PCL crystallization

Chatani et al. (Chatani et al. 1970) carried out a detailed X-ray study on the crystal structure of PCL. PCL crystals were described as having the PCL chains aligned parallel to each other, similar to polyethylene crystals. However, the molecules are slightly twisted and the carbonyl groups on two adjacent chains are offset. Generally, polyesters of the form [-\((\text{CH}_2)_z\text{CO-0}\)]_n crystallize with a monoclinic unit cell when \(z\) is even, but in an orthorhombic unit cell when \(z\) is odd. With PCL, \(z = 5\) and it crystallizes in an orthorhombic lattice of dimensions \(a = 7.47\ \text{Å}\), \(b = 4.98\ \text{Å}\), and \(c\) (fibril axis) = 17.05 Å. The three dimensional unit cell structure proposed for PCL was confirmed using electron diffraction studies by Hu and Dorset (Hu and Dorset 1990).

The effects of temperature, time and molecular weight on the crystallization of PCL was studied by Phillips et al. (Phillips et al. 1987; Phillips and Rensch 1989). They studied PCL samples of molecular weight 7000, 15 000, and 40 000 g/mole and found that the melting point increased with the molecular weight of PCL. In addition, the PCL was allowed to crystallize at various holding temperatures (Tc) between the range of 39° to 51° C. Below 39° C, the PCL samples would begin crystallizing while being quenched from the melt and above 51° C, the time needed for crystallization to occur was of the order of several days. Increasing the holding time of the PCL at Tc led to lamellar thickening, which was demonstrated by an increase in the melting point of the PCL crystals. Prior to impingement of spherulites, increasing the holding time did not affect the onset of PCL melting but did increase the Tm of PCL measured as the end of the melting endotherm. At longer times, spherulite impingement had occurred and both the onset and the end of the PCL melting endotherm occurred at higher temperatures as the holding times were increased. The thickness of the initial PCL crystals was about 24 repeat units long,
not counting the folds. A PCL sample with a higher molecular weight would have more folds initially.

In observing the crystallization of PCL under the microscope, banding in PCL spherulites was observed at low ΔTs (Phillips et al. 1987). Furthermore, the initial growth of PCL crystals was observed to be non spherulitic and was described as being acicular and "propeller like" in appearance. Eventually, branching of the fibres filled in the spaces and spherulites were formed. The initial growth rate of the acicular PCL crystals was faster than when the spherulites were fully formed and growing at a uniform rate (Phillips et al. 1987).

2.6.3 General mechanisms of polymer blend crystallization

Immiscible blends may be either microheterogeneous or macroheterogeneous. Microheterogeneous blends have separated phases. The domains of each phase are small (<1 μm) but can be visualized using electron microscopy (Mark et al. 1969). They may appear to have a single, broad Tg (Mark et al. 1969). Macroheterogeneous blends have distinct phase domains and are opaque, with two Tgs which may be shifted toward each other and are broader (Mark et al. 1969). Immiscible blends with two phases have either one phase dispersed within the other continuous phase, or contain two continuous phases in an interpenetrating network (Paul 1986).

The blending of miscible polymers can either increase or decrease Xc, overall crystallization rate, nucleation rate, and spherulite growth rate. This is because blending can affect the Tm°, Tg, and polymer diffusion, all of which can affect polymer crystallization.

In the case of polymer blends in which a semicrystalline polymer is blended with an amorphous polymer, the blend is completely miscible in the melt but phase separates on cooling.
as crystallization of the semicrystalline polymer occurs. The blend separates into distinct phases consisting of the crystalline phase, containing the semicrystalline polymer only, and the amorphous phase. The amorphous phase can be a homogeneous mixture of both polymers in the interlamellar regions, or it can be comprised of the pure amorphous polymer either between fibrils in the interfibrillar region, or between spherulites in the interspherulitic spaces (Defieuw et al. 1989a; Oudhuis 1994). Polymer chains which exit a crystalline lamella into the amorphous interlamellar region will have a greater free volume in the amorphous region than in the crystalline lamellae. Therefore, the chain density is greater in the lamellae than in the interlamellar space. As a result, only a fraction of the crystalline chains can proceed into the amorphous region in the form of loops or tie molecules, while the rest of the chains must reenter the lamellae as tight folds (Oudhuis et al. 1994). The presence of an amorphous polymer which is miscible with the semicrystalline polymer in the interlamellar region could lead to more perfect crystallites, since the free volume available for the semicrystalline polymer would be reduced, and an increase in the fraction of semicrystalline polymer chains undergoing tight folding would result (Oudhuis et al. 1994). The mechanical properties of the material may not be enhanced, however, since a reduction of tie molecules would also have occurred (Oudhuis et al. 1994). Lamellar thickness is inversely proportional to $\Delta T$ (Oudhuis et al. 1994). Therefore the thickness of lamellae will increase with the addition of amorphous polymer since the depression of melting point will reduce $\Delta T$ for any given $T_c$.

Crystal growth rates as a function of temperature show a maximum between $T_m$ and $T_g$ due to the competing contributions of $\Delta T$ and polymer viscosity (Sasaki et al. 1995). As a result, a blending process which affects $T_m^o$ and/or $T_g$ will affect the crystallization process of the
semicrystalline polymer. Changes in Tm° result in a change in ΔT which affects the nucleation and crystal growth rates.

Double melting peaks of a blended semicrystalline polymer with an amorphous polymer are often seen, due to the existence of amorphous polymer between crystalline lamellae. In this region, in which there is a relatively low semicrystalline polymer concentration, secondary crystallization takes place which shows a lower Tm. This could be due to either of the mechanisms described above including, thinner lamellae or thermodynamic melting point depression (Defieuw et al. 1989a; Defieuw et al. 1989c).

Polymer crystallite formation requires the formation of a critical nucleus on the growing crystal front, diffusion of polymer chains to the crystallization site, and the expulsion of non crystallizing components away from the crystallization front. The diffusion of polymers in the melt depends on several factors, including molecular weight, blend composition, interactions between dissimilar polymers, and the Tg of the polymers (Oudhuis et al. 1994). The Tg of the polymer near the growing front will be a function of the local composition of the blend at the growing front of the spherulite. The ability of the polymer chains to diffuse through the melt and crystallize will also be affected by the local polymer composition, since diffusion is critical for the crystallization process. The local Tg will be higher or lower than that of the pure crystalline polymer depending on the Tg of the amorphous polymer in the blend. As a polymer chain is incorporated into the crystal lamella, the local concentration of the semicrystalline polymer is decreased while the concentration of the amorphous component is enhanced. This will induce diffusion of both the semicrystalline and amorphous polymers from regions of high to low concentration. The diffusion distance and the extent to which the amorphous polymers are incorporated into the spherulite will be determined by growth and diffusion processes which are
in part dependent on $T_g$ (Oudhuis et al. 1994). The $T_g$ of the amorphous component is critical to the formation of polymer crystals in a blend, since it can inhibit or promote diffusion. For example, if the holding temperature for crystallization ($T_c$) was lower than the $T_g$ of the blend, then diffusion of chains would be frozen out and crystallization could not occur. Inhibition of crystallization of poly($\varepsilon$-caprolactone) by blending with poly(hydroxy ether of bisphenol A) was observed due to the high $T_g$ of the amorphous polymer (Defieuw et al. 1989c). Conversely, a lowering of the $T_g$ by the amorphous component in a blend with a semicrystalline polymer could enhance the diffusivity and mobility of the crystallizing chains possibly resulting in more perfect crystals (Oudhuis et al. 1994).

Crystallization from a phase separated blend in which the semicrystalline polymer is present in separate regions within the blend will result in spherulites of unequal sizes corresponding to the sizes of the individual regions of the semicrystalline polymer. The existence of spherulites of equal size is an indication of miscibility (Oudhuis et al. 1994). In miscible polymers, the spherulitic growth rate will be decreased in the presence of amorphous diluent due to the concentration of the amorphous component (Defieuw et al. 1989c).

The kinetics of crystallization in a blend have been described in the literature (Alfonso and Russell 1986; Sasaki et al. 1995). The kinetic analysis is based on the work of Turnbull and Fisher for the crystallization of pure polymer, which relates the rate of growth of the crystal $G$, to the work required to form a critical nucleus on the crystal surface ($\Delta F^*$), and by the energy needed to transport polymer segments across the solid-liquid interface ($\Delta E$) according to the equation:

$$G = G^0 e^{-\frac{\Delta E}{RT}} e^{-\frac{\Delta F^*}{k_B T}}$$

Equation 20
where $G^0$ is a constant, $T_0$ is the temperature at which motions necessary to transport segments across the liquid-solid interface cease, $T$ is the temperature of crystallization, and $k_B$ is the Boltzmann constant. This equation results in a bell shaped curve if growth rate is plotted against crystallization temperature and reflects the competing influences of $\Delta T$ and the diffusivity of the polymer chains. At low $\Delta T$ the crystallization process is nucleation controlled but at high $\Delta T$ values, the crystallization is diffusion controlled. Properties that affect the diffusion of the polymers, such as entanglements and branching will affect the kinetics of crystallization (Alfonso and Russell 1986; Braña et al. 1989; Shroff et al. 1996).

If a semicrystalline polymer is blended with a miscible amorphous polymer the equation becomes more complex (Alfonso and Russell 1986). Specific interactions between the polymer components in the liquid phase will alter the chemical potential of the liquid phase. This will alter the free energy necessary for the polymers to form a critical nucleus on the crystal surface and also the mobility of both the semicrystalline and amorphous polymers. Furthermore, as the crystal front advances, the non-crystallizing polymer must diffuse away to make room for the growing crystal. The ability of the crystal to grow must compete with the ability of the amorphous polymer to diffuse away. The slower of these two processes will dictate the kinetics of the process. The concentration of the semicrystalline component at the growth front will be decreased by an amount proportional to the volume fraction of the semicrystalline polymer. The $T_g$ of the amorphous polymer can alter the transport term associated with the solid-liquid interface. An equation for crystal growth rate that takes these considerations into account can be written as:

$$G_m = \frac{\phi_1 k_1 k_2}{k_1 + k_2} e^{-\Delta F^* / k_B T}, \quad \text{Equation 21}$$
where \( \phi_2 \) is the volume fraction of the semicrystalline component, \( T_c \) is the holding temperature for crystallization, \( k_1 \) is the rate of transport of the crystallizable segments across the liquid-solid interface, \( k_2 \) is the rate at which the amorphous component can be removed from the growth front, and \( \Delta F^*_{m} \) is the free energy of critical nucleus formation on the crystal surface modified by the presence of the amorphous component (Alfonso and Russell 1986).

### 2.6.4 Effect of blending on PCL crystallization

A number of studies have been conducted to study the crystallization of PCL when blended with different polymers. The morphology of PCL crystals and the kinetics of PCL crystallization when PCL was blended with other polymers differed, depending on the other polymer and whether this polymer was miscible with PCL, the polymer blend ratios, and the effect of blending on the physicochemical properties of the blend.

Blending PCL with an immiscible polymer would not be expected to affect the crystallization of the PCL since the PCL phase would exist in segregated domains. Blends of PCL with trimethylene carbonate were found to be immiscible since different blend compositions had no effect on the \( T_m \) and \( \Delta H \) of fusion of the PCL (Albertsson and Eklund 1994).

Reducing the chlorine content of chlorinated polyethylenes (CPE) reduces the miscibility of the CPE with PCL, resulting in phase separated blends (Defieuw et al. 1989a). Partially miscible blends of PCL with a CPE increased the spherulitic growth rate of PCL crystals within the PCL rich phase, compared to the growth rate of PCL crystals in a completely miscible PCL/CPE blend (Defieuw et al. 1989a).
PCL crystallization from blends with miscible polymers has been studied extensively. It has been found that the degree of crystallinity of PCL, normalized to the weight fraction of PCL in the blend, can be either increased or decreased with the addition of a blended polymer (Oudhuis et al. 1994). This was explained in terms of the change in Tg caused by the blending of the second polymer (Oudhuis et al. 1994; Defieuw et al. 1989b) or to bonding between the PCL and the second polymer (Zhong and Guo 1997). When the mobility of the PCL is restricted, either by raising the Tg above Tc, or by hydrogen bonding to the blended polymer, the ability of PCL to crystallize is diminished. If the Tg of the amorphous polymers is low enough that it does not cause the Tg of the blend to approach Tc, then it does not disturb the formation of lamellae and the degree of PCL crystallinity may be enhanced due to the reduction in the space available for amorphous PCL between lamellae (Oudhuis et al. 1994).

The growth rate of PCL spherulites is also affected by blending with miscible polymers. In a variety of PCL blending studies including PCL with poly(hydroxy ether of bisphenol A) (Defieuw et al. 1989c), CPE (Defieuw et al. 1989a), poly(vinyl chloride) (Ong and Price 1978b; Khambatta et al. 1976), and styrene-co-maleic anhydride (Defieuw et al. 1989b), the addition of the miscible polymer decreased the spherulitic growth rate of PCL crystals at a given temperature, Tc.

The morphology of PCL spherulites is also affected by blending. Pure PCL crystallizes as well defined spherulites which show a typical Maltese Cross birefringent pattern (Keith et al. 1989; Luyten et al. 1997). However, when blended with miscible polymers, the PCL spherulites can take on different morphological characteristics, which depend largely on the mobility of the blended amorphous polymer chains as PCL crystallization takes place. For example, some solution chlorinated polyethylenes, at high enough temperatures, are sufficiently mobile that
during PCL crystallization, the chains are rejected from the spherulite entirely and become segregated interspherulitically (Defieuw et al. 1989a). If the diffusion of the amorphous polymer chains is limited, then the chains will be segregated within the PCL spherulites. A common manifestation of this is the regular twisting of the PCL fibrils which leads to the appearance of concentric rings in the PCL spherulites when viewed under polarized light (Keith et al. 1989; Ong and Price 1978a). The width of these bands increases with the concentration of the amorphous polymer. This banding occurred even at low concentrations of amorphous polymer (<1%) (Keith et al. 1989) and was believed to be due to the adsorption of the amorphous polymer onto crystal boundaries, namely the growth face and fold surfaces.

Amorphous polymers which are segregated intraspherulitically can be interlamellar. This occurs when the diffusion of the polymer is retarded due to some interaction between the amorphous polymer and the PCL. This is reported to be the case for PCL blends with poly(hydroxy ether of bisphenol A) (Defieuw et al. 1989c), poly(vinyl methyl ether) and poly(styrene-acrylonitrile) (Oudhuis et al. 1994), and uncured novolac (Zhong and Guo 1997). Interlamellar segregation of the amorphous diluent can lead to an increase in the long spacing of the PCL crystals (Defieuw et al. 1989c).

Blends of PCL with polyvinyl chloride (PVC) have been extensively studied in the literature (Russell and Stein 1983; Ong and Price 1978a; Ong and Price 1978b; Khambatta et al. 1976; Keith et al. 1989). As PCL crystallizes, the PVC exists in the amorphous phase as a blend with PCL, between PCL lamellae. Ong and Price (Ong and Price 1978a) found that the blending of PVC with PCL did not alter the crystal lattice of the PCL as measured using X-ray analysis. They also quantitated the kinetics of the PCL crystallization using density analysis and measured an Avrami exponent of approximately 3 for blends of PCL and PVC (Ong and Price 1978b).
2.7 Mechanical properties of surgical paste

2.7.1 Mechanical properties of polymers

2.7.1.1 Viscosity

One important component in polymer characterization is the ability of the polymer to flow. This is measured by quantitating the resistance to flow, or viscosity, of the material (Rosen 1993). The two basic parameters which define viscosity are the shear stress (\(\tau\)) and shear rate (\(\gamma\)). Flow can be thought of as a plane of liquid moving over another plane. The difference in velocity, \(dv\), between two planes separated by an infinitesimal distance, \(dr\), is the shear rate, and the force required per unit area to bring about this flow is the shear stress. The viscosity of the material can then be defined as the shear stress divided by the shear rate:

\[
\eta = \frac{\tau}{\gamma}
\]

Equation 22

where \(\eta\) is the viscosity usually in units of poise (dyne s/cm² or g/cm/s).

In some materials, the viscosity is constant and independent of shear rate. A rheogram or a plot of shear stress versus shear rate, yields a straight line beginning at the origin, with a slope equal to the viscosity of the liquid. This type of flow behaviour is termed Newtonian flow (Martin 1993). Some materials exhibit what is called plastic flow. In this case, the materials behave elastically at low shear stress values, but above a yield value of stress, the materials flow at a constant viscosity. A rheogram for a plastic material would be a straight line which would not intersect the y-axis at the origin but rather at the yield value (Martin 1993).

In many materials, the viscosity is not independent of shear rate as it is in Newtonian systems. The viscosity of these materials depends on the shear rate. Materials whose viscosity decreases at increasing shear rates are called pseudoplastic, while materials whose viscosity
increases with increasing shear rate are called dilatant (Martin 1993). These systems can be characterized by using the equation:

\[ \psi^N = \eta' \gamma \]  

Equation 23

in which \( \eta' \) is the viscosity at shear rate \( \gamma \) and the exponent N can be used to characterize the system. A plot of \( \log \gamma \) vs \( \log \psi \) will result in a straight line of slope N and \( \gamma \)-intercept of \( \log \eta' \).

In Newtonian materials \( N = 1 \), for pseudoplastic materials \( N > 1 \), and for dilatant materials \( N < 1 \) (Martin 1993; Rosen 1993). Rheograms depicting the four types of flow behaviour as a function of shear rates are given in Figure 6A.

In polymeric systems at low shear rates, the materials behave in a Newtonian manner because the entanglements of the chain segments overcome any tendency toward molecular alignment. As the shear stress is increased, the polymer chains can begin to align and reduce the resistance to flow and the material exhibits pseudoplastic behaviour. At very high shear rates, the chains will theoretically be perfectly aligned and the resistance to flow will be constant at a minimum value and the material will once again exhibit Newtonian behaviour. This upper Newtonian region is difficult to see in practice, because the high shear rate conditions lead to mechanical breaking of the polymer chains and also because of the difficulty in maintaining constant temperature throughout the process (Rosen 1993).

Pseudoplastic behaviour is due to the loosening of polymer chain entanglements as a result of shear stress. When the stress is removed, the entanglements will reform but this process takes time. As a result, the viscosity of a pseudoplastic material may depend not only on the shear rate, but also on the shear history of the sample. Such materials are termed thixotropic (Rosen 1993; Martin 1993).
For given conditions of shear rate and shear stress, the viscosity of a material also depends on the temperature of the material. In general, for polymeric liquids, the viscosity decreases as the temperature (T) is raised and follows approximately an equation which is analogous to the Arrhenius equation:

$$\eta = Ae^{Ev/RT}$$  \hspace{1cm} \text{Equation 24}

in which A is a constant depending on the molecular weight and molar volume of the polymer, R is the gas constant, and Ev is an "activation energy" required to initiate flow between the polymer chains (Martin 1993).

### 2.7.1.2 Viscoelasticity

Polymers can neither be described as viscous fluids nor as elastic solids, but rather they are a combination of the two and are called viscoelastic (Rosen 1993). In contrast to pure viscous or elastic materials, the stress-strain properties of polymers are rate dependent. As a result, a stress-strain curve for polymeric materials must be constructed with time as a consideration.

Figure 6B shows the stress-strain curves of 3 types of materials. Curve A is of a material which exhibits a high initial modulus and fails at low strain values. Such materials are termed rigid and brittle. The term rigid, refers to the high initial modulus, which is the slope of the stress strain curve. It indicates that in response to stress, the material is not able to stretch and that at strain values of about 2%, the material fails. The area under the stress-strain curve, which represents the energy per unit volume required to cause failure, is relatively small compared to curves B and C and the material is called brittle.
Figure 6: A) Representations of rheograms for four materials which exhibit different flow properties as a function of shear rate. B) Representations of stress-strain curves for three different types of materials. a) Rigid, brittle b) Rigid, tough c) Flexible, tough plastic. Figure 6B adapted from: Rosen 1993.
Curve B represents a rigid and tough material. The high initial modulus is seen similar to curve A but instead of failing, the material reaches a yield point beyond which flow occurs. This flow serves to decrease the stress to a small degree and over time the strain is increased substantially. This ductile deformation of the material beyond the yield point imparts toughness on the material, since the area under the stress-strain curve is substantially greater than for the material depicted in curve A. The material exhibits two responses to the applied stress. The first is an instantaneous elastic deformation. Beyond the yield point however, the amount the material stretches depends on the length of time over which the stress is applied. This viscous flow is not recoverable. If the stress is removed before failure occurs, the material would not recover to its initial state but would show only partial recovery, corresponding to the initial elastic strain.

Curve C represents flexible and tough plastics. In this case the materials show a low initial modulus and so are not very stiff and deform readily in response to stress. If the stress is maintained beyond the yield point, these materials stretch to between (400-600%). This stretching is usually achieved as the material converts from one of low crystallinity to high crystallinity (Rosen 1993).

As seen in curves B and C in Figure 6B, the sample strain increased without changes in stress. This phenomenon is called creep and reflects the polymer's ability to stretch over time under a given stress, as the polymer chains are able to slip past each other and flow. Mathematically, the strain in the material (\( \delta \)) is a function of the load (L) and the time under constant load (t) or \( \delta = f(L,t) \). Upon removal of the load, the material will undergo some recovery but the strain which was due to viscous flow will not be recovered (Hearle 1982).
If the strain in the material is held constant, the converse of creep, namely stress relaxation can be observed. In this case, the load, or stress, will be a function of the strain and the time under constant strain, or \( L = f(\delta, t) \) where \( t \) is the time under constant strain. The stress may decay to zero or to a constant value. If the sample is released, only partial instantaneous recovery may be seen, with some permanent deformation observed (Hearle 1982).

Time dependence may also be observed if the rate of strain or stress is changed. That is, if \( L = f(\delta, d\delta/dt) \) or \( L = f(\delta, dL/dt) \). In general, increasing the strain rate will increase the initial modulus of the material because the entangled chains do not have time to slip past each other and the material seems stiffer.

When the stress on materials is removed, the material can undergo instantaneous recovery, followed by time dependent recovery, which may or may not return the material to its original form. If the load is reapplied during the time dependent recovery phase, the material will behave differently than it did when the load was first applied. Therefore the history of the sample is important in determining the mechanical behaviour of the sample. Dynamic tests can be conducted, whereby the material is subjected to repeated cycles of stress or strain. Material fatigue can occur when the material does not have time to fully recover from the previous load before the next load is imposed. After repeated challenge, the cumulative effect of the loads will cause the material to fail despite the fact that an isolated challenge may not have altered the material at all, had it had the time to fully recover (Van Krevelen 1976).

The temperature of the sample also influences mechanical behavior. A polymer sample will behave as a brittle solid below its \( T_g \), as a viscoelastic solid between \( T_g \) and \( T_m \), and it will undergo viscous flow above its \( T_m \). That is, the type of mechanical response seen with a
polymer depends on the temperature of testing and not on the polymer type (Hearle 1982; Rosen 1993).

2.7.2 Mechanical properties of blends

The tensile strength of immiscible blends may lie between the strengths of the individual components, or it may be lower than both because of poor interfacial adhesion between the phases (Paul 1986). Specific hydrogen bonding interactions have been shown, in one instance, to improve the mechanical properties of the blend but decrease the $X_c$ of the semicrystalline polymer (Lee et al. 1996).

2.8 Drug release from controlled release polymeric drug delivery systems

2.8.1 Erosion controlled release

Drug release can also occur as a result of the erosion of the polymer matrix if the rate of polymer erosion is faster than the rate of drug diffusion through the polymer. Polymer erosion can occur by cross-link cleavage, ionization of pendant groups and backbone cleavage (Langer and Peppas 1981; Hadgraft and Guy 1987). This erosion controlled mechanism was shown to play a part in the release of gentamycin from poly(d,l-lactide) cylinders (Zhang et al. 1994). Holland et al. reviewed the use of biodegradable polymers for the controlled release of macromolecules. One of the mechanisms for macromolecule release was through the erosion of the polymer matrix (Holland and Tighe 1986).

2.8.2 Diffusion controlled release

Drug release from implanted polymers occurs across a concentration gradient from within the polymer implant to the outside aqueous tissue fluids. The mechanisms by which the drug is
released depends on the nature of the drug and polymer. Higuchi (Higuchi 1961) described drug release from polymers based on diffusion of drug through the polymer down the concentration gradient. When diffusion is slow, due to large drug molecules or hydrophobic interactions between the drug and polymer, the release of drug may take place through an alternate mechanism.

The polymer may take up water from the aqueous external environment, which will cause the polymer to swell and allow the drug to diffuse out (Baker 1987). In some cases, water uptake by the polymer results in the formation of water channels within the polymer matrix. This in effect increases the surface area available for drug release and drug which dissolves in the water can be carried to the external environment. As drug is removed, the water channels expand to fill the space and new drug is exposed to the aqueous environment (Baker 1987).

The formation of channels and the diffusion of drug through the polymer matrix can be affected by the physicochemical properties of the polymer matrix. A process which decreases the free volume of the matrix, for example, an increase in crystallinity or the addition of an insoluble material into the matrix, will serve to decrease the permeability of the drug (Vandamme and Mukendi 1996a; Pitt et al. 1979b). Permeability of the drug or water within the polymer can be enhanced through processes which promote diffusion. These would include a decrease in Tg, which could be achieved through blending or copolymerization of other polymers, or by the addition of water soluble components which can dissolve out of the matrix when it is in an aqueous environment, leaving spaces in the matrix (Pitt et al. 1979b).
2.8.2.1 Monolithic solution

The drug is dissolved and uniformly dispersed in the polymer matrix. Release is achieved through diffusion and the release rate of drug is determined by the loading of drug (El-Arini et al. 1995), the nature of the polymer, and the geometry of the device (Higuchi 1963; Fu et al. 1976). A disadvantage of these systems is that release rates decline over time (Baker 1987).

2.8.2.2 Monolithic dispersion

The drug is distributed throughout the polymer at loadings above the solubility limit of the drug in the polymer. As a result, the drug is present in the polymer as a saturated solution and as solid particles. There are three types of monolithic dispersions depending on the volume fraction of drug in the polymer (Baker 1987; Hadgraft and Guy 1987).

At low drug loading levels (0-5%) the drug first dissolves in the polymer then diffuses to the surface of the device for release. This is termed a "simple monolithic dispersion". This model has been described by Higuchi who showed that drug release from polymers is directly proportional to the square root of time and depends on drug loading, the geometry of the device, the solubility of the drug in the polymer, and the diffusivity of the drug through the polymer. (Higuchi 1961; Higuchi 1963) At slightly higher drug loading levels (5-10%) cavities left at the surface of the polymer from the loss of drug are filled with fluid from the external environment and serve as preferred pathways for the release of drug from the polymer. The effect is that the overall apparent permeability of the drug through the polymer is increased. This is termed a "complex monolithic dispersion" (Baker 1987). When the drug loading in the polymer exceeds 20%, the fluid channels resulting from the loss of drug from the polymer become interconnected and form a continuous channel to the surface of the polymer. The main mechanism for drug
release is by diffusion through these channels and the rate of drug release is the solubility and diffusivity of the drug in the fluid filling the channels. This is termed a “monolithic matrix system” or simply “matrix system” (Baker 1987). This model of release through interconnecting pores has been shown by Bawa et al. (Bawa et al. 1985) to be the mechanism by which macromolecules are released from ethylene-vinyl acetate copolymer devices.

2.8.2.3 Mathematical models of drug release

Drug release from an ointment base containing a fine distribution of solid drug, into a perfect sink, has been described by Higuchi (Higuchi 1961) using the following equation:

\[ Q = \sqrt{Dt(2A - C_s)C_s} \]  
Equation 25

where \( Q \) is the amount of drug released after time \( t \) per unit exposed area, \( D \) is the diffusivity of the drug in the homogeneous matrix, \( A \) is the total amount of drug present in the matrix per unit volume, and \( C_s \) is the solubility of the drug in the matrix. According to this equation, if \( C_s \ll A \), the amount of drug released is proportional to the square root of time, diffusivity, drug concentration, and drug solubility.

In systems where the drug is dispersed as particles in the matrix and release of the drug is accompanied by the formation of water channels in the polymer, through which the drug may diffuse, then the equation must be modified. This is because the effective volume available for drug diffusion is reduced by the presence of pores and a tortuosity factor is also employed to account for lateral drift experienced by drug particles making their way through channels in the matrix (Higuchi 1963). The modified equation can be written as:

\[ Q = \sqrt{\frac{D\epsilon}{\tau}(2A - \epsilon C_s)C_s t} \]  
Equation 26
where $\tau$ is the tortuosity factor of the capillary system and $\varepsilon$ is the porosity of the matrix, which is the volume fraction of the overall matrix which is still available for diffusion and dissolution of drug. As a result, the diffusivity and solubility terms must be decreased by this factor. This equation is said to be valid on the assumptions that the drug particles are small relative to the diffusion distance and that $A > \varepsilon C_s$ by a factor of at least 3 or 4.

As drug is released from the system, the porosity changes and can be described by the following equation:

$$\varepsilon = \varepsilon_0 + KA$$

Equation 27

where $\varepsilon_0$ is the initial porosity and $K$ is a factor introduced to convert $A$ to its volume fraction and is equal to the specific volume of the drug. For the instances where the initial porosity is very small or where the fraction of the matrix volume occupied by drug is very large, $\varepsilon$ is approximately equal to $KA$ and substituting into equation 26 gives:

$$\frac{Q}{A} = \left(\frac{DK}{\tau}(2-KC_s)C_s t\right)^{1/2}$$

Equation 28

which means that the fraction of drug released at any time is independent of the drug concentration (Higuchi 1963).

In order to use the Higuchi equation to accurately predict drug release, an accurate determination of porosity and tortuosity must be made, and these factors are heavily dependent on processing variables (Desai et al. 1965). A simpler model to describe drug release has been given as:

$$Q/A = kt^n$$

Equation 29

where $k$ is a constant and $n$ is the diffusional exponent characteristic of the release mechanism (El-Arini and Leuenberger 1995; Peppas 1985; Ritger and Peppas 1987). Assuming one
dimensional release of drug from thin slabs under sink conditions, \( n = 0.5 \) for a diffusion release system. This analysis only applies to the initial fraction of drug release \( (Q/A < 0.6) \). The diffusion exponent depends on the geometry of the device, taking on values of 0.45 and 0.43 for cylindrical and spherical devices respectively (Ritger and Peppas 1987). Drug release controlled by factors other than diffusion will result in values for \( n > 0.5 \) up to a value of 1 which would be indicative of zero order release. To account for the lag time \( (l) \) in the time course of drug release, the term \( (t-l) \) has been substituted into the above equation in place of \( t \) (Ford et al. 1991).

3. PROJECT SPECIFIC AIMS

1. Characterize thermal properties of PCL:MePEG blends and paclitaxel loaded PCL:MePEG.
2. Characterize the PCL crystallization within PCL:MePEG blends and paclitaxel loaded PCL:MePEG.
3. Characterize mechanical properties of PCL:MePEG blends and paclitaxel loaded PCL:MePEG.
4. Determine the effect of time and storage condition on the surgical paste.
5. Measure and characterize the \textit{in vitro} release of paclitaxel from PCL:MePEG 80:20 surgical paste.
6. Determine if paclitaxel released from surgical paste has antiangiogenic activity.
7. Measure the biodistribution of paclitaxel in mice following subcutaneous injection of a paclitaxel loaded PCL:MePEG 80:20 pellet.
4. EXPERIMENTAL

4.1 Chemicals and Reagents

Paclitaxel was supplied by Hauser Chemicals, Boulder, CO. Radiolabelled paclitaxel ($^3$H-paclitaxel) was obtained in an ethanol solution with an activity of 1 mCi per mL from Amersham Life Science (Arlington Heights, IL). Poly($\varepsilon$-caprolactone) (PCL) was purchased from Polysciences (Warrington, PA.) and was labelled to be of molecular weight between 10k and 20k g/mole. Methoxypolyethylene glycol (MePEG), molecular weight 350, was from Union Carbide (Danbury, CT.).

Acetonitrile (ACN), methanol, dichloromethane (DCM), and chloroform were all HPLC grade from Fisher Scientific (Napean, Ontario, Canada). All buffers were made using deionized distilled water (d-$H_2O$).

Phosphate buffered saline (PBS), pH 7.4, was made by dissolving 8.22 g NaCl, 0.315 g sodium dihydrogen orthophosphate, 2.15 g sodium phosphate (Fisher Scientific, Fairlawn, NJ.), and 0.4 g albumin (Boeringer Mannheim, Germany) in 1 L d-$H_2O$.

Halothane and Vetbond were supplied by the Animal care facility and Ready Organic Scintillation Cocktail was from Beckman, Inc.

Nitrogen and prepurified helium gas were supplied by Praxair, Burnaby, BC, Canada.

4.2 Equipment

Water bath, Magni Whirl constant temperature bath from Blue M Electric Company (Blue Island, IL.)
Camera, Contax 167 MT (Tokyo, Japan)

Tissue Homogenizer, Polytron, model PT-2000 (Kinematica, Switzerland)

Plastic scintillation vials, 7 mL capacity, Fisher Scientific

Balances, Mettler Corporation models AE 163, AJ 100, and PJ 300

Hot plate/stirrer, Corning Corporation model PC-351

Centrifuges, Beckman GS-6 Centrifuge (Palo Alto, CA.) and an Eppendorf Centrifuge model 5415 C

Ovens, Thelco oven, Precision Scientific Company (Chicago, IL.)

Shel Lab oven, Johns Scientific Company (Portland, OR.)

Napco vacuum oven, Model 5831, Precision Scientific Company (Chicago, IL.)

Micro-centrifuge tubes, 1.5 mL capacity tubes with caps, Elkay Products (Shrewsbury, MA)

Pipettes, variable volume Pipetman from Gilson Company

4.3 Experimental Methods

4.3.1 Initial screening of polymers

Prior to selection of MePEG (molecular weight, 350) for blending with PCL, test blends of PCL with MePEG (M.W. 350, 550, 750), PEG (M.W. 200, 1500 and 8000) and propylene glycol were prepared.

4.3.2 Manufacture of surgical paste

Paclitaxel was blended with molten PCL samples at 60° C and loading levels of 1 to 30% paclitaxel. When MePEG was used in the formulation, the paclitaxel was first
mixed into the MePEG using a spatula, before this mix was incorporated into molten PCL. The PCL:MePEG ratio was 80:20. The molten pastes were allowed to cool to a solid at room temperature. Surgical paste samples were stored in glass screw cap vials at room temperature.

4.3.3 Solubility of paclitaxel

Four 1 mg samples of paclitaxel were placed in 50 mL Erlenmeyer flasks. The powder was dispersed in 5 mL of pre warmed d-H$_2$O, PBS buffer or PBS buffer with albumin by vortexing for 10 seconds prior to the addition of 45 mL of the appropriate dissolution medium at 37° C. Immediately after the addition of dissolution medium the first sample was taken (t = 0). Flasks were then placed in a 37° C water bath with circular shaking at a rate of 60 rpm. Various samples were taken from t = 0 to up to 26.5 hours and analyzed using liquid chromatography - mass spectroscopy.

When paclitaxel was suspended in d-H$_2$O, samples were filtered though 0.2 μm Acrodisc CR PTFE filters and diluted 1:1 with ACN prior to injection. When paclitaxel was suspended in PBS buffer (with or without albumin) a different extraction procedure had to be used because of the presence of the buffer salts and albumin. Samples were filtered as above. Into each 1 mL sample was added 4 mL of DCM. Vials were capped, shaken and centrifuged at 600 x g for 10 minutes to separate the layers. A glass pipette was used to remove 3 mL from the DCM layer. This was dried down under nitrogen and reconstituted with 0.5 mL of ACN:d-H$_2$O 1:1. The injection volume used was 20 μL, injected into a mobile phase of 40% d-H$_2$O and 60% ACN (with 2 mmol ammonium acetate to improve ionization), flowing at 150 μL/min. The column used was a Hewlett
Packard ODS Hypersil (C-18) column with dimensions of 5μm x 100 mm x 2.1 mm. Analysis was performed using a Fisons Quatro MS/MS which monitored the parent ion at 852 amu through the first MS. This was collisionally dissociated to a daughter ion at 521.1 amu to produce a peak. The dwell time was 0.5 seconds and the collision energy was 40 eV with a cone voltage of 22 V. The instrument was monitoring for negative ions.

Quantitation was achieved by integrating the paclitaxel peak and calculating the concentration based on the standard curve obtained from paclitaxel in ACN:d-H₂O 1:1 solutions at paclitaxel concentrations between 0.313 and 5 μg/mL. Storage of paclitaxel samples in ACN:d-H₂O 1:1 overnight in the freezer (-20° C) was found to have no effect on the resulting peak area for paclitaxel.

4.3.4 Formulation characterization

4.3.4.1 Density determination

The density of PCL, and a PCL:MePEG 80:20 blend were measured in triplicate using a Multi Pycnometer (Quantachrome, Syosset, NY).

4.3.4.2 Ternary solution test

Miscibility of PCL and MePEG was tested using the ternary solution test. Solutions of PCL in DCM and MePEG in DCM were made up at concentrations of 20% each. Five mL of each solution were mixed together by vortexing for 1.5 minutes in a 15 mL Kimax glass test tube with a PTFE lined screw cap. The tube was stored at room temperature and observed for phase separation over 72 hours.
4.3.4.3 X-Ray diffraction

Studies used a Rigaku Geigerflex X-Ray Diffractometer System (Rigaku Corporation, Tokyo, Japan) with a biplanar goniometer, interfaced with a Dexton PCII 286 computer by a D/max-B controller. Samples were exposed to Ni-filtered Cu Kα radiation at scanning rates of between 1 and 4° 2θ over a range of 4 to 35° 2θ, unless otherwise noted. The X-ray tube was operated at a potential of 37 kV and a current of 17 mA.

Powdered samples were packed into aluminum sample holders backed with a glass slide. Polymers were melted into sample holders in a 60° C oven and allowed to cool. Alternatively, a thin film of melted polymer was spread on a glass slide which was fixed on top of the aluminum sample holders with double sided scotch tape and allowed to cool.

X-ray powder diffraction (XRPD) patterns for paclitaxel were measured from 4 to 35° 2θ using a scan rate of 2° 2θ per minute using a tube voltage of 37 kV and 17 mA.

The thin film method was used to obtain the X-ray patterns of PCL and PCL:MePEG 80:20. Samples of PCL were melted at 60° C and allowed to crystallize at either 37° C, ambient temperature, or -20° C, and the X-ray patterns for each were obtained. Scans were from 5 to 55° 2θ at a rate of 2° 2θ per minute.

Paclitaxel was incorporated into PCL at concentrations of 2.4%, 20%, 42.3%, and 58.8%. Paclitaxel was also incorporated at 20% in PCL:MePEG 80:20. These were analyzed using the thin film method from 5 to 55° 2θ at 2° 2θ per minute.
4.3.4.4 Differential scanning calorimetry

Differential scanning calorimetry (DSC) was done using a Pyris 1 DSC (Perkin Elmer Corp., Norwalk, CT) which was cooled with liquid nitrogen using a Perkin Elmer Cryofill. The purge gas was prepurified helium (Praxair, Burnaby, BC, Canada) at a pressure of 20 psi. Unless stated otherwise, the samples were between 2 and 15 mg in crimped, but not hermetically sealed, aluminum pans (Rheometrics Scientific) using an empty pan as a reference. Results were analyzed using Perkin Elmer Pyris data analysis software.

4.3.4.4.1 Effect of sample size and scanning rate

Samples of PCL weighing 1.16, 5.91, 7.74, 9.82 and 11.65 mg were placed in DSC sample pans and analyzed using DSC. Samples were held at 90° C to eliminate thermal history. Samples were then programmed to go through cooling and heating cycles which consisted of cooling to 0° C at 40° per minute, isothermal hold for 3 minutes, then heating to 90° C followed by 10 minute hold at 90° C. The scan rate of the heating curves was increased with each cycle repetition and included values from 10 to 100° per minute.

4.3.4.4.2 Quenched samples

Blends of PCL:MePEG at PCL concentrations of 0, 10, 20, and 40% PCL were manufactured. Samples of about 10 mg (accurately weighed) were melted at 100° C for 10 min before quench cooling at 500° per minute to -100° C, where they were held isothermally for 3 minutes before heating at 40° per minute to 100° C.
4.3.4.4.3 Effect of different blend ratios

Blends of PCL:MePEG ranging from 0 to 100% PCL in increments of 10% were manufactured by blending at 60° C. Three samples from each blend composition were analyzed using DSC with average sample weights of 10.5 ± 1.1 mg (range of 8.2 to 13.2 mg). Each sample was held molten at 80° C for 10 minutes to eliminate thermal history, then cooled to -100° C at a rate of 40° per minute, held for 5 minutes and heated to 80° C at a rate of 40° per minute.

From these thermograms, the crystallization temperatures, Tg's and Tm's of the PCL phase in the blends, as a function of MePEG concentration were determined.

The effect of 20% paclitaxel loading in PCL and PCL:MePEG 80:20 on the Tg of the blends and on the melting endotherms for both MePEG and PCL was determined. Accurately weighed samples of about 10 mg were analyzed at scanning rates of 40° per minute.

4.3.4.4.4 Melting point depression

The effect of MePEG on the equilibrium melt temperature of PCL (Tm°) was investigated. Surgical pastes were manufactured by blending MePEG with PCL at concentrations of 0, 10, 20, and 30% MePEG. In addition, a paste consisting of 20% paclitaxel in PCL:MePEG 80:20 was also tested. Samples of about 5 mg (accurately weighed) were held isothermally at 80° C to eliminate thermal history. The samples were then cooled at 50° per minute to one of several predetermined holding temperatures (Tc) below Tm, where they were allowed to crystallize over time. Following crystallization, the samples were heated at 10° per minute through the melting transition. The Tm of the
PCL melting peak, taken as the peak of the melt transition, was determined. The Tm° of each blend was calculated by plotting Tm vs Tc for each blend to obtain the stability parameter and Tm° from the slope and y-intercept of each blend. For each blend, the Tm° of the PCL melting peak was determined in triplicate. The hold temperatures (Tc) used were from 25° C to 40° C in 3° intervals for all blends except 100% PCL. In the case of 100% PCL, the holding temperatures (Tc) used were from 35° to 45° C in 2° intervals.

4.3.4.5 Hot stage microscopy

A Mettler FP II hot stage was used to heat samples while viewing under the microscope. Samples were also held isothermally at temperatures above room temperature. An Olympus BH 2 stereo microscope (Japan) was used to view samples at magnifications of 40, 100 or 400X. The microscope had one polarizing lens in the light path between the sample and the eye pieces. Placing an additional polarizing lens below the sample, gave images of the sample between crossed polarizers. A micrometer was etched directly into one of the eye pieces to enable direct visual measurement of length.

Samples of PCL, PCL:MePEG 80:20, and 20% paclitaxel in PCL:MePEG 80:20 were observed using a hot stage microscope (magnification 50X) as they were heated from 30° C through the melting transition, at a rate of 10° C per minute. The temperatures at which the onset and completion of melting occurred, were recorded. A minimum of three scans were performed for each sample.

A sample of 20% paclitaxel in PCL:MePEG 80:20 was heated from 37° to 205° C at a rate of 10° per minute using the hot stage apparatus. Photographs were taken at
various points during the heating process and the temperatures at which melting began and was completed, were recorded.

A sample of PCL:MePEG 60:40 was analyzed on the hot stage in a similar manner except that a heating rate of 2°C per minute was used in addition to 10°C per minute. PCL:MePEG 20:80 blends were melted at 100°C and allowed to cool in the hot stage to ambient temperature. Photographic images were taken as the PCL crystals were forming.

4.3.4.6 Gel permeation chromatography

Gel permeation chromatography (GPC) was carried out at ambient temperatures using a Shimadzu LC-10AD HPLC (Kyoto, Japan) with either a 10^3 or 10^4 Å PLgel column and a Shimadzu RID-6A refractive index detector (Kyoto, Japan) using a detection cell temperature of 40°C. The mobile phase was chloroform flowing at 1 mL/min. The samples were 30 μL of a 0.2% solution of polymer in chloroform.

Polystyrene standards with known intrinsic viscosities ([η]) were used to generate a universal calibration curve which was constructed by plotting the log of the product of the inherent viscosity and molecular weight of each standard against the retention time. Polystyrene standards were from Pressure Chemical Company, Pittsburgh, PA, and spanned a range of molecular weights from 300 to 50 000 g/mole.

The molecular weights of PCL, MePEG and PCL:MePEG 80:20 blends were determined by GPC. The intrinsic viscosity [η] of PCL was determined using a Cannon-Fenske type viscometer (size 25) at 25°C ± 1°C. The relative viscosities of PCL solutions in chloroform, at concentrations of between 0.3 and 0.4% was measured, and the
approximation method of Solomon and Ciuta was used to convert this data into $[\eta]$ according to the equation:

$$[\eta] = \frac{\sqrt{2}}{c_x} \cdot \frac{t_x}{t_o} - 1 - \ln \left( \frac{t_x}{t_o} \right)$$

Equation 30

where $t_x$ is the time required for a solution in chloroform with concentration $c_x$ to flow through a viscometer and $t_o$ is the time for chloroform alone (Solomon and Ciuta 1962).

Molecular weight measurements for MePEG are given relative to polystyrene. All measurements were done in triplicate.

4.3.4.7 Mechanical testing

4.3.4.7.1 Viscometry

A Parallel Plate Rheometer (Rheometrics System IV, Rheometrics Inc., Piscataway, NJ) was used to measure the complex viscosity of polymer samples at 60° C. Polymer samples 1 mm thick, were placed between two parallel circular plates with a diameter of 25 mm. The lower plate was fixed but the upper plate was oscillated at different angular velocities while the instrument measured shear stress on the sample. Measurements were taken over a shear rate range of 0.1 to 100 rad/sec.

The complex viscosities of PCL:MePEG blends containing between 0% and 30% MePEG were measured on the parallel plate viscometer at 60° C and strain rates of between 0.1 and 100 rad/sec.
4.3.4.7.2 Strength testing

A CT-40 mechanical strength tester (Nottingham, England) interfaced with an Apple II Plus computer was used to measure the effect of MePEG on the strength of PCL. PCL containing 0%, 5%, 10%, or 20% MePEG (n = 3 at each blend ratio) were melted and cast as tablets with a diameter of 0.88 cm and an average thickness of 0.56 cm. Single variable ANOVA indicated that there was no statistically significant difference in the average thickness of the tablets at each blend ratio. Each sample tablet was placed on its edge between the two instrument platens, a lower platen which was stationary, and an upper platen which applied a downward force on the sample. The instrument measured displacement of the upper platen and applied force as a function of time, and from these data, the tensile strengths of the samples were calculated.

4.3.4.8 Scanning electron microscopy

Scanning electron micrographs were taken of samples mounted onto metal studs using double sided conductive tape and coated with a 100 Å film of gold-palladium (60:40) using a Hummer Sputter Coater. Images were taken using a Hitachi SEM (S-2300, Tokyo, Japan) with a 20 kV beam at magnifications from 100 to 10 000 X and downloaded to a computer using Quartz PCI image management system software.

Scanning electron microscope images were taken of surgical paste samples of PCL, PCL:MePEG 80:20 and 20% paclitaxel in PCL:MePEG 80:20.
4.3.4.9 Swelling study of PCL:MePEG paste

Samples of PCL containing between 0 and 20% MePEG were heated to 60° C and cast in moulds to produce paste cylinders with diameters of 7.8 mm and height 3.9 ± 0.4 mm. The cylinders were stored at 37° C in 10 mL of 10 mM PBS buffer with albumin (0.4 g/mL). At various times, the cylinders were removed from the buffer, blotted dry, weighed, and returned to the buffer. Percent weight change over time was recorded, as was the percent volume change in each cylinder at the completion of the experiment. When the samples reached constant weight, they were placed in a silica gel desiccator under vacuum, to dry to constant weight. The difference between sample weights before and after desiccation was taken as water loss from the polymer.

SEM images were taken of the interior of PCL and PCL:MePEG 80:20 pellets which had been swelled in PBS then dried in a silica gel vacuum desiccator to constant weight.

4.3.4.10 PCL crystallization in blends

4.3.4.10.1 Effect of MePEG on growth rate of PCL spherulites

The effect of MePEG on the growth rate of PCL spherulites was measured by manufacturing PCL:MePEG blends at PCL concentrations of 50%, 60%, 70%, 80%, 90%, and 100%. For each blend, a 10 mg sample was placed on a glass microscope slide with a coverslip and melted on the hot stage at 80° C for 5 minutes prior to transfer to the microscope stage at ambient temperature. These slides were observed between polarizing lenses through the microscope. Following nucleation, the growth rate of PCL spherulites
was measured using a stopwatch to time the growth of PCL crystals across micrometer divisions. From this, a growth rate of PCL spherulites was estimated. A minimum of 3 measurements were made for each blend ratio.

4.3.4.10.2 Microscopy of isothermal PCL crystallization in blends

Samples of PCL:MePEG blends containing between 10% and 100% PCL in increments of 10% PCL were made. For optical microscopy, a 10 mg sample of each blend was melted on a microscope slide with a coverslip at 85°C for 30 minutes. The samples were then placed in a 60°C oven for 15 minutes before being transferred to a 37°C oven for at least 2 hours to allow crystallization to take place. The resulting PCL crystals were photographed under microscope between polarized lenses at magnifications of 40, 100 and 400 X.

The effect of paclitaxel incorporation on the PCL crystals in PCL:MePEG 80:20 was investigated by manufacturing 1% and 20% loaded paclitaxel in PCL:MePEG 80:20 surgical pastes. About 10 mg of each sample was placed on a microscope slide, covered with a coverslip, and melted on a hot plate, prior to transfer to a 60°C oven for 15 minutes. Samples were then allowed to crystallize overnight in a 37°C oven. The resulting crystals were photographed under the microscope between crossed polarizing lenses at magnifications of 40, 100, and 400 X.

Scanning electron micrographs of the PCL:MePEG blends were taken by melting 10 mg of each sample on a SEM sample stub at 85°C for 30 minutes prior to transfer to a 60°C oven for 15 minutes. The samples were then transferred to a 37°C oven where the
blends were allowed to crystallize as a thin film on the stubs over at least 2 hours. The samples were then coated and analyzed at magnifications between 100 and 5000 X.

4.3.4.10.3 Crystallization kinetics

Blends of PCL:MePEG at concentrations of 0%, 10%, 20%, and 30% MePEG as well as 20% paclitaxel in PCL:MePEG 80:20 were manufactured and samples of about 5 mg (accurately weighed) were loaded into DSC pans. Samples were analyzed in triplicate. Samples were melted at 90° C for 10 minutes to eliminate thermal history. Samples were then cooled at 50° C per minute to 37° C where they were held isothermally and the heat flow in the samples was monitored over time. As the samples crystallized, an exothermic peak was recorded on the DSC. The total area of this peak was taken to correspond to 100% crystallization. The degree of PCL crystallization could be obtained by calculating the area under the curve for the crystallization of the PCL phase and normalizing it for the weight fraction of PCL in the blend. At any time point, the extent of crystallization could be calculated by dividing the area under the curve at that time, by the total area of the crystallization exotherm. Plots of the percent PCL crystallization versus time could thus be obtained. The data were then analyzed to give times at which 1, 5, 10, 15, 20, 25, 30, 35 and 40% crystallization were achieved. Avrami plots were constructed and the Avrami exponent was equal to the slope of the plots.
4.3.4.11 Degradation of surgical paste in buffer over time

Samples of PCL, PCL:MePEG 80:20, and 20% paclitaxel loaded PCL:MePEG 80:20, were manufactured and stored in PBS buffer containing sodium azide (1 g/L) at 37° C. About 3 mg spheres of sample were weighed and placed in 1.5 mL Eppendorf micro centrifuge tubes with 1 mL of buffer.

Analysis of samples was performed on freshly prepared samples (t = 0), and at 3, 6, 9 and 13 weeks following preparation. Changes in the MGPC of the samples were measured over time using GPC analysis as described above, using polystyrene standards. Four samples were measured for each sample at each time point.

4.3.4.12 Ageing study of surgical paste at different storage temperatures

Samples of PCL, PCL:MePEG 80:20, and 20% paclitaxel loaded PCL:MePEG 80:20, were manufactured and divided into four different batches. Three batches were stored dry at temperatures of 4°, 25°, and 37° C while the fourth was stored in PBS buffer containing sodium azide (1 g/L) at 37° C. The samples were stored as spherical pellets of about 3 mg each. In the case of the samples that were stored dry the pellets were produced by dropping molten paste onto glass slides. The samples stored in buffer were weighed and placed in 1.5 mL Eppendorf micro centrifuge tubes with 1 mL of buffer.

Analysis of samples was performed on freshly prepared samples (t = 0), and at 3, 6, 9 and 13 weeks following preparation. Samples were analyzed by DSC and by SEM. DSC analysis was carried out by cooling the samples to -110° C, holding for 5 minutes, then heating to 80° C at a rate of 40° C per minute. The Tg, and melt temperatures of the
polymers as well as the ΔH of fusion of PCL were measured. Four replicates were analyzed for each sample at each time point.

4.3.5 *In vitro* release studies

The high performance liquid chromatography (HPLC) system used was from Waters Corporation (Milford, MA) and consisted of a 600S controller, 717 autosampler, and 486 tunable absorbance detector which was set at 232 nm. Data analysis was performed using either a 746 data module (integrator) or Waters Millennium Software. The column was a Waters Nova-Pak C\textsubscript{18} column (3.9 x 150 mm) and the mobile phase consisted of a 58:5:37 mixture of ACN:MeOH:d-H\textsubscript{2}O which was premixed and filtered under vacuum using Millipore 0.45 μm HV filters (Millipore Corporation, Bedford, MA). The flow rate was 1 mL/min and the injection volume used was 20 μL, unless stated otherwise.

Unless otherwise noted, the *in vitro* release studies were performed in the following manner. Paclitaxel loaded paste samples were placed in glass Kimax tubes with PTFE lined plastic screw caps. The samples were suspended in 15 mL PBS buffer containing albumin 0.4 mg/L. The tubes were tumbled end over end in a 37° C oven at a rate of about 10 revolutions per minute. At various sampling times, 13 mL of supernatant were transferred to a second tube for analysis. Fresh buffer was replaced in the sampling tube to maintain sink conditions and the tube was placed back in the oven. Paclitaxel content in the supernatant was analyzed by pipetting 1 mL of DCM through the buffer and shaking for 10 seconds to extract the paclitaxel. The aqueous buffer was discarded and the organic phase was dried under N\textsubscript{2} gas at 60° C. The paclitaxel was reconstituted
in 1 mL of ACN:d-H$_2$O by vortexing for 30 seconds. This was injected into the HPLC for analysis. Quantitation was achieved by comparing the paclitaxel peak area obtained with standard curves of paclitaxel which had been constructed in ACN:d-H$_2$O 60:40 at concentrations of between 0.1 µg/mL and 20 µg/mL.

4.3.5.1 Effect of paclitaxel loading

The time course of paclitaxel release from surgical paste samples of PCL and PCL:MePEG 80:20 was investigated at 37°C at paclitaxel loadings of 1, 5, 10, 20 and 30%. The paste sample weights were approximately 2.5 mg (accurately weighed) with 4 tubes being measured for each sample.

4.3.5.2 Effect of MePEG loading

Paclitaxel release from 5 mg pellets containing 20% paclitaxel in blends of PCL and MePEG containing either 0, 5, 10, 20, or 30% MePEG was measured as described (4 tubes for each batch).

4.3.5.3 Long term release study

Paclitaxel release from 20% loaded 2.5 mg pellets of either PCL or PCL:MePEG 80:20 was measured in vitro for 90 days using the method described (4 tubes for each batch).

At the termination of the study, the residual amount of paclitaxel remaining in each pellet was measured by dissolving remaining pellets in 1 mL of DCM, drying under nitrogen and reconstituting in 60:40 ACN:d-H$_2$O and vortexing for 30 seconds. Samples were transferred to HPLC vials for analysis of paclitaxel content.
4.3.5.4 Effect of sterilization

Samples of 20% paclitaxel in PCL and in PCL:MePEG 80:20 were manufactured. The sample was split into two batches. One batch was sterilized using 30 KGy gamma irradiation from a $^{60}$Co source (Nordion International, Kanata, Ontario) while the other batch was not sterilized. An in vitro release study was conducted, comparing the release of paclitaxel from 2.5 mg pellets from the irradiated samples with those from the non sterilized samples (4 tubes for each batch).

4.3.6 Validation of paclitaxel assays in mouse tissues

4.3.6.1 HPLC assay

4.3.6.1.1 Specificity, Stability, and Recovery

Five C3H mice were sacrificed and the following tissues were harvested: blood, liver, lung, spleen and kidney and were stored at -20° C until analysis. Five samples of each tissue type were available for testing. Tissues were weighed, then spiked with paclitaxel, from a 200 µg/mL solution in ACN, at levels of 0, 5, 50 and 150 µg/g of tissue. The fifth tissue sample of each type was spiked with 50 µg/g paclitaxel and stored at -20° C for 18 days. Recovery was measured by analyzing each tissue for paclitaxel and comparing the values obtained with those for direct injections of 5, 50, or 150 µg paclitaxel made up to 3 mL in ACN.

Each tissue was placed in a 7 mL plastic scintillation vial and ACN was added to make a total volume of 3 mL. The tissues were homogenized using a Polytron and 1 mL of homogenate was transferred to 1.5 mL Eppendorf tubes for centrifugation at 15000 x g
for 5 minutes. The supernatants were transferred to HPLC vials for direct injection. The HPLC system was as described above except that the injection volume was 30 μL and the mobile phase was 50:50 ACN:d-H₂O at a flow rate of 1.5 mL/minute.

4.3.6.1.2 Precision, Accuracy, Linearity, Range and Limit of Detection

Forty female 5-month old C3H/HeN mice were anesthetized with a 100 μL Rompom® i.p. injection. The blood was removed through a syringe inserted directly into the heart. The blood samples (about 0.5 mL each) were stored untreated in pre-weighed 7 mL plastic scintillation vials and stored at -20° C.

The validation was conducted over 3 days. On each day, four standard curves were constructed by spiking blood samples with paclitaxel at levels of 0, 1, 10, 25, 50, 100, and 150 μg/g blood. The tubes were analyzed as described in Section 4.3.6.1.1 except that the flow rate was reduced to 1 mL/min and the volume of ACN added to each tissue sample was reduced to 2 mL. For each sample, a corrected value for the peak area was obtained, by dividing the peak area by the weight of the blood used and the volume of ACN added. The precision of the assay was evaluated by calculating the average value and % coefficient of variation (%CV) of the corrected peak areas obtained at each spike level both within one day (n = 4) and overall (3 days, n = 4 each day). The ruggedness of the assay was determined by performing an ANOVA on the precision data to test for statistically significant interday variation. To determine the accuracy and linearity of the assay, regression equations were calculated for each standard curve on each day. Linearity was satisfied if standard curves, which gave acceptable accuracy in measuring paclitaxel content of samples, had high r² values when fitted with linear regression
equations. The accuracy was measured by using one standard curve from each day as a standard curve for all the other samples run on that day. Three predicted values were obtained at each spiked concentration on each day. The average predicted values, %CVs, and bias could then be calculated within each day and overall. The ruggedness was again assessed using an ANOVA on the predicted values to test for interday variation.

The limit of detection for paclitaxel using this HPLC assay was calculated by injecting decreasing volumes of a 2 µg/mL paclitaxel in ACN solution into the HPLC until the signal to noise ratio obtained was less than 5.

### 4.3.6.2 \(^3\)H-paclitaxel assay

Samples containing radiolabelled drug were analyzed on a Beckman LS 6000 TA Scintillation Counter which registered counts per minute of tritium with a two minute analysis time.

A quantity of 200 µL of stock radioactive paclitaxel solution was made up to 10 mL with ACN to give a solution containing 2 µCi per 100 µL. This solution was used to make solutions containing 0.0001, 0.001, 0.01, 0.1, 1 and 2 µCi per 100 µL.

Four month old, female C3H mice weighing approximately 20 g were purchased through University Hospital and, following sacrifice in CO\(_2\) atmosphere, 1 mL of blood was removed by syringe from the heart and the following tissues were harvested: heart, lung, liver, spleen, kidney, and about 100 mg of muscle tissue. Tissues were placed individually in 7 ml plastic scintillation vials and were subsequently spiked with 100 µL standard \(^3\)H-paclitaxel (0.0001 to 1 µCi). The sampling method used was to add 2 mL ACN to each sample prior to homogenization using a Polytron. The homogenate was
transferred to 1.5 mL micro centrifuge tubes and centrifuged at 15000 x g for 5 minutes. Following centrifugation, 1 mL of supernatant was transferred to a clean scintillation vial and 5 mL of Ready Organic Liquid Scintillation Cocktail was added, prior to analysis on a Beckman LS 6000 TA Scintillation Counter.

To analyze the paclitaxel content of polymer, 100 mg of surgical paste (no drug) were spiked with 100 μL of one of the $^3$H-paclitaxel standards in scintillation vials. The spiked samples were dissolved with 0.5 mL of DCM and then diluted with 5 mL of Ready Organic Liquid Scintillation Cocktail before analysis.

The percent recovery was calculated by spiking clean empty scintillation tubes with 100 μL of a standard $^3$H-paclitaxel solution and adding 5 mL of Ready Organic Liquid Scintillation Cocktail. These tubes were read on the scintillation counter (n = 3) and the results were taken to be the value expected for 100 % recovery.

4.3.7 In vivo evaluation of formulation

4.3.7.1 CAM Assay

This study was reviewed and approved by the UBC Committee on Animal Care, protocol number A94-1633. This assay was performed as described by Dugan et al. (Dugan et al. 1991) with some modifications. Fertilized, domestic chicken eggs from Fitzsimmons Consulting and Research Services (Surrey, BC, Canada) were incubated (Humidaire model 21 incubator, New Madison, OH.) for four days prior to shell-less culturing. The egg contents were incubated at 37° C, 90% relative humidity and 3% CO$_2$ for 2 days (Forma Scientific model 3158 incubator, Marietta, OH.). On day 6 of
incubation, 3 mg aliquots of paste sample were placed directly on the CAM surface. After a 2 day exposure, the vasculature was examined using a stereomicroscope fitted with a Contax 35 mm camera. Antiangiogenic activity was defined as the presence of an avascular zone measuring at least 4 mm in diameter surrounding the paste pellet.

The samples tested on the CAM included PCL only (n=30), paclitaxel loaded PCL paste at loadings of between 0.05 and 20% (1.5 to 600 µg paclitaxel), and 20% loaded PCL:MePEG 80:20 (n=6).

4.3.7.2 Pilot study of paclitaxel distribution in mice following implantation of surgical paste

Fifteen female C3H mice between 42 and 56 days old were purchased through UBC Hospital. On day zero, the mice were anesthetized by placing them in a glass jar with gauze containing a few drops of Metofane. The mice were weighed, shaved behind the neck and injected with either control polymer (PCL:MePEG 80:20) or 20% paclitaxel loaded surgical paste subcutaneously in the back of the neck from syringes which were warmed to 55° C in a water bath and fitted with an 18G luer-lok needle. The injection sites were sealed with Vetbond® following the procedure. The amount of polymer injected was determined by weighing the syringes before and after injections were made.

At time points of 0.25, 2 and 17 days mice were sacrificed with CO₂ and blood, heart, lung, liver, spleen, kidney, and about 100 mg of muscle tissue were harvested along with the paste pellet. A total of nine mice were treated. Organs and tissues were analyzed for paclitaxel content using the HPLC assay described above.
The concentration of paclitaxel in the polymer pellets removed from the mice was measured using an HPLC assay. Three pieces of paclitaxel loaded polymer (between 1.7 and 7.2 mg) were analyzed for paclitaxel concentration from each sample of surgical paste isolated from mice. Each piece was analyzed in a separate tube by dissolving it in 2 mL of DCM and then adding 30 mL of ACN:d-H$_2$O 60:40. This results in a 2 phase mixture consisting of 17 mL of an ACN/paclitaxel/DCM phase and an aqueous phase containing trace amounts of paclitaxel.

Paclitaxel in the organic phase was measured by pipetting 100 µL into an HPLC vial containing 900 µL of ACN:d-H2O 60:40 (10 fold dilution), while paclitaxel in the aqueous phase was measured by direct injection. The injection volume was 20 µL and was quantitated using a standard curve constructed with paclitaxel in ACN:d-H$_2$O 60:40. The amounts of paclitaxel in each phase were added together and divided by the weight of the polymer fragment to determine the paclitaxel concentration.

In addition the pellets from mice 1-1, 1-5 and 2-4 were analyzed using SEM, GPC, and DSC.

4.3.7.3 Paclitaxel distribution study in mice following implantation of surgical paste

4.3.7.3.1 Preparation of paste

The control paste was made by blending 8.0 g PCL and 2.0 g MePEG at 60° C. The radioactive paste was prepared by blending 1.6 g MePEG with 2.0 g cold paclitaxel and 200 µL of stock $^3$H-paclitaxel (200 µCi). Into this mix was blended 7.8 g PCL at 60°. Both blends were loaded into 1 mL Luer-lok syringes.
4.3.7.3.2 Biodistribution study

This study was reviewed and approved by the UBC Committee on Animal Care, protocol number A95-0035. Four month old female C3H mice weighing about 20 g were ordered and housed at the Jack Bell Research Center. Mice were anesthetized with halothane, ear tagged, weighed, shaved behind the neck and injected with 125 mg of either control or paclitaxel loaded paste through an 18G needle. Some mice were pierced in the back of the neck but no polymer was injected (sham treatment group). Polymer syringes were pre heated to 60° C in a water bath to melt the polymer and allow injection. Injection wounds were sealed with Vetbond®.

At each time point (0.25, 2, 15, 30 days) 4 sham mice, 6 control and 10 treated mice were anesthetized with halothane, sacrificed by cervical dislocation, and weighed. The tissues, which included whole blood, lung, liver, spleen, kidney, muscle tissue from adjacent to paste pellet, as well as the paste pellet were harvested from each mouse and were placed in pre-weighed scintillation vials for analysis.

Prior to analysis, vials were weighed and then analyzed using the same method as in the validation section above. Quantification was achieved by making fresh standard curves using tissues spiked with radioactive paclitaxel using the method described above.
5. RESULTS

5.1 Solubility of paclitaxel

Three LCMS-MS standard curves and the corresponding regression equations for paclitaxel in ACN:H₂O 1:1 are shown in Figure 7. Coefficients of determination exceeded 0.994 for all three curves. The three standard curves were obtained at different times during the chromatographic analysis of paclitaxel in order to account for changes in the sensitivity of the LCMS-MS assay over time. Each standard curve was obtained by injection of the same set of standard solutions. The first standard curve was obtained immediately prior to analysis of the first sample. The second was obtained after half of the samples had been assayed (4 hours) and the third standard curve was obtained immediately after the last sample had been analyzed (8 hours).

The concentration of paclitaxel in d-H₂O at 37° C was found to rise to a peak of 3.6 ± 0.4 μg/mL in 3 hours before declining to an equilibrium value of 1.4 ± 0.9 μg/mL by 20 hours.

The dissolution profiles of paclitaxel in PBS buffer (± albumin) at 37° C are shown in Figure 8. In the case of paclitaxel dissolution in PBS buffer without albumin, the concentration reached a peak of 1.0 ± 0.3 μg/mL in 2.5 hours before declining to a value of 0.2 ± 0.1 μg/mL by 25 hours. The addition of albumin to the PBS resulted in the concentration of paclitaxel increasing to an equilibrium value of 1.4 ± 0.3 μg/mL at 2.5 hours.
Regression analysis parameters: $y = mx + b$, $r^2$

0 hours: $y = 26\ 526x + 6851$, $r^2 = 0.997$
4 hours: $y = 24\ 964x + 7661$, $r^2 = 0.998$
8 hours: $y = 24\ 362x + 10\ 033$, $r^2 = 0.994$

Figure 7: Standard curves of paclitaxel in acetonitrile-water 50:50. LCMS-MS parameters: 20 μL injection on a C$_{18}$ Hypersil column with a mobile phase of 2 mmol/L ammonium acetate in acetonitrile:water 60:40 flowing at 150 μL/min.
Figure 8: Dissolution profile at 37°C of paclitaxel in phosphate buffered saline, pH 7.4, with or without albumin (0.4 g/L). Samples were measured using LCMS-MS and are reported as the mean ± 1 S.D. of 4 samples.
5.2 Formulation characterization

5.2.1 Ternary solution test

No phase separation was observed following the mixing the of a 20% PCL in DCM solution with a 20% MePEG in DCM solution at ambient temperature which indicates that PCL and MePEG were miscible.

5.2.2 X-Ray diffraction patterns of paclitaxel and surgical paste

The X-ray diffraction data for PCL, and PCL:MePEG 80:20 from 5 to 35° 2θ are given in Figure 9. The X-ray diffraction pattern produced by PCL showed peaks between 20 and 25° 2θ indicating that the polymer is semicrystalline. Blending 20% MePEG into PCL did not change the pattern. No peaks were observed between 35° to 55° 2θ.

Samples of PCL were melted at 60° C and allowed to crystallize at either 37° C, ambient temperature, or -20° C. The different crystallization temperatures did not result in alterations to the X-ray patterns obtained. Therefore the temperature of crystallization from the melt does not appear to result in the formation of different crystal types and there is no evidence of polymorphic forms of PCL under these conditions.

Figure 10 shows the XRPD scans of as received paclitaxel, and paclitaxel loaded PCL and PCL:MePEG 80:20 which had been prepared by melting the polymer at 60° C and mixing in the paclitaxel with a spatula. The presence of paclitaxel peaks in the XRPD scans indicates that the drug is present in the crystalline form within the polymer matrix. At loadings of 2.4% (data not shown) paclitaxel in PCL, the X-ray scans did not show paclitaxel peaks but solid drug was visible in the sample as clumps of opaque material dispersed within the thin polymer film.
Tables 1 and 2 give the peak locations, d-spacings, and relative intensities of the major peaks in the XRPD scans of PCL, PCL:MePEG 80:20, and paclitaxel.

5.2.3 Thermal properties

5.2.3.1 Effect of sample size and heating rate on thermal properties and the peak melt temperature

The effect of heating rate on the onset of melting of PCL is shown in Table 3 for a 1.16 mg sample of PCL. At a scan rate of 20° per minute, the melting endotherm of PCL consisted of a double peak. Increasing the heating rate resulted in the elimination of the double melting peak. The onset of melting was lowered from 47.8° C to 44.6° C, while no change in the peak of the melting endotherm was observed with increasing heating rates from 20° to 100° per minute.

As the sample size was increased from 1.16 mg to 11.65 mg, the peak melting temperature increased from 49.9 ° to 55.2° C on heating and decreased from 22.2°C to 16.6° C on cooling when a heating/cooling rate of 40°/min was used. Sample size had no effect on the temperature of the onset of melting. As a result of these findings, similar sample sizes were used thereafter within each set of experiments.
Figure 9: Representative XRPD scans from 5° to 35° 2θ of PCL or PCL:MePEG 80:20.
Figure 10: Representative XRPD scans from 5° to 35° 2θ of paclitaxel and paclitaxel loaded surgical paste samples of PCL or PCL:MePEG 80:20.
Table 1: X-ray diffraction peak locations, d-spacings, and relative intensities of PCL and PCL:MePEG 80:20 surgical paste

<table>
<thead>
<tr>
<th>Peak location (°20)</th>
<th>d-spacings (Å)</th>
<th>I (rel)</th>
<th>Peak location (°20)</th>
<th>d-spacings (Å)</th>
<th>I (rel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.1</td>
<td>4.21</td>
<td>100</td>
<td>21.4</td>
<td>4.14</td>
<td>100</td>
</tr>
<tr>
<td>21.7</td>
<td>4.09</td>
<td>15.6</td>
<td>22.1</td>
<td>4.02</td>
<td>18.0</td>
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<tr>
<td>23.4</td>
<td>3.80</td>
<td>24.7</td>
<td>23.8</td>
<td>3.74</td>
<td>37.8</td>
</tr>
</tbody>
</table>

Table 2: X-ray diffraction peak locations, d-spacings, and relative intensities of paclitaxel (as received)

<table>
<thead>
<tr>
<th>Peak location (°20)</th>
<th>d-spacings (Å)</th>
<th>Intensity (rel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4</td>
<td>16.20</td>
<td>100</td>
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<tr>
<td>8.8</td>
<td>10.04</td>
<td>58.7</td>
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<tr>
<td>11.1</td>
<td>7.96</td>
<td>26.8</td>
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<tr>
<td>12.2</td>
<td>7.22</td>
<td>78.0</td>
</tr>
<tr>
<td>13.7</td>
<td>6.44</td>
<td>16.0</td>
</tr>
<tr>
<td>15.4</td>
<td>5.73</td>
<td>24.6</td>
</tr>
<tr>
<td>16.6</td>
<td>5.34</td>
<td>20.4</td>
</tr>
<tr>
<td>16.9</td>
<td>5.24</td>
<td>25.9</td>
</tr>
<tr>
<td>17.8</td>
<td>4.98</td>
<td>20.1</td>
</tr>
<tr>
<td>21.8</td>
<td>4.07</td>
<td>20.2</td>
</tr>
</tbody>
</table>
Table 3: Effect of DSC scan rate on the melting point of a 1.16 mg sample of PCL

<table>
<thead>
<tr>
<th>Heating rate (°C/min)</th>
<th>Tm&lt;sub&gt;onset&lt;/sub&gt; (°C)</th>
<th>Tm&lt;sub&gt;peak&lt;/sub&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>47.8</td>
<td>a</td>
</tr>
<tr>
<td>40</td>
<td>46.9</td>
<td>49.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>46.0</td>
<td>50.0</td>
</tr>
<tr>
<td>80</td>
<td>45.2</td>
<td>51.0</td>
</tr>
<tr>
<td>100</td>
<td>44.6</td>
<td>51.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> double melting peak (50.1° and 53.8° C)

<sup>b</sup> melting endotherm had a distinct shoulder
5.2.3.2 Effect of quench cooling on thermal properties

Representative DSC thermograms of quenched blends of PCL:MePEG are shown in Figure 11 for PCL concentrations of 0, 10, 20, 40, and 100% w/w. The thermogram of 100% MePEG is characterized by a glass transition (Tg) at -97° C followed by a recrystallization peak at -70° C. The MePEG then melts as a broad endotherm, displaying double peaks, between -60° and 0° C. The thermogram of the 100% PCL sample had a Tg at -70° C, although it is not clearly visible on the scale used for Figure 11, and a melting endotherm between 45° and 60° C which contained a double peak. Thermograms of PCL:MePEG blends are characterized by Tg’s in a range between -94° and -99° C, which are close to the Tg of 100% MePEG, followed by a recrystallization and melting of the MePEG component between -70° C and 0° C. The melting of PCL crystallites was observed between 30° and 55° C. No Tg corresponding to amorphous PCL was detected, although it may have been masked by the melting endotherm of MePEG.

5.2.3.3 Thermal properties of blends of PCL:MePEG

Representative cooling and heating DSC thermograms of the different PCL:MePEG blend ratios from 0 to 100% PCL are shown in Figure 12A and 12B respectively. As the 100% PCL sample was cooled, an exothermic peak corresponding to the crystallization of PCL was seen between 35° C and 5° C. The MePEG sample also showed a crystallization exotherm but this occurred between -20° and -80° C. Thermograms of PCL:MePEG blends resulted in crystallization exotherms for each component on cooling as first PCL, then MePEG crystallized. The peak temperatures of these exotherms are listed in Table 4.
Following a 5 minute hold at -100° C, the PCL:MePEG blends were heated at 40° per minute and representative thermograms are shown in Figure 12B. The melting of the PCL crystallites is demonstrated by the melting endotherm between 40° and 60° C. The 100% MePEG sample is characterized by the double endotherm due to the melting of the MePEG crystallites between -60° and 0° C. Blends containing both PCL and MePEG showed individual melting endotherms for the MePEG and PCL crystallites present in each sample. As the concentration of PCL in the blends was decreased the shape of the corresponding melting endotherms became a double peak. The effect that the blend ratios had on the melting points of the individual components is summarized in Table 5.

The Tg values reported in Table 5 are not clearly visible in Figure 12B because of the y-axis scale used. In Figure 13, representative thermograms of PCL:MePEG blends are shown with an expanded scale to illustrate the Tg regions. The glass transition on DSC is seen as a change in the baseline of the thermogram, and in Figure 13 the arrows point to the location of the onset of the glass transition. The top thermogram is that of 100% PCL and the onset of the Tg can be seen at about -72° C. The bottom thermogram is that of 100% MePEG and shows the glass transition beginning at about -95° C, which is followed by a recrystallization exotherm beginning at about -70° C. Blends of PCL and MePEG show Tg’s which are intermediate between those of 100% PCL and 100% MePEG.

The temperatures corresponding to the onset of Tg observed for each blend are plotted in Figure 14 as a function of blend composition. Theoretical Tg values were calculated using the Fox equation (Equation 12) for each blend concentration and are included in Figure 14 for comparison as the dotted line.
The melting point data for MePEG crystals as a function of blend composition, given in Table 5, show no evidence of an effect of PCL concentration on the melting point of the MePEG crystals (p = 0.53, single variable ANOVA). On cooling, the temperature at which MePEG crystallized decreased slightly with increasing PCL content in the blend as shown in Table 4.

The addition of increasing amounts of MePEG to PCL:MePEG blends reduced the melting point of PCL, taken as the end of the melting endotherm, from 59° C (0% MePEG) to 51° C (90% MePEG) as shown in Table 5. On cooling, a reduction in the crystallization temperature of PCL was also seen with the addition of MePEG from 19° to 6.4° C as the MePEG fraction was increased from 0% to 90% MePEG (Table 4). Since there were reductions in both Tm and Tc at each blend composition there was no statistical difference (p > 0.16, single variable ANOVA) in the degree of supercooling, which is calculated using the formula, Tm - Tc, and represents the driving force of crystallization, as a function of blend composition (Figure 15).

The heat of fusion (ΔHf) of PCL crystals was measured to be 69.2 ± 2.8 J/g. This corresponds to a degree of crystallinity of 49.6%, based on a ΔHf value of 139 J/g for a 100% crystalline sample of PCL (Pitt 1990). The area of the PCL melting peak, as the percentage of MePEG was increased in the blend, decreased in proportion to the decrease of the PCL weight percentage in the blend (Figure 16). The constant ΔHf per gram of PCL in the blend, indicated that the degree of crystallinity of PCL did not change with the presence of MePEG.

On cooling, the MePEG also crystallized as a separate phase. The ΔHf per gram of MePEG decreased linearly with MePEG concentration (Figure 17) indicating that the presence of PCL did not affect the crystallinity of the MePEG phase.
Figure 11: Representative DSC thermograms showing the effect of different blend ratios of PCL:MePEG on samples which had been quench cooled from the melt at 500° per minute and then heated at 40° per minute.
Figure 12A: Representative DSC thermograms showing the effect of different blend ratios of PCL:MePEG which were cooled from the melt at 40° per minute.
Figure 12B: Representative DSC thermograms showing the effect of different blend ratios of PCL:MePEG which were cooled to -100° C, then heated at 40° per minute.
Table 4: Thermal data for PCL and MePEG blends cooled from the melt using DSC, at a cooling rate of 40°C/min. Data represent the mean of three samples ± 1 S.D.

<table>
<thead>
<tr>
<th>PCL:MePEG</th>
<th>Peak of crystallization exotherm (°C)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MePEG</td>
<td>PCL</td>
<td></td>
</tr>
<tr>
<td>100:0</td>
<td>n/a</td>
<td>18.8 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>90:10</td>
<td>-55.6 ± 2.4</td>
<td>17.5 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>80:20</td>
<td>-48.1 ± 9.6</td>
<td>16.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>70:30</td>
<td>-35.4 ± 4.1</td>
<td>14.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>60:40</td>
<td>-35.6 ± 4.8</td>
<td>14.8 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>50:50</td>
<td>-37.4 ± 5.2</td>
<td>13.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>40:60</td>
<td>-33.2 ± 2.0</td>
<td>11.0 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>30:70</td>
<td>-33.4 ± 2.5</td>
<td>13.9 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>20:80</td>
<td>-34.1 ± 2.4</td>
<td>10.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>10:90</td>
<td>-33.4 ± 3.7</td>
<td>6.4 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>0:100</td>
<td>-31.4 ± 0.4</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Thermal data for PCL and MePEG blends heated from -100°C using DSC, at a heating rate of 40°/min. Data represent the mean of three samples ± 1 S.D.

<table>
<thead>
<tr>
<th>PCL:MePEG</th>
<th>Tg onset (°C)</th>
<th>Tm (end) (° C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MePEG</td>
</tr>
<tr>
<td>100:0</td>
<td>-71.9 ± 1.1</td>
<td>n/a</td>
</tr>
<tr>
<td>90:10</td>
<td>-85.1 ± 1.0</td>
<td>-0.3 ± 2.6</td>
</tr>
<tr>
<td>80:20</td>
<td>-88.0 ± 0.7</td>
<td>-1.6 ± 1.1</td>
</tr>
<tr>
<td>70:30</td>
<td>-90.1 ± 0.8</td>
<td>-1.8 ± 0.9</td>
</tr>
<tr>
<td>60:40</td>
<td>-90.7 ± 1.4</td>
<td>-0.2 ± 1.1</td>
</tr>
<tr>
<td>50:50</td>
<td>-93.0 ± 0.1</td>
<td>-1.1 ± 0.9</td>
</tr>
<tr>
<td>40:60</td>
<td>-93.5 ± 0.3</td>
<td>-2.4 ± 0.3</td>
</tr>
<tr>
<td>30:70</td>
<td>-94.1 ± 1.0</td>
<td>-0.9 ± 0.4</td>
</tr>
<tr>
<td>20:80</td>
<td>-94.4 ± 0.6</td>
<td>-1.4 ± 1.2</td>
</tr>
<tr>
<td>10:90</td>
<td>-94.6 ± 0.9</td>
<td>-0.8 ± 1.3</td>
</tr>
<tr>
<td>0:100</td>
<td>-95.8 ± 0.1</td>
<td>-0.8 ± 1.0</td>
</tr>
</tbody>
</table>
Figure 13: Representative DSC thermograms showing the glass transition (Tg) regions of PCL:MePEG blends at different PCL concentrations. The arrows represent the onset of the Tg for each blend. Scans were obtained by heating from -100° C at 40° per minute.
Figure 14: Effect of increasing the MePEG concentration on the observed Tg of blends of PCL and MePEG. Tg was measured using DSC at a heating rate of 40° C per minute. Data are presented as the mean of 3 determinations ± 1 S.D. The dotted line is the Tg values predicted by the Fox equation (Equation 12) assuming that both components were completely miscible and amorphous.
Figure 15: Effect of increasing MePEG concentration on the degree of supercooling attained before crystallization of PCL crystallites from PCL:MePEG blends. Both Tm and Tc were measured by DSC using scanning rates of 40° per minute. Tm was measured as the end of the melting endotherm of PCL and Tc was defined as the peak of the crystallization exotherm. Data are presented as the mean ± 1 S.D. of 3 determinations.
Figure 16: Change in the $\Delta H_f$ of PCL measured by DSC, corresponding to the PCL fraction in blends of PCL and MePEG, as a function of the weight fraction of PCL in the blend. Samples of about 10 mg were heated at 40° per minute through the melting point of PCL. Data represent the mean ± 1 S.D. of 3 determinations. The dotted line represents a linear decrease in $\Delta H_f$ from the value measured for 100% PCL to 0.

Figure 17: Change in the $\Delta H_f$ of MePEG measured by DSC, corresponding to the MePEG fraction in blends of PCL and MePEG, as a function of the weight fraction of MePEG in the blend. Samples of about 10 mg were heated at 40° per minute through the melting point of MePEG. Data represent the mean ± 1 S.D. of 3 determinations. The dotted line represents a linear decrease in $\Delta H_f$ from the value measured for 100% MePEG to 0.
5.2.3.4 Effect of paclitaxel on thermal properties of PCL:MePEG blends

A DSC thermogram of as received paclitaxel is shown in Figure 18A. The melting endotherm of paclitaxel has a peak at 221°C and a $\Delta H_f$ of 59.3 J/g. A representative DSC thermogram of 20% paclitaxel in PCL:MePEG 80:20 is shown in Figure 18B. The thermogram shows a single cooling/heating cycle. The top thermogram is a cooling curve from 75°C to -100°C while the bottom thermogram shows the heating thermogram from -100°C to 80°C. The cooling thermogram was characterized by an exothermic crystallization peak at 17.8°C representing the crystallization of PCL. This value is not significantly different from the temperature of crystallization values observed in blends of PCL:MePEG. The heating thermogram, shown as the lower scan in Figure 18B, is characterized by a double melting endotherm between 40°C and 60°C. The melting point of PCL in 20% paclitaxel in PCL:MePEG 80:20, calculated as the temperature at the end of the melting endotherm, was found to be 55.1°C ± 1.3°C which was not significantly different from the melting point of PCL in a PCL:MePEG 80:20 blend. The glass transition temperature of the PCL:MePEG 80:20 blend containing paclitaxel can be seen on the lower curve. The onset of this $T_g$ is at -72.0°C ± 4.0°C which is not significantly different from that of 100% PCL but is significantly greater than that of the PCL:MePEG 80:20 blend (Table 5). No glass transition temperature for MePEG can be seen.

The degree of supercooling obtained in these samples before crystallization occurred was 37.3°C which is slightly lower than that of the PCL:MePEG blends without paclitaxel.
5.2.3.5 Melting point depression of PCL by blending with MePEG

DSC was used to determine the melting point (Tm) of PCL crystallites as a function of the holding temperature at which crystallization took place (Tc) (data not shown). Representative Hoffman-Weeks plots, showing the change in Tm as a function of Tc for PCL crystals in PCL:MePEG blends containing 0, 10, 20, or 30% MePEG as well as 20% paclitaxel in PCL:MePEG 80:20, are given in Figure 19. The points plotted are the peak values of the melting endotherms against Tc as measured by DSC. From the slope and y-intercept of each plot the Hoffman-Weeks equation (Equation 13) was used to calculate the stability parameter (\(\phi\)) and the equilibrium melting point (Tm°) of PCL in the blend. Table 6 gives the stability parameters and equilibrium melting points calculated for each blend from the data. Analysis by ANOVA revealed no statistical difference in the stability parameters measured for the different blends (p > 0.19). The addition of MePEG to PCL decreased the equilibrium melting point of the PCL crystals from 59.1° C (100% PCL) to 55.2° C for the PCL:MePEG 70:30 blend. The equilibrium melting points determined for the PCL:MePEG blends and also for the 20% paclitaxel loaded PCL:MePEG 80:20 sample were not significantly different from each other, but all were significantly different from the Tm° of 100% PCL (ANOVA and Tukey test).

5.2.3.6 Calculation of the Flory interaction parameter

The Flory interaction parameter (\(\chi_{12}\)) was calculated from the data using the Nishi and Wang Equations (Nishi and Wang 1975), which are reproduced below as equations 15 and 16, and also by using group analysis.
Calculation of $\chi_{12}$ from Nishi and Wang equation

These calculations were made using the following values:

- Density of PCL = 1.185 g/cm$^3$ (measured using pycnometer)
- Molecular weight of PCL repeat unit = 114 g/mole (calculated from chemical formula)
- $M_n$ PCL = 14 900 g/mole (measured)
- Molar volume of PCL repeat unit ($V_{u2}$) = $114 \text{ g/mol} / 1.185 \text{ g/cm}^3 = 96.2 \text{ cm}^3/\text{mol}$
- Heat of fusion of PCL repeat unit ($\Delta H_{u2}$) = 139 J/g x 114 g/mol = 15 842 J/mol
- Density of MePEG (manufacturer's literature) = 1.1 g/cm$^3$ (label claim)
- Molecular weight of MePEG repeat unit = 44 g/mole (calculated from chemical formula)
- Molar volume of MePEG repeat unit ($V_{u1}$) = $44 \text{ g/mol} / 1.1 \text{ g/cm}^3 = 40 \text{ cm}^3/\text{mol}$
- $M_n$ MePEG = 350 g/mol (label claim)
- Universal gas constant ($R$) = 8.314 J/mol K or 1.987 cal/mol K
- Temperature ($T$) = 310 K

Figure 20 shows data plotted according to equation 15:

$$\frac{1}{T_m} - \frac{1}{T_m^*} = -\frac{B V_{2u}}{T_m^* \Delta H_{2u}} \left(\frac{\phi}{T_m^*}\right)^2$$  \hspace{1cm} \text{Equation 15}

where $T_m^0$ is the equilibrium melting point of 100% PCL, $T_m^*$ is the equilibrium melting temperature of PCL in a blend with MePEG at a MePEG concentration of $\phi_1$. From the slope of this plot and substituting values for $V_{2u}$, $\Delta H_{2u}$, $\phi_1$, given above, $B$ was calculated to be -10.6 J/cm$^3$. The y-intercept of this plot should theoretically be zero and as a result the low value obtained for the y-intercept (3 x $10^{-5}$ K$^{-1}$) was expected. The variable $B$ is the interaction energy density characteristic of the polymer pair. Substituting this value into equation 16:

$$\chi_{12} = \frac{B V_{1u}}{RT}$$  \hspace{1cm} \text{Equation 16}
gave a value for $\chi_{12}$ of -0.16 for PCL and MePEG.

**Calculation of $\chi_{12}$ from group analysis**

$\chi_{12}$ was also calculated from the solubility parameters of both PCL and MePEG using a table of chemical group contributions to molar attraction constants ($F$) from the literature (Martin 1993). According to this table, each methyl group contributes 133, ester groups contribute 303, and ether groups contribute 108 ($\text{cal/cm}^3)^{1/2}/\text{mole repeat unit}$. The sums of these contributions for PCL which consists of 5 methyl groups and 1 ester group, and MePEG, which consists of 2 methyl, and 1 ether group per repeat unit were calculated as follows:

**PCL:**
$$\Sigma F = (5 \times 133) + (1 \times 303) = 968 \text{ (cal/cm}^3)^{1/2}/\text{mole repeat unit}$$

**MePEG:**
$$\Sigma F = (2 \times 133) + (1 \times 108) = 374 \text{ (cal/cm}^3)^{1/2}/\text{mole repeat unit}$$

From these values, the solubility parameters ($\sigma$) of PCL and MePEG were calculated using:

$$\sigma = \Sigma F/V_u$$  \hspace{1cm} \text{Equation 31}

The solubility parameters calculated in this way were 10.06 ($\text{cal/cm}^3)^{1/2}$ for PCL and 9.35 ($\text{cal/cm}^3)^{1/2}$ for MePEG. Substituting into equation 17 gave an $\chi_{12}$ value for PCL and MePEG of 0.03.

**5.2.3.7 Hot stage microscopy**

Samples of PCL, PCL:MePEG 80:20 and 20% paclitaxel loaded PCL:MePEG 80:20 were observed on a hot stage through the microscope between crossed polarizing lenses. Crystalline regions of PCL or paclitaxel appeared as light regions because of their ability to refract the polarized light while the liquid regions appeared dark. As the samples were heated at 10° C per minute, the melting of crystals could be observed as the light regions gradually converting to dark. Both the temperatures at which the onset of melting occurred and the last
trace of crystalline material was visible were noted (melting range) and are reported here as the mean ± 1 S.D. of 3 measurements. The 100% PCL melting range was from 62.1 ± 2.3 °C to 65.6 ± 2.7 °C, the PCL:MePEG 80:20 was from 50.9 ± 2.4 °C to 55.2 ± 1.2 °C, and the 20% paclitaxel in PCL:MePEG 80:20 was from 51.1 ± 0.4 °C to 53.3 ± 0.4 °C.

A sample of 20% paclitaxel in PCL:MePEG 80:20 was heated on the hot stage and the thermal events observed using the microscope were photographed at various times and are shown in Figure 21. Figure 21A shows the sample at 37° C. The polymer matrix can be seen through the center of the photograph. At this temperature, PCL is crystalline and can be seen as the light regions. MePEG is a liquid at 37° C and as such could not be seen as a separate phase and is likely incorporated with the PCL crystals. The paclitaxel was not completely soluble in the matrix and some of the drug remained as a solid phase. Paclitaxel crystals can be seen in the upper left and lower right corners. The sample was heated at 10° per minute and Figures 21 B-D show the changes observed on heating. Figure 21B was taken at 51° C and shows the polymeric crystallites melting. At 60° C (Figure 21C) the polymer has completely melted but the paclitaxel crystals remain unchanged. As the sample was heated further, the paclitaxel began to dissolve in the molten PCL:MePEG 80:20 blend. Figure 20 D was taken at 165° C and shows the paclitaxel in the process of dissolving. At 204° C, paclitaxel dissolution was complete and the resulting melt was a homogeneous, one phase liquid.

Blends of PCL and MePEG and 20% paclitaxel loaded PCL:MePEG 80:20 were melted on the hot stage at 100° C for at least 5 minutes to remove the thermal history. The samples were then removed from the hot stage and allowed to cool to 37° C in the hot stage and held at 37° C while crystallization took place. The hot stage did not offer controlled cooling so it took approximately 10 minutes to cool to 37° C. Photographs of the PCL crystals were taken for
different blends at different stages of crystallization. Figure 22 A-D shows representative photographs of PCL crystals in 20% paclitaxel loaded PCL:MePEG 80:20 blends, and PCL:MePEG blends at blend ratios of 80:20, and 60:40, and a PCL:MePEG blend 20:80 (crystals growing after 1.5 hours at 37° C).
Figure 18: Representative DSC thermograms of A) as received paclitaxel and B) 20% paclitaxel loaded PCL:MePEG 80:20 surgical paste. The figure for the paste shows both the cooling from the melt and heating thermograms obtained using a scan rate of 40° per minute. No melting peak was seen for paclitaxel when the samples were heated to 230° C.
Figure 19: Representative Hoffman-Weeks plots showing the effect of increasing crystallization temperature (Tc) on the melting temperature (Tm) of PCL:MePEG blends and 20% paclitaxel loaded PCL:MePEG 80:20. The data were obtained using DSC.
Table 6: Effect of increasing MePEG concentration and paclitaxel loading in PCL:MePEG blends, on the stability parameters and equilibrium melting points of PCL crystals. Data represent the mean of 3 samples ± 1 S.D.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Stability parameter ($\phi$)$^a$</th>
<th>$T_m^\circ$ (°C)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>0.21 ± 0.05</td>
<td>59.1 ± 1.1</td>
</tr>
<tr>
<td>PCL:MePEG 90:10</td>
<td>0.24 ± 0.02</td>
<td>56.9 ± 0.6</td>
</tr>
<tr>
<td>PCL:MePEG 80:20</td>
<td>0.27 ± 0.02</td>
<td>56.2 ± 1.4</td>
</tr>
<tr>
<td>PCL:MePEG 70:30</td>
<td>0.26 ± 0.02</td>
<td>55.2 ± 0.6</td>
</tr>
<tr>
<td>20% paclitaxel in PCL:MePEG 80:20</td>
<td>0.26 ± 0.02</td>
<td>55.8 ± 0.6</td>
</tr>
</tbody>
</table>

$^a$ Determined from the slope of the Hoffman-Weeks plots
$^b$ Equilibrium melting point calculated from the y-intercept of the Hoffman-Weeks plot.
Figure 20: Melting point depression of PCL as a function of blend composition with MePEG, plotted according to Equation 15:

\[
\frac{1}{Tm^*} - \frac{1}{Tm} = -\frac{BV_{2u}}{\Delta H_{2u}} \times \phi^2
\]

Regression equation:
\[y = 0.0644x + (3 \times 10^{-5}), \quad r^2 = 0.9977\]
Figure 21: Photographs through crossed polarizers of the thermal events that occurred in a 20% paclitaxel loaded PCL:MePEG 80:20 sample as it was heated at 10°C per minute. A) 37°C with material was in the solid phase with paclitaxel crystals and polymer matrix visible; B) 51°C, the polymer matrix is melting; C) 60°C, polymer completely melted but the paclitaxel crystals remained; and D) 165°C, paclitaxel crystals dissolving in the polymer.
Figure 22: Representative photographs of crystals which formed from the melt (5 minutes at 100°C) following cooling to 37°C over 10 minutes. A) 20% paclitaxel loaded PCL:MePEG 80:20 blend, arrows point to paclitaxel crystals in polymer matrix; B) PCL:MePEG 80:20; C) PCL:MePEG 60:40; and D) PCL:MePEG 20:80 showing crystallization still in progress.
5.2.4 Gel permeation chromatography

The universal standard curve for a $10^3$ Å column using chloroform as the mobile phase flowing at 1 mL/min at 25° C is shown in Figure 23. It was constructed by measuring the retention time of polystyrene standards of known intrinsic viscosity between 300 and 50k g/mole, through the GPC. The log of the product of intrinsic viscosity and molecular weight of each sample was plotted against the retention time of the sample to generate the curve.

The intrinsic viscosity of PCL was determined by measuring the relative viscosity ($\eta_{\text{rel}}$), which is defined as the ratio of the time taken for the polymer solution to flow through the measurement area of a viscometer at a given temperature to that of the solvent alone. This value was then used to calculate the intrinsic viscosity ($[\eta]$) using the approximation of Solomon and Ciuta (Equation 30). Relative viscosity of PCL was measured in triplicate using PCL solutions in chloroform of 0.39, 0.30 and 0.40%. The relative viscosity of the PCL was found to be $1.14 \pm 0.01$ which was used to calculate a $[\eta]$ value of $0.34 \pm 0.02$ dL/g for PCL.

The retention times of PCL and PCL:MePEG 80:20 samples in the GPC could be converted to the molecular weight of PCL using the measured $[\eta]$ value and the universal standard curve. The molecular weight of MePEG could only be reported relative to polystyrene since the $[\eta]$ of MePEG was not known. Using the $10^3$ Å column, the molecular weight of PCL alone was found to be 19 443 g/mole, and for the PCL:MePEG 80:20 blend, the PCL peak corresponded to a molecular weight of 20 126 g/mole while the MePEG peak corresponded to a molecular weight of 641 g/mole relative to polystyrene. Note that the molecular weight values calculated using the universal standard curve from the GPC represent the $M_{\text{GPC}}$, and are not expected to be equal to the Mn nor the Mw of the sample.
PCL was injected through the 10 Å column and analysis of the resulting PCL peak allowed calculation of the Mn, Mw, and PDI of the PCL sample using Equations 2 and 3. These were found to be 14 900 g/mole, 47 100 g/mole, and 3.15 respectively. Representative GPC scans of PCL, MePEG and PCL:MePEG are shown in Figure 24.

5.2.5 Mechanical testing

5.2.5.1 Rheology

The effect of MePEG on the viscosity of PCL (from 0% to 30% MePEG) at 60° C determined using the parallel plate rheometer is shown in Figure 25. Figure 25A shows the complex viscosity measured as a function of radial strain rate. At low strain rates, there was a relatively large error in calculating viscosities because small errors in the strain rate are magnified in the calculations. At higher strain rates however, the viscosity of the polymers was independent of strain rate, indicating that the samples exhibited Newtonian behaviour at radial strain rates up to 100 radian per second. Figure 25B is a plot of complex viscosity of the paste as a function of MePEG concentration in PCL:MePEG blends and it shows that MePEG reduces the melt viscosity of PCL in a concentration dependent manner.

5.2.5.2 Compression testing

The compressive strength of PCL was decreased by the addition of MePEG to the polymer. The addition of 5%, 10%, and 20% MePEG to PCL resulted in a reduction in the compressive strength of tablets from 179.4 ± 34.2 (MePEG absent) to 151.6 ± 29.5, 85.8 ± 20.6, and 26.7 ± 2.7 N/cm² respectively. As MePEG loadings were increased, the tableted samples were observed to fail in a changing manner. With no MePEG present, PCL tablets did not
deform on compression but rather split sharply in half. As MePEG was added to the samples, the tablets were observed to undergo deformation on compression and at the point of failure could be seen to crumble as they split.

5.2.6 Scanning electron microscopy

Representative scanning electron micrographs of PCL:MePEG blends are shown in Figure 26 A-D. The micrographs show the isothermal crystallization that occurred in the blends which were melted and then stored at 37°C to allow crystallization to take place. Figure 26A is the micrograph of a 100% PCL sample. Details are not clearly visible but the outline of circular PCL spherulites, which were about 50 μm in diameter, can be seen. The detail visible in this image is representative of all thin film PCL samples. Figures 26B and C show the solidification of PCL:MePEG 80:20 and 50:50 blends, respectively. The structures seen in these micrographs are the PCL spherulites which form from the blends. The spherulites appear to have grown as two dimensional discs which originated from a central nucleation point and then grew radially outward. Figure 26D is an image of the PCL:MePEG 20:80 blend. Again detail is lacking in the image but the “feathery” outline of a PCL crystal can be seen.

5.2.7 Swelling study of PCL:MePEG paste

When cylinders of PCL were stored in PBS buffer at 37°C for 15 days, their weight increased by 0.8 ± 0.4%, indicating minimal swelling. Under the same conditions, the samples containing PCL with MePEG lost weight due to the diffusion of water soluble MePEG out of the polymer blend into the aqueous buffer. Steady state was achieved after 1 week but measurement was continued for 15 days. Weight losses were 1.8 ± 0.6%, 3.3 ± 0.5%, and 5.8 ± 1.1%, for the
samples containing 5, 10, and 20% MePEG in PCL, respectively. The magnitude of these weight losses were the net result of MePEG diffusing out of, and water diffusing into, the pellets.

Following swelling, the samples initially loaded with 0, 5, 10, and 20% MePEG were dried to remove water which had diffused into the pellets. The samples removed from the buffer after 15 days were found to contain 0.2 ± 0.4%, 3.7 ± 0.7%, 8.5 ± 1.2%, and 18.6 ± 0.6% water respectively. A rough estimate of the initial weight percentage of MePEG in the PCL:MePEG blends was made by adding the percentage weight lost due to MePEG diffusing out, to the percentage weight of water which had diffused into the pellets. In this way the initial MePEG weight percentage in the pellets was found to be 7%, 14.3%, and 24.4% for the samples loaded with 5, 10, and 20% MePEG, respectively. SEM images of the interior of PCL and PCL:MePEG 80:20 samples which had been stored in PBS buffer at 37°C for 15 days before being dried to constant weight, are shown in Figure 27 A and B, magnification 5000X. The 100% PCL sample (Figure 27A) did not show evidence of channels within the matrix. The interior of the PCL:MePEG 80:20 sample (Figure 27B) resembled the interior of a sponge. Empty water channels can be seen between crystalline PCL fibrils.

The PCL cylinders showed insignificant volume changes while the cylinders containing MePEG decreased in volume by 3.2%, 7.7%, and 6.2% for the 5, 10 and 20% MePEG samples respectively. These volume changes were all significantly different from zero at p < 0.025 using a paired two sample t-test.
Figure 23: Universal GPC standard curve using polystyrene standards of known molecular weight and intrinsic viscosity. Samples were run on a $10^4$ Å PLgel column with chloroform as a mobile phase flowing at 1 mL/min at 25° C. The regression equation for the standard curve along with the coefficient of determination are given in the figure.

Regression equation:

$$y = -1.071x + 9.853$$

$$R^2 = 0.991$$
Figure 24: GPC traces of A) PCL, B) MePEG, and C) PCL:MePEG 80:20. The PCL elutes first at 5.6 minutes while the MePEG elutes at 8.2 minutes. Chromatographic conditions: $10^3$ Å column with mobile phase of chloroform flowing at 1 mL per minute and refractive index detection.
Figure 25: A) Complex viscosity as a function of tangential strain rate as measured by a parallel plate rheometer for different blends of PCL:MePEG at 60° C. B) Effect of MePEG blending on the complex viscosity of PCL paste samples measured within the Newtonian regions of the samples at a tangential strain rate of 100 rad/sec.
Figure 26: Scanning electron microscope images of PCL:MePEG blends which had crystallized at 37°C and which contained A) 100:0; B) 80:20; C) 50:50; and D) 20:80 PCL:MePEG ratios. Magnification was 1000X.
Figure 27: Scanning electron microscope images of the interior of A) PCL pellet and B) PCL:MePEG 80:20 pellet incubated in PBS buffer at 37° C for 15 days and dried to constant weight. Magnification was 5000X.
5.2.8 PCL crystallization

5.2.8.1 Effect of MePEG on growth rate of crystals

Blends of PCL and MePEG were melted, mounted onto a microscope slide with a coverslip, and allowed to solidify at room temperature while being viewed under the microscope between crossed polarizing lenses. The growth rates of PCL crystals were determined by measuring the time taken for growing PCL crystals to go across micrometer divisions etched into the eyepiece of the microscope. Growth rates as a function of MePEG concentration are shown in Figure 28. The presence of MePEG did not affect the growth rate of PCL crystals (p > 0.34, using single variable ANOVA) crystallizing isothermally at room temperatures.

5.2.8.2 Microscopy of PCL crystallization in blends

Blends of PCL and MePEG, over a full range of compositions, were melted onto microscope slides with a coverslip, and allowed to solidify at room temperature or 37°C. The samples were viewed under the microscope between crossed polarizing lenses. Representative photographic images of the resulting PCL spherulites are shown in Figure 29 A-E. Figure 29A shows crystallization of 100% PCL. The spherulites had expanded to cover the entire field of view and exhibited a well defined Maltese cross pattern.

Figures 29 B-E are photographic images showing the effect on the resulting PCL spherulites, of differing PCL:MePEG blend ratios of 80:20, 50:50, 30:70 and 10:90, respectively. In all cases, the PCL spherulites grew to fill the entire field of view. Figures 29 D and E were taken before crystallization was complete. Increasing MePEG content resulted in larger spherulites. In addition, the crystalline PCL, which can be seen as light regions on the
photographs, changed in appearance as MePEG was added. The diluted PCL crystallite fibers appeared more diffuse and less densely packed in Figures 29 C and D than in Figure 29A. Banding patterns could be seen as concentric rings in the PCL spherulites as more MePEG was added. These are visible in Figures 29 C-E. The banding, which is due to periodic twisting of the PCL lamellae, is most clearly visible in Figure 29E which is a photograph of growing spherulites at low magnification of a 10:90 blend.

5.2.8.3 Avrami exponent

Blends of PCL:MePEG with MePEG concentrations of 0, 10, 20 and 30% were cooled from a melt and allowed to crystallize isothermally at 37° C in the DSC. Heat flow in the samples was monitored over time, and as PCL crystallization took place, an exotherm was recorded on the DSC. For each sample, the total area under the crystallization exotherm was measured and taken to correspond to complete crystallization of PCL in a given sample. At any time during the course of PCL crystallization, the fraction of the area under the crystallization exotherm up to that time, divided by the total area of the exotherm, was taken to represent the fraction of total crystallization which had taken place at that time point.

Representative scans showing the heat released as the samples were held at 37° C over time are given in Figure 30A. The 100% PCL sample showed a sharp crystallization exotherm which began almost immediately and was completed by 6 minutes. As the PCL content was decreased from 90% PCL to 70% PCL, a longer lag time, or time taken before initiation of the crystallization exotherm, was observed. In addition, the peak height of the exotherms was decreased while the length of time taken for crystallization to be completed was increased. Figure 30B shows a representative calculation of the fraction of crystallization that occured over
time in the PCL:MePEG 80:20 curve. The total area under the curve represents a 100% extent of
crystallization. The crystallization exotherm is divided by slices which correspond to fractional
areas of between 0.01 and 0.4 of the total area under the exotherm. The time at which these
slices occurred was calculated by the DSC software and is included on the table in Figure 30B.

The effects of MePEG and paclitaxel on the time course of PCL crystallization are shown
in Figure 31 where the fraction of crystallization which had taken place in each blend is plotted
against the length of time that the blends were held at 37°C. All the curves are characterized by
a lag phase before crystallization occurs followed by a period of linear increase in crystallinity.
The time to onset of crystallization was calculated by extrapolating the linear portion of each
curve back to the x-axis and the time at the intersection point was taken as the onset time of
crystallization. The slope of this linear portion was measured to be the crystallization rate of the
samples. The 100% PCL sample can be seen to have the shortest onset time and the steepest
linear slope indicating the fastest crystallization rate. As MePEG was added, the onset times
were increased and the crystallization rates were decreased in a concentration dependent manner.
Figure 31 also includes the crystallization profile of 20% paclitaxel loaded PCL:MePEG 80:20.
The addition of paclitaxel did not alter the crystallization profile from that seen with the
PCL:MePEG 80:20 blend alone, as the two curves are almost superimposed on each other. The
onset times and crystallization rates were measured in triplicate for each blend ratio and the
results are summarized in Table 7.

The Avrami exponent was calculated for each sample by plotting log(ln(1/θ)) against the
log time according to the Avrami equation (Equation 18). For each blend ratio tested, the
Avrami exponent was calculated using triplicate samples, and using only the crystallization times
for up to crystallization fractions of 0.4. This was done to avoid measurement of crystallization data following impingement of spherulites. Representative Avrami plots are given in Figure 32 and show that all blends exhibit linear relationships from which Avrami exponents can be calculated as the slope of the best fit linear regression lines (not shown in figure). At a holding temperature of 37° C, the blends of PCL:MePEG 70:30 showed a large variation in crystallization profiles leading to large errors in onset time and crystallization rates measured. As a result the Avrami plots for PCL:MePEG 70:30 at 37° C were not reproducible, and data for crystallization at 34° C are included in Figure 32 instead. The log k and Avrami exponents calculated for PCL crystallization as a function of blend composition at 37° C and for the PCL:MePEG 7:3 blend at 34° C are summarized in Table 8.
Figure 28: Effect of MePEG on the growth rate of PCL spherulites, measured by timing the progression of the spherulite growth fronts across micrometer markings under the microscope. Samples had been melted at 80°C then transferred to the microscope stage at room temperature and observed for crystallization. Data represent the mean of at least 3 determination ± 1 S.D.
Figure 29: Representative photographs of PCL crystals formed isothermally from a melted blend with MePEG using different blend ratios of PCL:MePEG.  A) PCL 100:0 at 37\(^\circ\) C; B) PCL:MePEG 80:20 at 37\(^\circ\) C; C) PCL:MePEG 50:50 at 37\(^\circ\) C; D) PCL:MePEG 30:70 at ambient temperature; and E) PCL:MePEG 10:90 at ambient temperature.
Figure 30: DSC thermograms showing exothermic crystallization peaks measured as PCL crystals formed at 37° C. A) Crystallization peaks of PCL in blends with MePEG at different PCL concentrations. B) PCL crystallization from PCL:MePEG 80:20 blend showing the times taken to achieve various percentages of crystallization.
Figure 31: Time course of the percentage of PCL crystallization at different blend compositions of PCL and MePEG, as well as 20% paclitaxel loaded PCL:MePEG 80:20. Samples were cooled rapidly from the melt at 80°C to 37°C and held isothermally. PCL crystallization was then monitored over time and the mean ± 1 S.D. of 3 samples is given for each blend.
Table 7: Effect of MePEG and paclitaxel on the onset and rate of crystallization of PCL in blends which were melted at 80° C for 5 minutes, cooled at 50° per minute and held isothermally at 37° C until PCL crystallization was completed.

<table>
<thead>
<tr>
<th>Blend composition</th>
<th>Onset time (min)(^a)</th>
<th>Crystallization rate(^b) (fraction crystallization achieved/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>0.7</td>
<td>0.54</td>
</tr>
<tr>
<td>PCL:MePEG 90:10</td>
<td>1.3</td>
<td>0.32</td>
</tr>
<tr>
<td>PCL:MePEG 80:20</td>
<td>2.2</td>
<td>0.20</td>
</tr>
<tr>
<td>PCL:MePEG 70:30</td>
<td>3.7</td>
<td>0.09</td>
</tr>
<tr>
<td>20% paclitaxel in PCL:MePEG 80:20</td>
<td>2.5</td>
<td>0.26</td>
</tr>
</tbody>
</table>

\(^a\) Onset time calculated as the intersection point of extrapolated linear portion of the fraction of crystallization achieved over time plot (Figure 31) with the x-axis.

\(^b\) Crystallization rate calculated as the slope of the linear portion of the fraction of crystallization achieved over time plot (Figure 31).
Figure 32: Representative Avrami plots for crystallization of PCL from blends with MePEG as well as from a 20% paclitaxel loaded PCL:MePEG 80:20 sample. Samples were cooled rapidly from the melt at 80° C to 37° C and crystallization was monitored by measuring the exothermic crystallization transition using the DSC. Data was plotted using the Avrami equation (Equation 18).
Table 8: Values of log k and Avrami exponents (n) for PCL isothermal crystallization as a function of blend composition and holding temperature (Tc)

<table>
<thead>
<tr>
<th>Blend composition</th>
<th>Tc (° C)</th>
<th>Log k $^a$</th>
<th>Avrami exponent (n)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL 100:0</td>
<td>37</td>
<td>-0.7 ± 0.2</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>PCL:MePEG 90:10</td>
<td>37</td>
<td>-1.6 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>PCL:MePEG 80:20</td>
<td>37</td>
<td>-2.5 ± 0.2</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>PCL:MePEG 70:30</td>
<td>37</td>
<td>-2.8 ± 1.1</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td>20% paclitaxel in PCL:MePEG 80:20</td>
<td>37</td>
<td>-2.8 ± 0.2</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>PCL:MePEG 70:30</td>
<td>34</td>
<td>-2.0 ± 0.4</td>
<td>3.1 ± 0.3</td>
</tr>
</tbody>
</table>

$^a$ Values represent the mean of 3 samples ± 1 S.D.
5.2.9 Degradation of surgical paste in buffer

Samples of PCL, PCL:MePEG 80:20 and 20% paclitaxel loaded PCL:MePEG 80:20 were manufactured and stored in PBS buffer at 37° C for 13 weeks. At 3 week intervals, samples of each paste were analyzed using the GPC to observe changes in the molecular weight ($M_{GPC}$).

Representative GPC scans of the PCL paste formulations are given in Figure 33 A-E. Figures 33 A-C show the GPC chromatograms of the three starting materials, PCL, PCL:MePEG 80:20 and 20% paclitaxel loaded PCL:MePEG 80:20 respectively, which had been stored at 37° C in a dry state. In all three samples the PCL peak is seen at 5.7 minutes. The PCL:MePEG 80:20 sample (Figure 33 B) also has a peak corresponding to the elution of MePEG at 8.4 minutes, while the 20% paclitaxel loaded PCL:MePEG 80:20 chromatogram (Figure 33 C) has both paclitaxel and MePEG co-eluting at 8.4 minutes. The solvent peak is seen at 9.9 minutes.

When PCL:MePEG 80:20 was stored in PBS buffer at 37° C, the GPC scan of the sample after 1 month of storage is shown in Figure 33 D. It is different from the control sample (Figure 33 B) in that the PCL peak has been shifted to 5.8 minutes, indicating a decrease in the PCL molecular weight, and the MePEG peak is no longer seen. The disappearance of the MePEG peak from the PCL:MePEG 80:20 scans which were stored in buffer indicates that the MePEG is no longer in the sample and has diffused from the paste into the aqueous medium. Analysis of 20% loaded PCL:MePEG 80:20 samples which had been stored in buffer (Figure 33 E) as compared to control samples which were stored dry (Figure 33 C) revealed that the PCL eluted at a later time, 5.8 minutes, but no disappearance of the MePEG peak could be detected because the MePEG and paclitaxel co-elute, and the presence of paclitaxel likely accounted for the peak at 8.5 minutes.
The changes in the M\textsubscript{GPC} of the PCL alone, blended with MePEG (80% PCL) or in a 20% paclitaxel in PCL:MePEG 80:20 formulation stored at 37° C in PBS buffer, as a function of time are given in Table 9. Over the course of 13 weeks the M\textsubscript{GPC} of PCL in the surgical paste samples was significantly reduced when the samples were stored in PBS buffer at 37° C. This effect was greatest in the 100% PCL sample and least in the 20% paclitaxel loaded PCL:MePEG 80:20 sample.

5.2.10 Ageing of surgical paste at different storage temperatures

Samples of PCL, PCL:MePEG 80:20, and 20% paclitaxel loaded PCL:MePEG 80:20 were stored at temperatures of 4°, 25°, and 37°, and 37° C in PBS buffer over 13 weeks. At 3 week intervals, the melting points, and degree of crystallinity of the samples were measured by DSC.

Figures 34 A-F show the effect of storage conditions on the melting point (peak of melting endotherm) and percent crystallinity of PCL as a function of time for PCL, PCL:MePEG 80:20, and 20% paclitaxel in PCL:MePEG 80:20. Figures 34 A-C show an increase in the melting point of PCL over time for all samples and storage conditions. In the case of the 100% PCL sample, the T\textsubscript{m} increased gradually over the 13 weeks at all temperatures but the increase was significantly greater in the samples stored at 37° C than in those stored at 4° and 25° C. Statistical analysis using an ANOVA and Tukey test showed that there was no significant difference between the T\textsubscript{m}'s of the two samples stored at 37° C and between the samples stored at 4° and 25° C.

The PCL:MePEG 80:20 and 20% loaded PCL:MePEG 80:20 samples (Figures 34 B and C) also showed an increase in T\textsubscript{m} for all samples. The samples stored at 37° C showed a sharp
rise in Tm in the first 3 weeks followed by a gradual increase over the remaining 10 weeks. In contrast the samples stored at 4\(^\circ\) or 25\(^\circ\) C showed a gradual increase in Tm throughout the entire 13 week course of the study. In all cases, the order of storage conditions which caused the greatest increase in PCL Tm, ranked from the most change to the least was: 37\(^\circ\) C in buffer > 37\(^\circ\) C > 25\(^\circ\) C > 4\(^\circ\) C. In the case of the PCL:MePEG 80:20 sample (Figure 34B) the Tm's of the samples at 13 weeks were all significantly different from each other. In the 20% paclitaxel loaded PCL:MePEG 80:20, the Tm's of the samples stored at 25\(^\circ\) C and at 37\(^\circ\) C (dry) were not different but both of these were significantly different from the Tm's of the samples stored at 4\(^\circ\) and 37\(^\circ\) C in buffer. Statistical significance was determined using an ANOVA and Tukey test.

Changes in the degree of crystallinity (Xc) in the PCL component of PCL, PCL:MePEG 80:20, and 20% paclitaxel loaded PCL:MePEG 80:20 over 13 weeks under different storage conditions are shown in Figures 34 D-F, respectively. In all samples and under all storage conditions a statistically significant increase in Xc was measured after 13 weeks. The samples stored either at 37\(^\circ\) C in buffer or at 4\(^\circ\) C showed the smallest increases in Xc. The samples stored at 37\(^\circ\) C dry and 25\(^\circ\) C showed similar increases in Xc.

The effects of time and dry storage temperature on the Tg of the PCL:MePEG 80:20 and 20% paclitaxel loaded PCL:MePEG 80:20, and on the peak temperature of the melting endotherm of MePEG in the PCL:MePEG 80:20 blends are summarized in Table 10. Analysis of the initial samples showed that the Tg of the PCL:MePEG 80:20 blends was -87.0\(^\circ\) C, while the MePEG component melted at -13.3\(^\circ\) C. The 20% paclitaxel loaded PCL:MePEG 80:20 blend had a Tg of -73.4\(^\circ\) C.

When stored at 4\(^\circ\) C, no significant change in the Tg of PCL:MePEG 80:20 blends was observed over 13 weeks. In the 20% paclitaxel loaded paste, the Tg was increased to -37\(^\circ\) C after
6 weeks and was not observed at 9 weeks. The melting point of MePEG decreased to -16.3°C at 13 weeks which was significantly different from the initial value (p < 0.05, Student’s t-test).

At 25°C there was no significant change in the Tg’s of PCL:MePEG 80:20 or 20% paclitaxel loaded PCL:MePEG 80:20, and no change in the melting point of MePEG in the PCL:MePEG 80:20 blends.

Storage at 37°C resulted in a significant decrease (p < 0.05 ANOVA, Tukey test) in the Tg of the PCL:MePEG 80:20 blend to -91.1°C and in the melting point of the MePEG component to -14.2°C after 13 weeks. No difference in the the Tg of 20% paclitaxel loaded PCL:MePEG 80:20 paste was observed following storage at 37°C for 13 weeks.

No melting transitions for MePEG were observed in any of the 20% paclitaxel in PCL:MePEG 80:20 samples.

The samples which were stored at 37°C in PBS buffer did not show any evidence of glass transitions or MePEG melting peaks.
Figure 33: Representative GPC scans of paste samples which had been stored for 1 month at 37°C. A) PCL stored dry; B) PCL:MePEG 80:20 stored dry; C) 20% paclitaxel loaded PCL:MePEG 80:20 stored dry; D) PCL:MePEG 80:20 stored in PBS buffer; and E) 20% paclitaxel loaded PCL:MePEG 80:20 stored in PBS buffer. Chromatographic conditions: 10³ Å column with mobile phase of chloroform flowing at 1 mL per minute with refractive index detection. PCL eluted at 5.8 minutes and MePEG and paclitaxel both eluted at 8.5 minutes.
Table 9: Change in molecular weight of the PCL component in samples of PCL, PCL:MePEG 80:20 and 20% loaded PCL:MePEG 80:20 following 13 weeks of storage in PBS buffer at 37°C.

<table>
<thead>
<tr>
<th>Time</th>
<th>M&lt;sub&gt;GPC&lt;/sub&gt; (g/mole)</th>
<th>PCL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PCL:MePEG 80:20&lt;sup&gt;a&lt;/sup&gt;</th>
<th>20% paclitaxel loaded PCL:MePEG 80:20&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
<td>20.2k ± 0.1</td>
<td>20.5k ± 0.2</td>
<td>20.4k ± 0.2</td>
</tr>
<tr>
<td>13 weeks</td>
<td></td>
<td>14.2k ± 0.1*</td>
<td>17.3k ± 1.5*</td>
<td>18.9k ± 1.5*</td>
</tr>
</tbody>
</table>

* Statistically significant difference from initial value (t-test, p < 0.05)

<sup>a</sup> Reported M<sub>GPC</sub> values are the mean of 3 determinations ± 1 S.D.
Figure 34: Ageing of surgical paste, under different storage conditions as shown by the melting point of the PCL component (Tm) and the degree of crystallinity of PCL (Xc) measured using DSC. A) Tm of PCL; B) Tm of PCL:MePEG 80:20; C) Tm of 20% paclitaxel loaded PCL:MePEG 80:20; D) Xc of PCL; E) Xc of PCL:MePEG 80:20 and F) Xc of 20% paclitaxel loaded PCL:MePEG 80:20. Data represent the mean of 4 determinations ± 1 S.D.
Table 10: Thermal properties (Tg and the MePEG Tm peak) of PCL:MePEG 80:20 and 20% paclitaxel in PCL:MePEG 80:20 stored under dry conditions at different temperatures. Data represent the mean of 3 measurements ± 1 S.D.

<table>
<thead>
<tr>
<th>Time</th>
<th>PCL:MePEG 80:20</th>
<th>20% paclitaxel in PCL:MePEG 80:20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tg (°C)</td>
<td>MePEG Tm peak (°C)</td>
</tr>
<tr>
<td>Initial</td>
<td>-87.0 ± 0.7</td>
<td>-13.3 ± 0.4</td>
</tr>
<tr>
<td>4°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 weeks</td>
<td>-89.3 ± 1.3</td>
<td>-12.4 ± 0.8</td>
</tr>
<tr>
<td>6 weeks</td>
<td>-90.2 ± 2.5</td>
<td>-12.4 ± 1.5</td>
</tr>
<tr>
<td>9 weeks</td>
<td>-90.5 ± 2.5</td>
<td>-14.0 ± 1.5</td>
</tr>
<tr>
<td>13 weeks</td>
<td>-89.9 ± 2.7</td>
<td>-16.3 ± 1.8</td>
</tr>
<tr>
<td>p value*</td>
<td>0.31</td>
<td>0.009</td>
</tr>
<tr>
<td>25°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 weeks</td>
<td>-88.3 ± 1.2</td>
<td>-11.6 ± 2.2</td>
</tr>
<tr>
<td>6 weeks</td>
<td>-89.8 ± 2.2</td>
<td>-12.4 ± 0.8</td>
</tr>
<tr>
<td>9 weeks</td>
<td>-88.1 ± 2.7</td>
<td>-14.0 ± 0.3</td>
</tr>
<tr>
<td>13 weeks</td>
<td>-87.9 ± 1.6</td>
<td>-14.1 ± 1.8</td>
</tr>
<tr>
<td>p value*</td>
<td>0.38</td>
<td>0.09</td>
</tr>
<tr>
<td>37°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 weeks</td>
<td>-87.3 ± 1.1</td>
<td>-11.5 ± 0.5</td>
</tr>
<tr>
<td>6 weeks</td>
<td>not observed</td>
<td>-10.2 ± 0.5</td>
</tr>
<tr>
<td>9 weeks</td>
<td>-86.4 ± 1.0</td>
<td>-12.4 ± 1.7</td>
</tr>
<tr>
<td>13 weeks</td>
<td>-91.1 ± 2.5c</td>
<td>-14.2 ± 1.3 d, e</td>
</tr>
<tr>
<td>p value*</td>
<td>0.005</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* p value calculated using a single variable ANOVA comparing data from initial sample with those of each time point

- a Statistically different (p < 0.05) initial time point in group (t-test)
- b Statistically different (p < 0.05) from initial and 3 week time points in group (t-test)
- c Statistically different (p < 0.05) from all other time points in group (Tukey test)
- d Statistically different (p < 0.05) from initial time point (Tukey test)
- e Statistically different (p < 0.05) from 6 week time point (Tukey test)
5.3 In vitro release studies

In all in vitro release studies the sampling intervals were chosen so that sink conditions in the sampling vial would not be exceeded. However, on the first day of release studies it is likely that sink conditions were exceeded despite sampling five times. It was noted that when analyzing paclitaxel samples stored in buffer a second peak was visible on the HPLC. This peak was determined to be due to 7-epitaxol, an epimer of paclitaxel. The 7-epitaxol peak area was never more than 10% of that of the paclitaxel peak, and was not counted in quantitation of paclitaxel released. Previous work in our laboratory had shown that paclitaxel was stable in buffer for at least 3 days. Therefore sampling intervals were maintained in all in vitro release studies at 3 days or less. The exception to this was during the long term release study (Section 5.3.3, Figure 39), when sampling intervals of up to 4 days were used during the latter stages of the study.

5.3.1 Effect of paclitaxel loading

The effect of paclitaxel loading on the time course of drug release from either PCL or PCL:MePEG 80:20 surgical paste are shown in Figure 35A and B. Insets in the figures represent the first day of drug release on an expanded scale. The release curves are characterized by an initial burst phase over approximately 1 day, followed by a period of slow sustained release. The total amounts of paclitaxel released after 14 days from a 2.5 mg paste pellet (M_{14}), the initial release rate (V_{o}, defined as the drug release rate during the first 12 hours of the study) and the sustained release rate (V_{10}, determined by calculating the slope of the cumulative release curve over the last 10 days of the study) are shown in Table 11 for each formulation. Total paclitaxel release after 14 days increased with paclitaxel loading for both PCL and PCL:MePEG 80:20
surgical paste. Both the initial and sustained release rates also increased with paclitaxel loading for both types of surgical paste. The values obtained for paclitaxel release from PCL:MePEG 80:20 surgical paste were compared with those obtained from PCL surgical paste at equivalent paclitaxel loadings using the Student's t-test. The initial paclitaxel release rate from PCL:MePEG 80:20 was found to be significantly less than from PCL at loadings above 10% paclitaxel. There was no difference found in the sustained release rates of paclitaxel from both types of surgical paste except at 1% paclitaxel loading, where the release rate from PCL:MePEG 80:20 was less than from PCL alone. The cumulative release of paclitaxel was found to be lower from the PCL:MePEG 80:20 surgical paste than from PCL at all loadings but the difference was only significant from the 1% and 20% paclitaxel loaded pastes.

The cumulative release of paclitaxel from PCL and PCL:MePEG 80:20 is shown as a function of the square root of time in Figure 36 for the 20% loading of drug. The model describing drug release through a diffusion mechanism predicts that drug release should be linearly related to the square root of time (Equation 23). Best fit linear regression equations calculated for the data after 1 day for each formulation, are included in Figure 36. After 1 day the release rate of paclitaxel is linear with the square root of time. The percentage (of total loading) of drug released at 14 days for each polymer, at different paclitaxel loadings is shown in Figure 37. Analysis of the percent release data as a function of the initial paclitaxel loading revealed that for the PCL surgical paste, the 1% paclitaxel loaded samples released a significantly higher percentage of paclitaxel than did the 5% loaded samples. The 5% loaded samples released a greater percentage of paclitaxel than the 10% loaded samples but there was no statistically significant difference in the percentage of paclitaxel released from the 10%, 20% and 30% loaded samples of PCL surgical paste. The PCL:MePEG 80:20 surgical paste, showed no
statistical difference between the percent of paclitaxel released from the 1% and 5% loaded samples. Both the 1%, and 5% loaded samples released a significantly higher percentage of paclitaxel than the 10% loaded samples, which also released a significantly higher percentage of paclitaxel than the 20% loaded samples. There was no statistically significant difference between the percentage of paclitaxel released from the 20% and 30% loaded PCL:MePEG 80:20 surgical paste. Percentage release data was compared using a single variable ANOVA and t-tests. Statistical significance was defined at p < 0.05.

5.3.2 Effect of MePEG loading

In vitro release studies were conducted using 5 mg pellets of 20% paclitaxel loaded surgical paste samples of varying PCL and MePEG blend ratios. The effect of MePEG concentration on the time course of paclitaxel release from PCL:MePEG pastes is shown in Figure 38. The upper curve in Figure 38 represents the cumulative release of paclitaxel from a 100% PCL surgical paste over 14 days. The total amount of paclitaxel released from this formulation was significantly greater than the cumulative amount of paclitaxel released from any of the formulations containing MePEG over 14 days. Analysis of the cumulative release of paclitaxel over 14 days from the formulations containing between 5% and 30% MePEG in the polymer matrix, showed no statistical difference in their cumulative paclitaxel release (ANOVA, p > 0.6).

5.3.3 Long term release study

The cumulative in vitro release of 20% loaded paclitaxel normalized to 2.5 mg PCL or PCL:MePEG 80:20 pellets is shown in Figure 39 over 90 days. The curves are characterized by
a burst phase over approximately one day followed by a period of slow sustained released which continued for the entire duration of the study. The cumulative paclitaxel released from the PCL sample after 90 days ($M_{90}$) was $104.0 \pm 38.0 \ \mu g$ per 2.5 mg pellet, which was significantly higher than from the PCL:MePEG 80:20 sample ($40.6 \pm 11.2 \ \mu g$ per 2.5 mg pellet). This difference was found both in the initial release rate ($V_0$), $13.2 \pm 4.8 \ \mu g$/day for PCL and $6.4 \pm 2.1 \ \mu g$/day for PCL:MePEG 80:20, and in the release rate over the last 60 days of the study ($V_{60}$), which was determined to be $0.6 \pm 0.3 \ \mu g$/day for PCL and $0.2 \pm 0.1 \ \mu g$/day for the PCL:MePEG 80:20 sample. The value for $V_{60}$ was calculated as the slope of the best fit regression line through the data points from day 30 to day 90 ($p < 0.03$, Student’s t-test).

5.3.4 Effect of sterilization

The effect of sterilization by 30 kGy of $^{60}$Co gamma irradiation on the release profiles of 20% paclitaxel loaded PCL and PCL:MePEG 80:20 are shown in Figures 40 A and B, respectively. The release profiles were characterized by an initial period of rapid paclitaxel release over about 1 day, followed by a period of slower release. Sterilization did not cause a significant difference in the cumulative release of paclitaxel from either polymer as p values of 0.78 and 0.23 were calculated using a t-test for the PCL and PCL:MePEG 80:20 blends, respectively.
Figure 35A: Effect of paclitaxel loadings on the cumulative release profile of paclitaxel from PCL surgical paste pellets which were tumbled end over end at 37° C in phosphate buffered saline with albumin, normalized to 2.5 mg pellets. The inset shows the burst phase release data over the first day of the study on an expanded scale. Data represent the mean of 4 samples with error bars shown only in the positive direction (1 S.D.) for clarity.
Figure 35B: Effect of paclitaxel loadings on the cumulative release profile of paclitaxel from PCL:MePEG 80:20 surgical paste pellets which were tumbled end over end at 37°C in phosphate buffered saline with albumin, normalized to 2.5 mg pellets. The inset shows the burst phase release data over the first day of the study on an expanded scale. Data represent the mean of 4 samples with error bars shown only in the positive direction (1 S.D.) for clarity.
Table 11: Data for paclitaxel released over 14 days from either PCL or PCL:MePEG 80:20 surgical paste pellets normalized to 2.5 mg, at different paclitaxel loadings. Values represent mean of 4 samples ± 1 S.D.

<table>
<thead>
<tr>
<th>Loading</th>
<th>V₀ᵃ (μg/2.5 mg paste/day)</th>
<th>V₁₀ᵇ (μg/2.5 mg paste/day)</th>
<th>M₁₄ᶜ (μg from 2.5 mg paste)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCL</td>
<td>PCL:MePEG</td>
<td>PCL</td>
</tr>
<tr>
<td>1%</td>
<td>5.0±0.6</td>
<td>4.2±0.3</td>
<td>0.2±0.04</td>
</tr>
<tr>
<td>5%</td>
<td>18.1±2.8</td>
<td>19.7±5.0</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>10%</td>
<td>24.1±7.8</td>
<td>13.1±3.1*</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>20%</td>
<td>46.3±8.2</td>
<td>18.4±5.4*</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>30%</td>
<td>76.2±24.0</td>
<td>19.9±4.8*</td>
<td>1.7±0.7</td>
</tr>
</tbody>
</table>

ᵃ Initial release rate defined as slope of release curve over first 12 hours of release study
ᵇ Sustained release rate defined as slope of cumulative release curve over last 10 days of study
ᶜ Cumulative paclitaxel released in 14 days
* Values are statistically different from PCL alone (p < 0.05, Student’s t-test)
Regression analysis parameters (after day 1): \( y = mx + b, r^2 \)
PCL: \( y = 7.8x + 17.6, r^2 = 0.983 \)
PCL:MePEG 80:20: \( y = 6.9x + 3.6, r^2 = 0.992 \)

Figure 36: Cumulative release of paclitaxel, normalized to 2.5 mg pellet pellet weight, from 20% paclitaxel loaded PCL and PCL:MePEG 80:20 as a function of the square root of time. Data represent the mean of 4 samples. Error bars have been omitted for clarity.
Comparisons of percentage of drug released within each surgical paste group
(statistically significant differences, p < 0.05, denoted by "*"):
PCL: 1% > 5% > 10% ~ 20% ~ 30%
PCL:MePEG 80:20: 1% ~ 5% > 10% > 20% ~ 30%

Figure 37: Effect of paclitaxel loading on the percent of paclitaxel released from 2.5 mg PCL, and PCL:MePEG 80:20 pellets, which were tumbled end over end in phosphate buffered saline with albumin at 37° C for 14 days. Data represent the mean of 4 determinations with error bars of 1 S.D.
Figure 38: Time course of paclitaxel released from 20% paclitaxel loaded PCL:MePEG blends at varying blend ratios. Release was measured from pellets which were tumbled end over end at 37° C in phosphate buffered saline with albumin, and is reported per mg of paste. Data represent the mean of 4 determinations (n = 5 for PCL:MePEG 100:0) with error bars (1 S.D.) shown in the positive direction only for clarity.
Figure 39: Time course of cumulative paclitaxel release from 20% paclitaxel loaded PCL and PCL:MePEG 80:20 pellets. Release was measured from pellets which were tumbled end over end at 37° C in phosphate buffered saline with albumin and was normalized to release from 2.5 mg pellets. Data represent the mean of 4 determinations ± 1 S.D.
Figure 40: Time course of cumulative paclitaxel release from 20% paclitaxel loaded A) PCL and B) PCL:MePEG 80:20 surgical paste samples. Release was measured from pellets which were tumbled end over end at 37°C in phosphate buffered saline and was normalized to release from 2.5 mg pellets. For each surgical paste formulation the effect of sterilization by ⁶⁰Co gamma irradiation on the paclitaxel release profiles was compared. Data represent the mean of 4 determinations ± 1 S.D.
5.4 In vivo evaluation of formulation

5.4.1 CAM assay

Figures 41 A and B show representative photographs of control PCL and 5% paclitaxel loaded PCL paste discs, respectively which had been placed on the CAM for 48 hours. The discs, which weighed approximately 3 mg each, were placed on the developing CAM and their effect on the development of vasculature in the CAM was observed visually. Figure 41 A shows that PCL paste discs alone did not alter development of vasculature. The pattern of blood vessels which developed underneath the PCL discs is identical to the blood vessel pattern throughout the photograph. In contrast, Figure 41 B shows that the paclitaxel loaded PCL disc significantly altered the CAM vasculature in the region adjacent to the disc, producing an avascular zone around the disc which had a diameter of about 4 mm. The paclitaxel induced avascular zone contained areas of altered blood flow continuity, disrupted vascular networks, and sparse blood vessel remnants. At the periphery of this zone, blood vessels are characterized by their altered “elbowing” appearance.

The antiangiogenic activities of paclitaxel released from PCL paste samples loaded with between 0.05 and 20% paclitaxel and 20% paclitaxel in PCL:MePEG 80:20 are given in Table 12. Inhibition of angiogenesis was observed for all formulations containing 0.25% paclitaxel and greater (7.5 μg of paclitaxel and greater). Surgical paste loaded with 0.1% (3.0μg) paclitaxel showed only partial angiogenesis inhibition with only 1 out of 8 samples giving a positive result for antiangiogenic activity. No antiangiogenic activity was seen for surgical paste samples loaded with 0.05% paclitaxel.
Figure 41: Representative optical micrographs showing the effect of 3 mg surgical paste disks on chick chorioallantoic membrane (CAM). The disks were placed on the CAM after 6 days of shell-less culturing and the images were taken 2 days later. A) Control surgical paste (PCL without paclitaxel) showing normal blood vessel architecture of CAM located about the surgical paste disk. B) 5% paclitaxel loaded PCL showing induced avascular zone measuring approximately 6 mm in diameter characterized by blood stasis and vessel disruption. Magnification = 8X.
Table 12: Inhibition of angiogenesis by paclitaxel loaded surgical paste discs using the chick chorioallantoic membrane assay.

<table>
<thead>
<tr>
<th>% Paclitaxel in polymer</th>
<th>Polymer</th>
<th>Weight paclitaxel in polymer (µg)</th>
<th>Antiangiogenesis (positive results/tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>PCL</td>
<td>1.5</td>
<td>0/9</td>
</tr>
<tr>
<td>0.1</td>
<td>PCL</td>
<td>3.0</td>
<td>1/8</td>
</tr>
<tr>
<td>0.25</td>
<td>PCL</td>
<td>7.5</td>
<td>4/4</td>
</tr>
<tr>
<td>0.5</td>
<td>PCL</td>
<td>15</td>
<td>4/4</td>
</tr>
<tr>
<td>1</td>
<td>PCL</td>
<td>30</td>
<td>8/8</td>
</tr>
<tr>
<td>5</td>
<td>PCL</td>
<td>150</td>
<td>4/4</td>
</tr>
<tr>
<td>10</td>
<td>PCL</td>
<td>300</td>
<td>5/5</td>
</tr>
<tr>
<td>20</td>
<td>PCL</td>
<td>600</td>
<td>12/13</td>
</tr>
<tr>
<td>20</td>
<td>PCL:MePEG 80:20</td>
<td>600</td>
<td>4/6</td>
</tr>
<tr>
<td>0</td>
<td>PCL &amp; PCL:MePEG 80:20</td>
<td>0</td>
<td>0/30</td>
</tr>
</tbody>
</table>

* Positive angiogenesis inhibition defined as avascular zone surrounding surgical paste disc of at least 4 mm in diameter.
5.4.1.1 HPLC assay

5.4.1.2 Specificity, stability, and recovery

Representative HPLC chromatograms of paclitaxel recovered from mouse tissues are shown in Figure 42. At about 1 minute a solvent front peak was observed in all chromatograms consisting of the acetonitrile and other components which passed through the column without interacting with the stationary phase. Paclitaxel eluted at 2.1 minutes when a mobile phase flow rate of 1.5 mL was used (Figure 42 A) and at 4 minutes when a mobile phase flow rate of 1 mL per minute was used (Figure 42 B). Blank mouse tissue samples, no paclitaxel added, including whole blood, lung, liver, spleen and kidney, were run using a mobile phase flow rate of 1.5 mL per minute and gave chromatograms which did not exhibit any deviations from the baseline at 2.1 minutes except for the blood and liver samples. In the case of the blood sample, a peak was observed at 2.1 minutes. In the case of the liver sample, the solvent front peak was large and had not yet completely passed through the detector at 2.1 minutes. As a result the presence of a peak at 2.1 minutes could not be determined since the absorbance level detected at 2.1 minutes had not returned to baseline levels.

The mouse tissues were spiked with either 5, 50, or 150 μg paclitaxel per gram of tissue. A sample of each tissue was also spiked with 50 μg paclitaxel per gram of tissue and stored at -20° C for 18 days. A percent recovery of paclitaxel from these tissues was calculated by running these samples through the assay and comparing the paclitaxel peak area obtained with those from control runs, in which the paclitaxel was present but no tissue was used. Table 13 shows the percent recovery data for each organ tested at the different paclitaxel loadings. The
percent recovery of paclitaxel from blood, liver and kidney at paclitaxel loadings of 5 μg/mL were all greater than 100%. This discrepancy was thought to be due to the assay being unreliable at low paclitaxel loading levels. Recovery of samples spiked at 50 μg/g dropped by about 30% over 18 days at -20° C indicating that the paclitaxel was not stable in the mouse tissue over this time.

The observation that the chromatograms of blank tissues did not show a peak at 2.1 minutes indicates that the assay is specific for paclitaxel in lung, spleen and kidney. Subsequent runs were conducted using a flow rate of 1 mL/min so that the paclitaxel would elute at a later time and not experience interference from the large solvent front peak seen with the liver samples. The paclitaxel peak observed in blank blood samples was due to contamination of the Polytron and will be addressed in the next section.

### 5.4.1.3 Precision, accuracy, linearity, range, and limit of detection

Using a flow rate of 1 mL per minute, the retention time for paclitaxel elution was 4.05 ± 0.01 (mean ± 1 SD) minutes and ranged from 4.017 minutes to 4.067 minutes. The precision data are given in Table 14. Acceptable assay precision was taken to be a %CV at the lowest concentration measured, which was < 20% and < 15% at all other concentrations. These criteria were only met using this HPLC assay at paclitaxel concentrations > 10 μg/g whole blood. As a result, all further calculations were performed on samples at concentrations > 10 μg/g. The ANOVA tests indicated statistically significant interday variation in precision data (at 10 and 25 μg/g) which meant that fresh standard curves would need to be constructed on each sampling day.
The assay was linear with the average $r^2$ value for the 12 standard curves run being $0.9939 \pm 0.0075$, and a range of 0.9727 to 0.9995. The accuracy data (Table 15) show significant interday variation as well. Bias values are included for each concentration each day. At 10 $\mu$g/g the bias averages 20.6% but at all other concentrations the bias is less than 10% with the exception of the 100 $\mu$g/g sample on day 1 (10.15%).

The blank blood samples showed paclitaxel peaks which were thought to be due to contamination of the Polytron with paclitaxel. This was confirmed by processing blank blood samples again following a thorough cleaning of the Polytron. These samples did not show peaks eluting where paclitaxel would be expected (Figure 42C).

Following Polytron cleaning it remained to be confirmed that the cleaning procedure did not adversely affect the precision data obtained. Fresh blood samples were spiked with either 10, 50, or 150 $\mu$g/g paclitaxel (n = 3) and analyzed with the assay. All % CV values obtained were less than 10% indicating that the assay met acceptable precision criteria between 10 and 150 $\mu$g/g (Table 16) and was acceptable for use in analyzing paclitaxel in mouse blood within the limits stated above.

The limit of detection for paclitaxel was found by injecting continually decreasing amounts of paclitaxel into the HPLC until the signal to noise ratio was less than 5. The limit of detection for paclitaxel was found to be less than 0.02 $\mu$g.
Figure 42: Representative HPLC chromatograms for paclitaxel in mouse tissue. A) Paclitaxel in kidney spiked at 150 μg paclitaxel/g kidney tissue with a mobile phase flow rate of 1.5 mL/min; B) paclitaxel in whole mouse blood spiked at 150 μg paclitaxel/g whole blood using a mobile phase flow rate of 1 mL/min; and C) sample of blank whole mouse blood using a mobile phase flow rate of 1 mL/min.
Table 13: Percent recovery of paclitaxel from mouse tissues using an HPLC assay. Values were calculated by dividing the paclitaxel peak areas obtained from tissues which had been spiked with paclitaxel with those from paclitaxel samples without mouse tissues present. Chromatographic parameters: 30 μL injections were made into a C-18 column with a mobile phase of ACN:water 50:50 flowing at 1.5 mL per minute and a uv detector set at 232 nm. Values listed represent the average of 2 injections from single samples.

<table>
<thead>
<tr>
<th>Paclitaxel loading</th>
<th>Blood</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μg/g</td>
<td>109.4</td>
<td>0.0</td>
<td>185.4</td>
<td>0.0</td>
<td>119.7</td>
</tr>
<tr>
<td>50 μg/g</td>
<td>86.0</td>
<td>90.1</td>
<td>sample lost</td>
<td>66.4</td>
<td>70.3</td>
</tr>
<tr>
<td>150 μg/g</td>
<td>60.9</td>
<td>54.1</td>
<td>88.0</td>
<td>72.1</td>
<td>63.6</td>
</tr>
<tr>
<td>50 μg/g (18 d)a</td>
<td>67.7</td>
<td>49.7</td>
<td>67.0</td>
<td>41.0</td>
<td>48.2</td>
</tr>
</tbody>
</table>

* a Samples were spiked with 50 μg/g paclitaxel then stored at -20° C for 18 days prior to analysis.
Table 14: Inter- and intra-day precision of an HPLC standard curve of paclitaxel in whole mouse blood.

<table>
<thead>
<tr>
<th>Paclitaxel Concentration (µg/g)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>All days</th>
<th>ANOVA p value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Area&lt;sup&gt;a&lt;/sup&gt;</td>
<td>%CV&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Peak Area</td>
<td>%CV</td>
<td>Peak Area</td>
</tr>
<tr>
<td>0</td>
<td>432403</td>
<td>-</td>
<td>514804</td>
<td>-</td>
<td>68677</td>
</tr>
<tr>
<td>1</td>
<td>32314</td>
<td>52.37</td>
<td>46217</td>
<td>26.89</td>
<td>172289</td>
</tr>
<tr>
<td>10</td>
<td>315903</td>
<td>18.29</td>
<td>465432</td>
<td>2.06</td>
<td>471251</td>
</tr>
<tr>
<td>25</td>
<td>1008117</td>
<td>8.27</td>
<td>1208832</td>
<td>2.43</td>
<td>1165346</td>
</tr>
<tr>
<td>50</td>
<td>2163632</td>
<td>6.61</td>
<td>2289910</td>
<td>12.36</td>
<td>2530149</td>
</tr>
<tr>
<td>100</td>
<td>4504214</td>
<td>6.90</td>
<td>4669362</td>
<td>9.55</td>
<td>5192666</td>
</tr>
<tr>
<td>150</td>
<td>7724899</td>
<td>10.30</td>
<td>7360154</td>
<td>5.57</td>
<td>7850337</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peak area is the average calculated from four standard curves at each concentration for each day (n=16 for all days)

<sup>b</sup> %CV is the ratio of the standard deviation to the average, expressed as a percentage. A value less than 20% at the lowest concentration and less than 15% at all other concentrations indicated sufficient precision

<sup>c</sup> ANOVA p values indicate the level of significance for single factor ANOVA tests between each day’s peak areas at each concentration. P < 0.05 indicates a significant difference between peak areas on different days.
Table 15: Accuracy of HPLC standard curves of paclitaxel in mouse whole blood.

<table>
<thead>
<tr>
<th>Paclitaxel Concentration (µg/g)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured value</td>
<td>%CV</td>
<td>Bias (%)</td>
</tr>
<tr>
<td>10</td>
<td>13.39</td>
<td>7.03</td>
<td>3.39</td>
</tr>
<tr>
<td>25</td>
<td>26.70</td>
<td>5.20</td>
<td>1.70</td>
</tr>
<tr>
<td>50</td>
<td>48.21</td>
<td>6.88</td>
<td>-1.79</td>
</tr>
<tr>
<td>100</td>
<td>89.85</td>
<td>3.23</td>
<td>-10.15</td>
</tr>
<tr>
<td>150</td>
<td>154.88</td>
<td>11.81</td>
<td>4.88</td>
</tr>
</tbody>
</table>

<sup>a</sup> Measured value is the average (n = 3 for each day) of values of concentration calculated from one standard curve on that day.

<sup>b</sup> %CV is the ratio of the standard deviation to the average, expressed as a percentage. A value less than 15% indicates sufficient accuracy at each concentration.

<sup>c</sup> Bias is the ratio of the deviation of measured value from the actual concentration measured, expressed as a percentage.

Table 16: Precision of HPLC standard curves for paclitaxel in whole mouse blood that were generated after the Polytron instrument was cleaned.

<table>
<thead>
<tr>
<th>Paclitaxel Concentration (µg/g)</th>
<th>Peak Area</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>638441</td>
<td>3.80</td>
</tr>
<tr>
<td>50</td>
<td>3189384</td>
<td>4.76</td>
</tr>
<tr>
<td>150</td>
<td>9766210</td>
<td>8.73</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peak area is the average calculated from 3 standard curves generated in one day.

<sup>b</sup> %CV is the ratio of the standard deviation to the average, expressed as a percentage. A value less than 15% indicates sufficient precision at each concentration.
5.4.2 Pilot study of paclitaxel distribution in mice from surgical paste

A pilot biodistribution study was conducted using only 9 animals to measure the distribution of paclitaxel in mice following subcutaneous implantation of 20% paclitaxel loaded PCL:MePEG 80:20. The HPLC assay was used to measure the paclitaxel concentrations in the mouse tissues. The mouse identification numbers, weight, treatment group and paste dose are summarized in Table 17. The mice all weighed about 20 g with the exception of mouse 2-1 (25 g). Paste dose administered to each animal was calculated as the difference in syringe weight before and after polymer injection. The paste dose of 303 mg reported for animal 1-4 reflects the sum of the weight of polymer injected and spilled. It is not known how much polymer was injected into the animal.

Paclitaxel could only be detected in the liver (2.0 to 6.7 µg/g) and muscle tissue which had been adjacent to the paste pellet (Table 18). Of the three mice which served as controls (PCL:MePEG 80:20 was injected with no drug) one was sacrificed after 2 days and two were sacrificed after 17 days. Paclitaxel was detected in the muscle tissue on which the paste pellets were injected. In the first two days, the levels of paclitaxel measured in the muscle varied between 223 and 1692 µg/g muscle tissue. There was no detectable difference between the levels measured at 0.25 days and the levels of paclitaxel measured at 2 days. By 17 days however, the paclitaxel measured had dropped to 6.8 µg/g in one animal and was not detected at all in the other.

The values reported here for paclitaxel in the liver are below the level of quantitation for the HPLC assay (10 µg/mL). Detection of paclitaxel in tissues outside the paste implant suggests that paclitaxel is released from the formulation in vivo. Paclitaxel was present in the muscle...
tissue at high levels for at least two days but these levels had decreased to below the minimum level of quantitation for the assay by two weeks. Detection of paclitaxel in the liver but not the blood was felt to be due to the low sensitivity of the assay and to the fact that the liver is the major organ of paclitaxel metabolism and some collection of drug may have occurred here.

In order to quantitate the levels of paclitaxel found in the mouse tissues accurately, a more sensitive assay for paclitaxel in whole blood and mouse tissues was needed. To achieve this, subsequent biodistribution studies were conducted with a radiolabel assay using tritium labelled paclitaxel (\(^3\)H-paclitaxel).
Table 17: Initial data for pilot study of biodistribution of paclitaxel from injected pellets of surgical paste

<table>
<thead>
<tr>
<th>Mouse ID</th>
<th>Weight (g)</th>
<th>Treatment group</th>
<th>Paste dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>19.5</td>
<td>Control</td>
<td>76</td>
</tr>
<tr>
<td>1-2</td>
<td>21.1</td>
<td>Control</td>
<td>32</td>
</tr>
<tr>
<td>1-3</td>
<td>22.0</td>
<td>Control</td>
<td>45</td>
</tr>
<tr>
<td>1-4</td>
<td>18.5</td>
<td>20% paclitaxel</td>
<td>303*</td>
</tr>
<tr>
<td>1-5</td>
<td>19.1</td>
<td>20% paclitaxel</td>
<td>78</td>
</tr>
<tr>
<td>2-1</td>
<td>25.0</td>
<td>20% paclitaxel</td>
<td>10</td>
</tr>
<tr>
<td>2-2</td>
<td>19.9</td>
<td>20% paclitaxel</td>
<td>57</td>
</tr>
<tr>
<td>2-3</td>
<td>19.7</td>
<td>20% paclitaxel</td>
<td>49</td>
</tr>
<tr>
<td>2-4</td>
<td>19.7</td>
<td>20% paclitaxel</td>
<td>43</td>
</tr>
</tbody>
</table>

*Luer-lok failure, most of the surgical paste used spilled and was not injected
Table 18: Distribution of paclitaxel in mouse liver and muscle tissues following surgical paste administration via subcutaneous injection of either control PCL:MePEG 80:20 or 20% paclitaxel loaded PCL:MePEG 80:20. Paclitaxel was quantified using an HPLC assay.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Treatment group</th>
<th>Time sacrificed (days)</th>
<th>Liver (µg/g tissue)</th>
<th>Muscle (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Control</td>
<td>17</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1-2</td>
<td>Control</td>
<td>2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1-3</td>
<td>Control</td>
<td>17</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1-4</td>
<td>20% paclitaxel</td>
<td>0.25</td>
<td>6.7</td>
<td>223.7</td>
</tr>
<tr>
<td>1-5</td>
<td>20% paclitaxel</td>
<td>0.25</td>
<td>4.8</td>
<td>1691.6</td>
</tr>
<tr>
<td>2-1</td>
<td>20% paclitaxel</td>
<td>2</td>
<td>2.2</td>
<td>423.2</td>
</tr>
<tr>
<td>2-2</td>
<td>20% paclitaxel</td>
<td>2</td>
<td>2.0</td>
<td>1363.0</td>
</tr>
<tr>
<td>2-3</td>
<td>20% paclitaxel</td>
<td>17</td>
<td>4.8</td>
<td>6.8</td>
</tr>
<tr>
<td>2-4</td>
<td>20% paclitaxel</td>
<td>17</td>
<td>3.0</td>
<td>ND</td>
</tr>
</tbody>
</table>

* The muscle tissue harvested was located immediately adjacent to the injected paste pellet and represents the site of action of the paclitaxel.
5.4.3 Characterization of surgical paste samples following implantation in mice

Characterization of the paste pellets recovered from the mice included, analysis of residual paclitaxel content, GPC, and DSC.

GPC chromatograms from the three PCL:MePEG 80:20 polymer pellets which had been implanted into mice for either 2 or 17 days are shown in Figure 43. In all three chromatograms, a single peak was observed at 5.6 minutes which corresponds to the PCL. No evidence of a decrease in the molecular weight of PCL is seen since the retention time for the PCL did not change between 2 and 17 days, and no MePEG peak was observed in any of the chromatograms.

DSC analysis was performed on paste samples that were recovered from mice (Figure 44). Peaks corresponding to the crystallization of PCL at about 20° C on cooling and the melting of PCL at 50° C on heating were observed. No MePEG peaks were detected, and a glass transition for PCL was observed at -64° C, although this is not visible on the scale used in Figure 44.

Table 19 gives the percentage of paclitaxel remaining in each polymer sample which had been recovered from the mice. Two residual paclitaxel percentage values are reported for each sample. The first reflects the measured percentage of paclitaxel in the recovered pellet per weight of paste sample recovered. The second value is a corrected value which accounts for the MePEG which is assumed to have diffused from the paste sample in vivo. It is based on the fact that MePEG made up 16% of the original weight of the injected paste.
Figure 43: GPC chromatograms of PCL:MePEG 80:20 paste pellets removed from mice following 2 days implantation (A) and 17 days implantation (B and C). The PCL peak can be seen at 5.6 minutes and no MePEG peak was observed. Chromatographic conditions: $10^3$ Å column with mobile phase of chloroform flowing at 1 mL per minute with refractive index detection.
Figure 44: DSC thermograms of PCL:MePEG 80:20 (± 20% paclitaxel) pellets removed from mice after 2 and 17 days. Both cooling and heating runs were done using a scan rate of 40° per minute.
Table 19: Percentage of paclitaxel remaining in surgical paste pellets which were recovered from mice following subcutaneous implantation. Reported values represent the mean paclitaxel percentage in 3 paste fragments from a single pellet.

<table>
<thead>
<tr>
<th>Sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time (days)</th>
<th>% paclitaxel ± 1 S.D.</th>
<th>Corrected&lt;sup&gt;b&lt;/sup&gt; % paclitaxel ± 1 S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>0.25</td>
<td>21.6 ± 0.6</td>
<td>18.0 ± 0.5</td>
</tr>
<tr>
<td>2-1</td>
<td>2</td>
<td>23.8 ± 4.2</td>
<td>20.0 ± 3.7</td>
</tr>
<tr>
<td>2-2</td>
<td>2</td>
<td>26.0 ± 1.9</td>
<td>22.0 ± 1.7</td>
</tr>
<tr>
<td>2-3</td>
<td>17</td>
<td>20.3 ± 1.6</td>
<td>16.9 ± 1.4</td>
</tr>
<tr>
<td>2-4</td>
<td>17</td>
<td>21.1 ± 0.6</td>
<td>17.6 ± 0.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Initial surgical paste samples were 20% paclitaxel loaded PCL:MePEG 80:20.

<sup>b</sup> Assumes MePEG had diffused out of formulation. Paclitaxel concentration calculated using polymer weight which would have been present had MePEG not diffused out.
5.4.4 $^3$H-paclitaxel assay

An assay for paclitaxel distribution studies in mice, which was more sensitive than the HPLC assay was required and a radiolabel assay using $^3$H-paclitaxel was developed. Table 20 shows the precision data obtained using the assay for $^3$H-paclitaxel from paste and mouse tissues at different spiked levels of radioactivity. The percent coefficients of variation are never greater then 15% for each tissue at each loading level.

The accuracy was determined by calculating regression equations of standard curves for each organ/tissue. The linearity of each curve was determined by calculating $r^2$ values and the last standard curve was used to give a set of predicted values using the measurements from each individual vial from the other standard curves. The linearity of the standard curves was confirmed by the $r^2$ values of all curves being greater than 0.999. Tables 21 through 28 give average predicted values, %CV, % bias and % recovery values for each organ. The % bias was calculated as the deviation of the predicted value from the true value, as a percentage of the true value. The % recovery value reported is the percentage of the counts obtained from mouse tissues, compared to the counts obtained when an equal amount of $^3$H-paclitaxel was analyzed with no mouse tissue present.

The data show that the radio assay for $^3$H-paclitaxel in mouse tissue is specific, precise, accurate, linear, and gives reasonable recovery. The ruggedness of the assay in terms of interday variability was not assessed since standard curves would be used on each day that tissues were analyzed.
Table 20: Precision of $^3$H-paclitaxel assay in mouse tissues and polymer paste. Samples were spiked with different amounts of $^3$H-paclitaxel corresponding to the radioactivity levels shown in the table. The %CV obtained from analysis of 4 tissues spiked at each radioactivity level are given.

<table>
<thead>
<tr>
<th>Radioactivity ($\mu$Ci)</th>
<th>%CV in $^3$H-paclitaxel data obtained from mouse tissues and the polymer paste</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td>0.0001</td>
<td>9.84</td>
</tr>
<tr>
<td>0.001</td>
<td>8.74</td>
</tr>
<tr>
<td>0.01</td>
<td>3.58</td>
</tr>
<tr>
<td>0.1</td>
<td>1.07</td>
</tr>
<tr>
<td>1</td>
<td>0.64</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Table 21: Ability of a standard curve of $^3$H-paclitaxel in whole mouse blood to predict the amount of radioactivity in three whole blood samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by extracting the $^3$H-paclitaxel into acetonitrile and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity ($\mu$Ci)</th>
<th>Average predicted value ($\mu$Ci)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>6.96</td>
<td>4.54</td>
<td>44.55</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0010</td>
<td>6.30</td>
<td>3.33</td>
<td>38.63</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0102</td>
<td>3.07</td>
<td>2.30</td>
<td>36.33</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1015</td>
<td>0.98</td>
<td>1.46</td>
<td>37.29</td>
</tr>
<tr>
<td>1</td>
<td>0.9786</td>
<td>0.78</td>
<td>-2.14</td>
<td>36.56</td>
</tr>
</tbody>
</table>
Table 22: Ability of a standard curve of $^3$H-paclitaxel in mouse heart tissue to predict the amount of radioactivity in three heart samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by extracting the $^3$H-paclitaxel into acetonitrile and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity (μCi)</th>
<th>Average predicted value (μCi)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>7.37</td>
<td>10.80</td>
<td>50.09</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0010</td>
<td>3.60</td>
<td>2.02</td>
<td>42.56</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0106</td>
<td>2.47</td>
<td>6.01</td>
<td>42.87</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1025</td>
<td>1.35</td>
<td>2.49</td>
<td>42.74</td>
</tr>
<tr>
<td>1</td>
<td>0.9927</td>
<td>1.31</td>
<td>-0.73</td>
<td>42.03</td>
</tr>
</tbody>
</table>

Table 23: Ability of a standard curve of $^3$H-paclitaxel in mouse lung tissue to predict the amount of radioactivity in three lung samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by extracting the $^3$H-paclitaxel into acetonitrile and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity (μCi)</th>
<th>Average predicted value (μCi)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>2.66</td>
<td>11.45</td>
<td>51.25</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0010</td>
<td>1.59</td>
<td>1.60</td>
<td>43.43</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0099</td>
<td>1.13</td>
<td>-0.58</td>
<td>42.40</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0980</td>
<td>0.96</td>
<td>-2.01</td>
<td>42.98</td>
</tr>
<tr>
<td>1</td>
<td>0.9858</td>
<td>0.60</td>
<td>-1.42</td>
<td>43.84</td>
</tr>
</tbody>
</table>
Table 24: Ability of a standard curve of $^3$H-paclitaxel in mouse liver tissue to predict the amount of radioactivity in three liver samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by extracting the $^3$H-paclitaxel into acetonitrile and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity (μCi)</th>
<th>Average predicted value (μCi)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>0.00</td>
<td>19.77</td>
<td>46.07</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0010</td>
<td>5.13</td>
<td>-3.13</td>
<td>34.96</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0107</td>
<td>0.66</td>
<td>6.57</td>
<td>38.30</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1009</td>
<td>1.30</td>
<td>0.88</td>
<td>37.27</td>
</tr>
<tr>
<td>1</td>
<td>0.9924</td>
<td>0.38</td>
<td>-0.76</td>
<td>37.20</td>
</tr>
</tbody>
</table>

Table 25: Ability of a standard curve of $^3$H-paclitaxel in mouse spleen tissue to predict the amount of radioactivity in three spleen samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by extracting the $^3$H-paclitaxel into acetonitrile and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity (μCi)</th>
<th>Average predicted value (μCi)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>5.57</td>
<td>-17.55</td>
<td>42.77</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0009</td>
<td>4.48</td>
<td>-10.31</td>
<td>38.50</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0101</td>
<td>1.07</td>
<td>0.80</td>
<td>43.66</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0987</td>
<td>1.41</td>
<td>-1.32</td>
<td>43.76</td>
</tr>
<tr>
<td>1</td>
<td>0.9802</td>
<td>1.90</td>
<td>-1.98</td>
<td>44.08</td>
</tr>
</tbody>
</table>
Table 26: Ability of a standard curve of $^3$H-paclitaxel in mouse kidney tissue to predict the amount of radioactivity in three kidney samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by extracting the $^3$H-paclitaxel into acetonitrile and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity ($\mu$Ci)</th>
<th>Average predicted value ($\mu$Ci)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>2.26</td>
<td>2.45</td>
<td>47.95</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0010</td>
<td>3.70</td>
<td>-3.53</td>
<td>41.56</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0098</td>
<td>7.08</td>
<td>-2.03</td>
<td>42.54</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0922</td>
<td>8.80</td>
<td>-7.84</td>
<td>41.42</td>
</tr>
<tr>
<td>1</td>
<td>0.9345</td>
<td>5.14</td>
<td>-6.55</td>
<td>42.20</td>
</tr>
</tbody>
</table>

Table 27: Ability of a standard curve of $^3$H-paclitaxel in mouse muscle tissue to predict the amount of radioactivity in three muscle samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by extracting the $^3$H-paclitaxel into acetonitrile and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity ($\mu$Ci)</th>
<th>Average predicted value ($\mu$Ci)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>12.85</td>
<td>6.01</td>
<td>52.86</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0010</td>
<td>2.62</td>
<td>2.77</td>
<td>43.62</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0104</td>
<td>1.03</td>
<td>4.01</td>
<td>43.36</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1017</td>
<td>2.60</td>
<td>1.71</td>
<td>43.84</td>
</tr>
<tr>
<td>1</td>
<td>1.0154</td>
<td>1.65</td>
<td>1.54</td>
<td>44.27</td>
</tr>
</tbody>
</table>
Table 28: Ability of a standard curve of $^3$H-paclitaxel in PCL:MePEG 80:20 paste to predict the amount of radioactivity in three paste samples of known radioactive content. The standard curve was generated as a function of the amount of radioactivity added. Radioactivity was measured by dissolving the paste pellet in dichloromethane and measuring the radioactivity using a scintillation counter. The recovery of radioactivity, compared with the counts obtained in samples which did not contain tissue are also reported.

<table>
<thead>
<tr>
<th>Radioactivity (μCi)</th>
<th>Average predicted value (μCi)</th>
<th>% CV</th>
<th>% bias</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>6.85</td>
<td>3.35</td>
<td>59.17</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0011</td>
<td>1.18</td>
<td>6.86</td>
<td>56.09</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0103</td>
<td>1.89</td>
<td>3.38</td>
<td>54.47</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1034</td>
<td>0.97</td>
<td>3.40</td>
<td>55.91</td>
</tr>
<tr>
<td>1</td>
<td>1.0434</td>
<td>6.11</td>
<td>4.34</td>
<td>57.18</td>
</tr>
<tr>
<td>2</td>
<td>1.9868</td>
<td>1.10</td>
<td>-0.66</td>
<td>61.53</td>
</tr>
</tbody>
</table>
5.4.5 Distribution study in mice

The weight gain of the mice by treatment category up to day 30 is shown in Figure 45. There was no difference in growth between the mice in the different treatment groups (single variable ANOVA).

On day 15, it was noted that the mice who had received paclitaxel loaded paste had open sores at the injection site which surrounded the now exposed polymer implant. This was believed to be due to the delay in wound healing caused by paclitaxel. The application of anesthetic cream to the open sores on the mice was initiated on an “as needed” basis following this day. By day 30, the sores on the paclitaxel treated mice had healed over but on sacrifice, only six of the 10 mice still had paste pellets remaining and these were all less than 5 mg.

The decrease in paclitaxel concentration in the paste pellets recovered from the animals is shown in Figure 46. By day 15, the drug concentration had dropped to 60% of its initial value. Data for day 30 were not included because the large percentage error associated with weighing the paste fragments, resulted in large errors in estimating the paclitaxel content of the paste. The measured paclitaxel concentration in the paste at day 15 was significantly lower than the initial paclitaxel concentration (Student’s t-test; p < 0.004).

The concentrations of paclitaxel detected in each of the tissues at different times are shown in Table 29. In the blood, paclitaxel was detected at 6 hours at a level of 10.5 µg/g, and this decreased to 3.3 µg/g by day 2. No paclitaxel was detected in blood on days 15 or 30. In liver, the paclitaxel concentration increased from 6 hours to 2 days (15.9 µg/g to 30.7 µg/g). By days 15 and 30 the paclitaxel levels in the liver had dropped to 0.5 and 0.2 µg/g, respectively. The muscle tissue immediately adjacent to the surgical paste pellet was analyzed for paclitaxel
content and represented the site of action for the drug. Paclitaxel concentration was above 10 μg/g in the muscle tissue at all time points (up to 30 days). The level of paclitaxel detected decreased over that time period from 70.8 μg/g at 6 hours, to 11.4 μg/g at 30 days. No paclitaxel was detected in the heart, lung, spleen, or kidney of any of the mice.
Figure 45: Change in weight observed in mice treated with $^3$H-paclitaxel labelled surgical paste pellets. The mice were divided into 3 groups. Sham group, (n = 4), underwent procedure but no paste injected; control group, (n = 6) paste matrix injected, no drug; and treatment group, (n = 10) $^3$H-paclitaxel labelled PCL:MePEG 80:20 injected (20% paclitaxel loaded). Injections were made with 125 mg surgical paste, subcutaneously in the back of the neck.
Figure 46: Concentration of $^3$H-paclitaxel, expressed as the percent of $^3$H-paclitaxel remaining, measured in surgical paste pellets which were surgically removed following implantation in mice. $^3$H-paclitaxel was measured using a radioassay and points reflect the mean of 10 samples ± 1 S.D.
Table 29: Concentration of $^3$H-paclitaxel detected in mouse tissues following implantation of paste. Concentrations are expressed in units as μg/g tissue and are calculated as if all the radioactivity represented paclitaxel and not a metabolite. Data are expressed as the mean of 10 samples ± 1 S.D.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Concentration of $^3$H-paclitaxel (μg/g tissue)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Whole blood</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>-</td>
</tr>
<tr>
<td>Muscle</td>
<td>-</td>
</tr>
<tr>
<td>(adjacent to paste)</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
</tr>
<tr>
<td>Spleen</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ It was not known whether the radioactivity detected came from $^3$H-paclitaxel or from a tritium labelled metabolite of paclitaxel. For the purposes of the table only, the concentrations were calculated as if all the radioactivity was generated by $^3$H-paclitaxel. ND = not detected
6. DISCUSSION

6.1 Solubility of paclitaxel

In water, paclitaxel was found to have an apparent solubility of 3.6 μg/mL at 37° C giving rise to a supersaturated solution which recrystallized as a more stable form of paclitaxel with a lower solubility (1.4 μg/mL). This stable form of paclitaxel has been reported to be paclitaxel dihydrate (Liggins et al. 1997).

The presence of albumin in PBS buffer stabilized the dissolved paclitaxel (Figure 8). Without albumin, the paclitaxel concentration rose to a peak after about 2 hours in PBS buffer at 37° C, and then crystallized as a more stable form as observed for dissolution in water. With albumin present, the paclitaxel concentration in solution was stable at 1.4 μg/mL for 24 hours. Paclitaxel is bound by plasma proteins (eg. albumin) in vivo, and a large fraction (>90 - 97%) of the drug is transported within the blood in the bound state (Suffness 1995). The presence of albumin in the suspending medium enhanced the solubility of the paclitaxel in the anhydrous form and prevented the precipitation of paclitaxel in to a less stable form in the time frame studied.

6.2 Physicochemical characterization of formulation

6.2.1 X-ray diffraction of PCL:MePEG blends

The X-ray diffraction scans for PCL and PCL:MePEG 80:20 are shown in Figure 9. The peak locations and d-spacings are given in Table 1 and agree with those reported for PCL in the literature (Ong and Price 1978a) but with an extra peak at about 21.7° 2θ (d-
This was the least intense of the three peaks identified, and was located very close to the most intense, or base peak, at 21.1° 2θ (4.21 Å). Some of the X-ray scans performed on PCL and PCL blends (data not shown) did not have a peak at 21.7° 2θ, but rather a distinct shoulder was visible on the base peak at 21.1° 2θ. Blending of PCL with MePEG did not change the X-ray pattern of PCL indicating that at 25° C, the temperature at which the X-ray measurements were obtained, only the PCL existed in the crystalline form. A similar observation was made when PCL was blended with polyvinyl chloride with no change to the location of peaks in the X-ray pattern (Ong and Price 1978a).

The X-ray powder diffraction pattern of paclitaxel is shown in Figure 10 with scans of paclitaxel loaded PCL and PCL:MePEG 80:20 blends. In the paclitaxel loaded polymer patterns, peaks were observed for both paclitaxel, and PCL crystals. The peak locations and corresponding d-spacings are given in Table 3. The X-ray powder diffraction pattern of paclitaxel is not currently listed in the International Powder Diffraction Files but the pattern in Table 3 corresponded to the X-ray pattern reported for anhydrous paclitaxel (Liggins et al. 1997). Formulations of paclitaxel in PCL or PCL:MePEG 80:20 consisted of paclitaxel crystals incorporated in the semicrystalline polymer as shown by the presence of both paclitaxel peaks, and PCL peaks, in the X-ray scans of the blends. Increasing the paclitaxel content in the blend increased the relative intensities of the paclitaxel peaks, to the PCL peaks, due to changes in the relative fractions of paclitaxel and PCL crystals in the blends.

When paclitaxel was blended into molten PCL at a loading of 2.4%, paclitaxel, crystals were visible in the sample. There was no evidence of the presence of a crystalline
paclitaxel phase using X-ray analysis, indicating that this technique could not be used to measure the solubility of paclitaxel in PCL.

6.2.2 Thermal analysis of PCL:MePEG blends

6.2.2.1 Effect of heating rate and sample size on thermal properties and peak melt temperature

The heating rate and sample size were shown to have some effects on the thermal events measured for PCL by DSC (Table 3). As the heating rate was increased, the melting endotherm of PCL polymer was increased to slightly higher temperatures and the double melting peak for PCL, which was seen for PCL at low heating rates, was not apparent. The change in the shape of the melting endotherms with heating rate are an indication that the overall melting reaction cannot be an equilibrium transition because of the time dependence observed (Wunderlich 1990). The double melting endotherm can be explained to be due to the melting of metastable crystallites at a temperature below the melting point of more stable PCL crystals. The melt from the metastable crystals then underwent recrystallization to more stable PCL crystals. Further heating resulted in the melting of the more stable crystals and two melting endotherms were observed (Sweet and Bell 1972; Todoki Kawaguchi 1977; Nichols and Robertson 1992). This was not seen at higher heating rates because insufficient time was available for the recrystallization of more stable crystallites to occur (Wunderlich 1990). At a heating rate of 20° per minute, sufficient time was available for the crystallization of more stable PCL crystallites, which subsequently melted at a higher temperature (53.8° C).
Sample size affected the peak of both the melting and crystallization temperatures but not the onset of melting. The peak temperature of both the melt and crystallization transitions reflects the temperature at which the largest fraction of the polymer is undergoing the transition. Heat being added to, or removed from, the polymer must travel through the polymer from/to the instrument. The thermal lag introduced by samples of different size was manifested as an increase in the peak of the melting endotherm or a decrease in the peak of crystallization exotherm (Wunderlich 1990). Sample size did not affect the onset of melting. This is because the onset of melting reflects the first melting of the polymer sample which is in intimate contact with the sample pan, and this would not change with sample size.

6.2.2.2 Effect of quench cooling on thermal properties of PCL:MePEG blends

It has been reported that PCL crystallization cannot be prevented by quench cooling (Phillips et al. 1987). In the case of PCL:MePEG blends, quench cooling, even at low PCL fractions did not inhibit PCL crystallization (Figure 11). Although the volume fraction of PCL in the melt was reduced when blended with MePEG, the viscosity of the blend was also reduced, and PCL was still able to crystallize despite the rapid cooling.

After quench cooling, a small recrystallization peak was seen just prior to the melting endotherm of MePEG. MePEG (MW 350 g/mole), is made up of relatively small molecules which gives them greater mobility by reducing the likelihood of chain entanglements. As a result, MePEG crystallization also occurred following quench cooling of PCL:MePEG blends.
Quench cooling of polymer blends has been used as a means of inhibiting crystallization of polymeric components. A completely amorphous blend can then be studied, which may give more information about the miscibility of the blended polymers (Avramova 1995; Shibayama et al. 1995). In the case of PCL:MePEG blends, quench cooling did not inhibit PCL or MePEG crystallization, and therefore the composition of the amorphous phase was not identical to the initial blend composition. The Tg's observed for the quench cooled blends were a function of the composition of the amorphous phase which was unknown. Only one Tg was observed for each blend studied, which would indicate that the polymers were miscible at the concentrations present in the amorphous phase (Pitt 1990; Mark 1969). A second Tg, corresponding to the PCL component, was not observed. However, the melting endotherm of MePEG may have masked this transition.

6.2.2.3 Effect of blend ratios on thermal properties of PCL:MePEG blends

At all blend ratios of PCL and MePEG tested, the blend appeared homogeneous, both by visual inspection and under the microscope in the melt, but on cooling in the DSC, phase separation occurred. Crystallization exotherms for both PCL (~ 15° C) and MePEG (~ -30° C) were seen as the samples were cooled (Figure 12A). The peak temperatures of the crystallization exotherms of PCL and MePEG are listed in Table 4. For both blend components, the temperature of the peak of the crystallization exotherms decreased with the addition of the other polymer, indicating that the presence of the second polymer delayed crystallization. This could be due to either a decrease in the onset or rate of nucleation or a decrease in the growth rate of the crystals.
Thermal events on heating PCL:MePEG blends are shown in Figure 12B and Table 5. Heating the PCL:MePEG blends resulted in three distinct thermal events. A glass transition was followed by melting of first MePEG, then PCL crystals.

A single glass transition was observed at all blend ratios, between the Tg of each polymer alone. The single Tg indicated that the polymers were completely miscible. In support of the miscibility of PCL and MePEG was the microscopic and macroscopic visual observation that PCL:MePEG mixtures melted into a single homogeneous, transparent phase.

Examination of the glass transitions of the PCL:MePEG blends revealed that enthalpy relaxation occurred in blends of high MePEG concentration. The enthalpy relaxation peak can be seen clearly in the DSC scan of 100% MePEG as an endotherm just prior to the establishment of the baseline following the glass transition (Figure 13). Enthalpy relaxation is due to the development of regions containing short range order on storage below the Tg of a polymer (Wunderlich 1990). The time required for enthalpy relaxation to occur is inversely proportional to the mobility of the atoms within the polymer and therefore, molecules of low molecular weight would likely undergo enthalpy relaxation more rapidly than long chain polymers. This explains the presence of enthalpy relaxation peaks in the MePEG sample but not in the samples of higher PCL content.

Additional evidence in support of PCL and MePEG miscibility was obtained in the ternary solution test. In this test, the PCL and MePEG were dissolved in a common solvent and mixed together. If the two polymers were not miscible than we would expect them to have different solubilities in the common solvent (DCM) which would result in phase separation into a MePEG rich solution and a PCL rich solution in DCM. No phase separation
occurred and this can be taken as evidence for the miscibility of PCL and MePEG in a 1:1 ratio at room temperature (Domb 1993).

As a result of the phase separation which occurred in the PCL:MePEG blends on cooling, the composition of the amorphous phase, at temperatures close to the Tg did not reflect the initial composition of the blend. Since the Tg values of PCL:MePEG blends were dependent on the composition of the amorphous phase, the observed Tg’s deviated from those predicted by the Fox equation (Figure 14). Liu and Donovan noted that the Fox equation was developed for blends which were completely amorphous and may therefore not be valid for semicrystalline polymer blends (Liu and Donovan 1995).

PCL forms miscible blends with a variety of polymers because it contains both a nonpolar segment and a polar ester bond linkage capable of forming hydrogen bonds (Zhong and Guo 1997; Pitt 1990). The relatively polar MePEG molecules have polar ether groups and a terminal hydroxyl group which could form hydrogen bonds with PCL to give the polymers miscibility (Zhong and Guo 1997).

The melting of a polymer crystallite in a blend can be affected by interactions of the crystalline polymer chains with the solvent polymer chains, or the crystallite size and its perfection in the semicrystalline polymer (Liu and Donovan 1995; Alfonso and Russell 1986). This could theoretically be manifested as an increase or a decrease in the Tm or ΔH of the melting transition (Nishi and Wang 1975). Figure 12B and Table 5 showed that on heating, the MePEG melting endotherm was not affected by the presence of PCL. No change in the Tm of the MePEG crystals (Figure 12B) or the ΔH of fusion of MePEG, normalized
for MePEG weight (Figure 17), was detected, indicating that the MePEG crystals formed independently from PCL.

The melting of the PCL crystals was affected by blending with MePEG. Melting point depression was observed with the addition of MePEG but there was no change in $\Delta H$ of PCL melting (Figure 16). Therefore, less energy was needed to melt the PCL but the degree of PCL crystallinity remained unchanged. The melting point depression observed with the PCL crystals was approximately equivalent to the corresponding freezing point depression observed in the blends. That is, the degree of supercooling attained before PCL crystallization was induced (critical $\Delta T$) during DSC cooling, was similar for all the PCL:MePEG blends tested (Figure 15). Nucleation and crystallization of PCL crystals only occurred following attainment of a critical $\Delta T$. MePEG delayed PCL nucleation and crystallization by reducing the melting point. As a result, the critical $\Delta T$ required for PCL crystallization could only be attained if the crystallization temperature was also reduced.

6.2.2.4 Effect of paclitaxel on the thermal properties of PCL:MePEG blends

The melting point of paclitaxel was shown to be at 221°C (Figure 18A) and a $T_g$ of paclitaxel quenched from the melt has been reported at 153°C (Liggins 1997). When blended with PCL or PCL:MePEG 80:20, the melting peak of paclitaxel was no longer observed on DSC scans (Figure 18B). Paclitaxel crystals were visible in the surgical paste at room temperature, and were shown to be crystalline using X-ray analysis. When heated in the DSC however, the paclitaxel was no longer present as a crystalline form at 220°C. It was determined that on heating, paclitaxel dissolved in the polymer matrix at temperatures below 220°C. Figure 21 A - D is a series of photographs showing the thermal events which
occurred as a 20% paclitaxel loaded PCL:MePEG 80:20 sample was heated at 10° per minute. In Figure 21A both the polymer and paclitaxel crystals can be seen to make up the paste matrix. As the sample was heated, the polymer matrix melted and this was followed by the dissolution of the paclitaxel crystals into the molten polymer at elevated temperatures (Figure 21 B-D). This visual evidence served to explain the absence of a paclitaxel melting endotherm on heating paclitaxel loaded PCL:MePEG samples using DSC.

When paclitaxel loaded PCL:MePEG 80:20 was cooled from the melt and then subsequently heated through the melting of the polymer, no effect on the temperatures at which the PCL crystallized and melted was seen (Figure 18B). However, crystallization of MePEG was inhibited by the addition of paclitaxel since the cooling and heating curves revealed no peaks corresponding to MePEG crystals (Figure 18). The other major effect was that the Tg of the blend had been shifted from -88° C to -72° C. The increase in Tg relative to a PCL:MePEG blend without paclitaxel, was due to the paclitaxel blending with MePEG or MePEG:PCL to give a single amorphous phase characterized by this higher Tg. The Tg of this blend occurred over the same temperature range as the Tg of PCL and it was not possible to determine whether the amorphous PCL phase was separated from the MePEG/paclitaxel amorphous phase. The interaction of paclitaxel and MePEG may be due to hydrogen bonding between the paclitaxel and MePEG molecules. Although paclitaxel is hydrophobic, it contains 4 groups which are capable of forming hydrogen bonds and these may interact with the hydroxyl groups of the MePEG (Balasubramanian et al. 1994).

There are three possible states in which a drug may exist in the amorphous phase of a polymer: 1) crystalline drug particles dispersed in the polymer, 2) drug dissolved (miscible)
in the polymer in which it either stiffens or plasticizes the polymer chains, and 3) non-
crystalline drug is dispersed in the polymer matrix (Mumper and Jay 1992). Cases 1 and 3
represent drug-polymer phase separation in the amorphous regions of the matrix and no effect
of drug loading on Tg would be expected. Case 2 represents miscible dispersions in which
the polymer chains are stiffened by interactions with the drug molecules or plasticized by
interruption of polymer-polymer interactions by the presence of drug molecules.

Incorporation of paclitaxel into the PCL:MePEG 80:20 surgical paste likely caused a
stiffening of the MePEG chains since paclitaxel increased the Tg of the blend. The stiffening
may have resulted from the dissolution of paclitaxel into the amorphous phase of the surgical
paste and increased H bonding with the MePEG.

6.2.2.5 Melting point depression

The method of Nishi and Wang (1975) was employed to determine whether the
melting point depression of PCL due to the addition of MePEG was a function of PCL
lamellar thickness, or whether there was a change in the equilibrium melting point of PCL.
Nishi and Wang studied the case of a semicrystalline polymer blended with an amorphous,
non crystallizing polymer. The method assumes miscibility of the polymers, since otherwise
no melting point depression would be observed. In this work, the MePEG was considered to
be the amorphous polymer even though it is semicrystalline. This assumption was
considered to be acceptable because the MePEG did not undergo crystallization over the
temperature range studied.

The holding temperatures (Tc) employed in the study of isothermal crystallization of
PCL were similar to those used by Phillips and Rensch (1989) who were studying PCL
crystallization. They found that a Tc below 39° C resulted in PCL nucleation before Tc could be reached, and a Tc above 51° C resulted in long nucleation times of the order of several days.

The equilibrium melting points (Tm*) of the PCL crystals in PCL:MePEG blends were measured as a function of holding temperature (Tc) in Hoffman-Weeks isothermal crystallization experiments (Figure 19). MePEG caused a reduction in the equilibrium melting point of PCL in the blend, equal to Tm° - Tm*, where Tm° is the equilibrium melting point of 100% PCL. This indicated that the observed melting point depression was due, at least in part, to the thermodynamic effects of mixing PCL with MePEG (Table 6). This was supported by the negative $\chi_{12}$ value calculated using the Nishi and Wang method (-0.16) and the $\chi_{12}$ value calculated using group analysis (0.03) (Table 7) and suggested an interaction between PCL and MePEG. Calculation of $\chi_{12}$ using solubility parameters calculated from group contributions has been done for miscible poly(2,6-dialkyl-4-vinyl phenol) blends (Coleman et al. 1996). The low (or negative) value determined for $\chi_{12}$ was an indication that there was a low activation energy associated with the mixing or interaction of the two materials.

At 37° C, the addition of MePEG to PCL reduced the ΔG of PCL melting through its interaction with PCL chains. The reduction of ΔG is usually attributed to a reduction in the enthalpy of mixing between the polymer chains of the semicrystalline polymer and the amorphous diluent molecules. However, an increase in ΔS of mixing may also contribute to a reduction in ΔG (Nishi and Wang 1975; Liu and Donovan 1995; and Lee et al. 1997). A decrease in ΔG of PCL melting would lead to a reduction in the Tm* of the PCL in the blend.
There was no difference in the stability parameters of the PCL crystals as a function of the amount of MePEG added in the blend indicating that the presence of MePEG did not affect the morphology of the PCL crystals. If the melting point depression due to the addition of MePEG to PCL, was an effect of MePEG on the PCL lamellae, different stability parameters for the PCL crystals would be expected. Furthermore the Hoffman-Weeks plots would extrapolate to the same Tm° (Nishi and Wang 1975).

Literature values of the Tm° of PCL, over a similar molecular weight range as the one used here, range from 63° C (Defieuw et al. 1989c) to 69° C (Philips et al. 1987). The latter measurement of Tm° was made by observing the melting of the PCL crystals under polarized light, and the Tm was taken as the temperature at which the last birefringent species disappeared. The measured value for Tm° for PCL given in Table 6 (59.4° C) was slightly lower than that measured by Defieuw. The difference may be due to the fact that the peak of the melting endotherm was used as a measure of Tm in this work instead of the end of the melting endotherm since the peak Tm could be determined with greater accuracy. It is also possible that lower Tm° values were obtained because the holding times were not well controlled in our work. The Tm’s of the samples were measured soon after the primary crystallization peak had occurred. Philips and Rensch (1989) reported that PCL showed significant lamellar thickening for up to 3 days at a given Tc, which resulted in an increase in Tm. Our samples were held at Tc until only the primary crystallization was completed, and probably reflected the melting of the PCL lamellae that formed initially. The samples were never held at Tc for more than 1.5 hours and did not have time to anneal.
Paclitaxel did not change the stability parameters of PCL crystals formed under isothermal conditions from blends of PCL:MePEG 80:20 (Table 6) indicating that the morphology of the PCL crystals were unaffected by the incorporation of paclitaxel to the blend. Dilution of PCL with paclitaxel and MePEG caused less melting point depression than MePEG alone. When PCL was diluted to 64% of the total blend composition (20% paclitaxel in PCL:MePEG 80:20), the melting point depression was equivalent to the dilution of PCL to between 70 and 80% of the total blend composition with MePEG alone. That is the combination of paclitaxel and MePEG caused less melting point depression than MePEG alone. This implies that the amorphous phase consisting of paclitaxel and MePEG has a higher $\chi_{12}$ value with PCL than the MePEG:PCL alone, since a higher $\chi_{12}$ is reflected in a higher $\Delta G$ of the reaction. Melting point depression of PCL in a blend is less extensive, and therefore the $\Delta G$ of the reaction is less thermodynamically favorable, when the amorphous phase is paclitaxel and MePEG compared to MePEG alone. Since the $\Delta S$ of the PCL melting is not likely to be different, regardless of the amorphous phase (Nishi and Wang 1975), the difference in $\Delta G$ is likely due to a higher $\chi_{12}$ with the paclitaxel/MePEG amorphous phase.

6.2.2.6 Hot stage microscopy

The melting ranges of PCL and PCL:MePEG 80:20 blends were confirmed visually using the hot stage. The melting temperatures for 100% PCL were higher than those measured using the DSC. This was thought to be due to the time delay in visually observing a melting event, and to the lower heating rate used. Heating PCL at $10^5$ per minute gave the chains time to recrystallize after the initial melting of the metastable PCL crystallites, before the melting temperature of the more stable crystallites was reached. When scanning at lower
heating rates, higher Tm's can be observed due to crystalline lamellae having more time to realign into more perfect crystallites during the scan (Wunderlich 1990). This effect was not as prominent in the PCL:MePEG 80:20 blends. As discussed in Section 6.2.3.3, MePEG caused a delay in the crystallization of PCL which may have allowed the PCL crystals to form more stable lamellae.

Photographic images of the PCL crystals formed from a 20% paclitaxel loaded PCL:MePEG 80:20 blend following crystallization from the melt at 37° C (Figure 22A) showed evidence of paclitaxel crystals (see arrows). Nucleation of polymer crystals appeared to have occurred on the surface of the paclitaxel crystals, followed by polymer crystal growth away from the paclitaxel crystals. This phenomenon of polymer crystal nucleation on dispersed solid surfaces was reported for the nucleation of polyethylene glycol crystals on the surface of oxazepam particles (Gines et al. 1996). The ability of the paclitaxel crystals to act as heterogeneous nucleation sites for PCL crystallization provides an explanation as to why the ΔT of PCL crystallization in paclitaxel loaded PCL:MePEG blends was less than for PCL:MePEG blends alone. In section 6.2.3.3, it was determined that a minimum ΔT had to be attained before PCL crystal growth could occur on the heterogeneous nuclei present in PCL:MePEG blends. When paclitaxel crystals are present however, they probably serve as nucleation sites for PCL crystals. These sites are more stable than those present in PCL:MePEG blends and therefore, a lower ΔT is sufficient to drive the PCL crystallization process.

Evidence of banding of the PCL crystals was also apparent in Figure 22A. Although not fully understood, banding is a common effect seen in PCL blends with a non crystallizing
polymer. It is thought to be due to the twisting of fibrils as they grow outward from the
center of the spherulite. As this happens, the crystallographic axes of the fibrils is being
twisted. As a result, the orientation of the crystals periodically changes from one which
refracts light to one which is not birefringent and so, when viewed under polarized light the
banding image is seen (Keith et al. 1989). Banding has been induced in PCL spherulites by
PVC at PVC concentrations as low as 0.5% at a Tc of 40° C (Keith et al. 1989).

PCL crystallization from a PCL:MePEG 80:20 blend (paclitaxel absent) was shown in
Figure 22B. In contrast to PCL crystallization in paste containing paclitaxel, all the
spherulites originated from central nucleation sites and radiated outward, and the banding
effects were less evident. In the absence of the relatively large paclitaxel crystal surface as a
nucleation site, PCL nucleation probably occurred at smaller heterogeneous sites in the melt
with the result that the PCL crystals grew outwards from a central nucleation site and
assumed a circular appearance. Banding was less in evidence with this blend composition.
A possible explanation for this is that the composition of the amorphous phase may
determine the banding pattern. The amorphous phase in the paclitaxel loaded PCL:MePEG
80:20 was characterized by a higher Tg and was more viscous than an amorphous phase
containing MePEG alone. Furthermore, the concentration of PCL in the 20% paclitaxel in
PCL:MePEG 80:20 paste was 64%. This dilution effect of PCL by paclitaxel may also have
contributed to the banding observed.

PCL spherulites produced from a PCL:MePEG 60:40 blend (Figure 22C) appeared to
have originated from central heterogeneous nucleation sites and some evidence of banding
was observed. In both Figures 22 B and C the boundaries between adjacent spherulites can
be seen as “straight lines” half way between the spherulite centers. This has been described as evidence that the growth rates of the spherulites within each blend were identical and that nucleation occurred at approximately the same time (Hearle 1982).

PCL crystallization in progress from a blend of PCL:MePEG 20:80 (Figure 22D) showed the PCL crystals radiating outward from a central nucleation site. The coarse appearance of the spherulites (large space between PCL fibrils) was due to the large dilution of PCL with MePEG. Banding was evident in the spherulites as the large volume of amorphous MePEG caused the growing PCL fibrils to twist.

6.2.3 Mechanical testing of PCL:MePEG blends

Addition of MePEG to PCL caused a reduction in the Tg of the blend and in the Tm of the PCL. This would produce a less viscous paste because, at any temperature above the Tg, the blend would be relatively closer to the Tm of PCL and farther from its Tg.

The effect of MePEG on the melt viscosity of blends with PCL was measured and showed that blending MePEG with PCL reduced the viscosity of the melt at 60° C (Figure 25). At each blend concentration, the viscosity was independent of strain rate within the range of strain rates tested. The relatively small MePEG molecules probably plasticized PCL in the melt by increasing the free volume of the PCL, allowing the PCL chains to slip past each other more easily.

Compression test data showed that the paste was more readily deformed with the addition of MePEG (Section 5.2.5.2). It was found that the PCL:MePEG paste was more apt to crumble under stress than PCL alone. A possible explanation for this is that the PCL
crystals occupy a lower volume fraction of the material with the addition of MePEG. Since the PCL crystals impart the bulk of the material strength, dilution of the PCL crystals with MePEG resulted in a reduction in the modulus of the blend.

6.2.4 Application of mechanical test data to clinical use of surgical paste

The decrease in melt viscosity would facilitate the spreading of the molten paste in the surgical tumour resection site in practice. The form with which the surgical paste failed (crumbling) would present a problem clinically if the solid paste were to crumble in the body and fall away from the site of action. However, in tumour resection surgery, the area which had been occupied by the tumour could be packed with an inert material which would hold the paste in place. The effect that MePEG blending had on the deformation properties of the paste would result in the paste having a greater ability to deform when stressed by the natural movements of the body. The reduced strength of the paste would also decrease the likelihood of the paste causing injury to adjacent tissue. Examples of the tensile strength of various human tissues include: 78 to 170 N/cm$^2$ for various tissues within arteries; 47 to 110 N/cm$^2$ for the transverse and longitudinal directions of the kidney calyx; 130 N/cm$^2$ for the transverse direction of spinal dura mater; and 430 to 550 N/cm$^2$ for the transverse and longitudinal directions of stomach tissue (Duck 1990). The tensile strength of PCL surgical paste was reduced from 179.4 N/cm$^2$, which is greater than the strength of the tissues mentioned, to 26.7 N/cm$^2$, which is less than the strength of the tissues mentioned, by the addition of 20% MePEG. Thus the likelihood that the surgical paste could cause mechanical damage to these tissues would be diminished.
6.2.5 PCL crystallization

6.2.5.1 Effect of MePEG on growth rate of PCL spherulites

Since the blending of MePEG caused a reduction in the Tm of the PCL crystals, and the same Tc was used for all samples, ΔT was different for all blends tested, and therefore, the nucleation rate would likely be different. As a result, only the effect of MePEG on the growth rate of PCL spherulites was considered in this section.

Dilution of PCL with MePEG did not affect the linear growth rate of spherulites growing at room temperature at PCL concentrations greater than 50% (Figure 28). Blending MePEG with PCL likely caused competing effects with regard to the spherulitic growth rate of PCL spherulites. The reduction in melt viscosity induced by MePEG blending would be expected to increase the growth rate of the spherulites by allowing the amorphous PCL chains to diffuse to the crystalline growth face more easily. However, in order for the PCL to crystallize, MePEG molecules must diffuse away from the growth face which would be expected to retard the growth of the spherulite compared to PCL alone. Hence, there was no net effect on the radial growth rate of the PCL spherulites.

6.2.5.2 Optical microscopy of PCL crystallization in blends

Evidence suggested that MePEG was not incorporated into the PCL crystal lattice of PCL crystals which formed from PCL:MePEG blends. From Figure 29 A-C and at all blend ratios tested, the PCL crystals appeared to be volume filling. In volume filling, the spherulites grow until they impinge on one another, regardless of the MePEG concentration (up to 90%). This suggested that the MePEG was not expelled from the growing spherulites,
otherwise it would form a separate region between the spherulites and would be visible under polarized light as dark regions (proportional in area to the MePEG blend concentration) between the PCL spherulites. Hence, it seems likely that MePEG was located in the amorphous regions within the spherulites. Direct evidence for this is given by the photographs in Figure 29 C-E in which banding in the PCL spherulites was observed. The presence of bands, in spherulites formed from a blend, has been cited as evidence that the amorphous polymer is located within the spherulite, but does not by itself indicate whether it is located in the interlamellar or interfibrillar regions (Luyten et al. 1997).

Within the PCL spherulites, the MePEG may be located either in the interlamellar, or interfibrillar spaces (see Figure 5A). If the MePEG was located in the interlamellar regions it would take up some of the available space between PCL lamellae. The result would likely be an increase in the degree of PCL crystallinity due to the reduction in the number of PCL chains which could exit the crystalline lamellae and proceed into the amorphous regions in the form of tie molecules or large loops. A similar situation was found when PCL was blended with poly(vinyl methyl ether) (PVME). In this case the PVME was found to distribute in the interlamellar regions of the PCL spherulites resulting in an increase in the Xc of PCL in accordance with the same model proposed above for PCL:MePEG blends (Oudhuis et al. 1994). The PCL crystals that formed under these conditions would have a lower Tm however, because the greater proportion of tight loops would increase the surface free energy of the crystal, resulting in lamellae with lower stabilities depending on the concentration of MePEG present. Since neither the Xc nor the stability parameters of the PCL were affected by blending with MePEG, and the melting point depression was explained
in terms of thermodynamic considerations, it seems likely that the MePEG was excluded into the interfibrillar regions (see Figure 5A).

The average size of the PCL spherulites increased as the concentration of PCL decreased (Figure 29). This is a function of a reduction in the number of nucleation sites per unit volume at lower PCL concentrations, due to the decreased ΔT. When the MePEG fraction was low, there was a greater density of nucleation sites present and therefore the space available, before impingement of spherulites occurred, was reduced. As the MePEG concentration was increased, the number of PCL spherulites per unit volume was reduced and the spherulites had more room available for crystal growth.

The spherulites were approximately uniform in size at each blend ratio. This would imply heterogeneous, athermal nucleation which occurs as a function of ΔT. Heterogeneous nucleation occurs as crystals form on preexisting nucleation sites such as impurities or in cases when imperfect melting of spherulites had occurred before the cooling was initiated. In this case, the nucleation occurred at roughly the same time and the number of stable nuclei did not change as crystal growth occurred. This would result in spherulites of roughly equal size if a uniform distribution of nucleation sites, and similar growth rates of each spherulite can be assumed.

6.2.5.3 Avrami exponent

To minimize the effect of crystallization times, analysis was only conducted below a 40% extent of crystallization. Representative crystallization thermograms of different PCL:MePEG blends, shown in Figure 30A, depict the different time courses of PCL
crystallization at different blend ratios. For all blends tested, the time course of crystallization involved a lag phase followed by linear crystal growth. This lag phase is thought to be due to the requirement that heterogeneous nuclei in the sample require time to develop into a structure on which crystals can grow (Wunderlich 1990).

A possible interpretation for the Avrami exponent calculated for PCL crystallization required assumptions about the nucleation of the crystals. Theoretically, four temperature regions can be described below the Tm of a polymer. The first region includes about 10 to 30° below Tm and in it, no nucleation occurs. About 30° - 50° below Tm is an athermal region in which heterogeneous nucleation occurs but the number of nuclei remain constant and no homogeneous nucleation is possible. As the temperature is decreased further (40° - 80° below Tm), smaller, or less perfect heterogeneous nuclei can grow in time to critical size, resulting in thermal nucleation as the number of nuclei increase with time. Below a certain temperature, homogeneous nucleation occurs. This is a thermal process as new nuclei are formed so rapidly that often no spherulitic structures can be seen with the microscope.

As noted in section 6.2.2.6, when viewed under microscope following isothermal crystallization at room temperature, it appeared that the spherulites had formed athermally. They were of approximately equal size and the boundaries between them were the perpendicular bisectors of the line joining the centers. This observation was more pronounced as the MePEG fraction was increased. At high MePEG concentrations the nuclei formation was strictly athermal, but at lower MePEG concentrations some thermal nucleation may have been taking place. At the Tc employed (room temperature) the ΔT of 100% PCL was in the region where some thermal nucleation took place. With the addition of
sufficient amounts of MePEG in the blend, the $\Delta T$ was reduced to the point where the system exhibited strictly athermal nucleation.

The addition of MePEG delayed the onset of crystallization of PCL (Table 7) and is thought to be primarily due to a decrease in $\Delta T$ by MePEG lowering the Tm° of PCL. At all blend ratios, the number of nuclei reached a limiting value (assuming the athermal nucleation model proposed above), but the nuclei density decreased as the MePEG fraction was increased. Therefore, although the radial spherulite growth rate was not affected by MePEG concentration, the number of spherulites decreased. As a result, the overall crystallization rate, which is the sum of the nucleation rate and the growth rate of crystals, shown in Table 7, and graphically in Figure 31, was decreased as MePEG was added.

The Avrami exponent ($n$) can be used to describe polymer crystallization but is limited in its ability to accurately characterize the mechanisms and geometric forms of crystallization. The Avrami equation in thermal analysis is often only a good representation of the data, rather than a precise analysis in terms of crystallization mechanism (Wunderlich 1990). Information on nucleation and morphology would be needed in order to fully interpret $n$. The constant $k$ in the Avrami equation depends on crystal geometry and nucleation mechanism. Theoretically, the value calculated as the Avrami exponent should be an integer. In practice, fractional values for $n$ are often encountered (Wunderlich 1976) and the data tend to deviate from theory at long crystallization times due to a number of unfulfilled assumptions (Wunderlich 1976) including:

i) volume of polymer does not remain constant as crystallization proceeds
ii) linear growth rate not always constant with time (may be due to local temperature gradients due to exothermal crystallization)

iii) rate of appearance of nuclei may not be constant (dependent on availability of heterogeneous nucleation sites)

iv) crystal morphology may not be precisely spherical (or circular)

v) actual crystalline substructure frequently branches (higher n is needed to describe this process)

vi) two stage crystallization can occur which relates to the formation of crystal nuclei within, but independent from, an existing spherulite (ie. nucleation and crystal growth occurs in the interfibrillar space of a spherulite but the resulting crystal is not a branch of any of the surrounding fibrils)

vii) following initial crystal formation the crystallites can anneal over time to form more stable crystals.

The Avrami plots of the blends are shown in Figure 32. The slopes represent the Avrami exponents while the y-intercept values represent log k. The Avrami exponent, n, as well as the constant, log k, are summarized in Table 8 for the PCL:MePEG blends tested. It can be seen that for the PCL:MePEG data at 37° C, the order of log k values was 100% PCL > 90% PCL > 80% PCL. Since k represents geometry and nucleation factors including the number of spherulites per unit volume, this observation supports the previous evidence discussed above that the addition of MePEG resulted in a reduction in nuclei per unit volume.
The calculated Avrami exponent, approximately 3, indicates that the crystals were
grown as spherulites at all concentrations of MePEG tested. The Avrami exponent was
calculated for the PCL:MePEG 70:30 sample at 37° C but this temperature was apparently
too high to make accurate measurements because the ΔT of the sample was low, and so the
crystallization rate was decreased, resulting in increased error in measuring the amount of
crystallinity developed in the sample over time. The data collected at 34° C was used to
generate an Avrami exponent and confirmed the value of approximately 3.

The fractional Avrami exponent found in this work may be attributed to some of the
unfulfilled assumptions mentioned above. The weight fraction was used, rather than the
volume fraction of crystallization in the calculations. Although it avoids the problem of the
change in total volume as crystallization proceeds it can still introduce errors in the order of
between 0.15 to 0.25 in estimates of n (Wunderlich 1976). The rate of nuclei formation may
not have been constant since some thermal nucleation appears to have occurred in the
samples at low MePEG concentration and exhaustion of heterogeneous nuclei occurred in the
samples with high MePEG concentrations. Since k is partially a nucleation term, if
nucleation density changed during the course of the study then the calculated value of n
would also be affected.

According to Wunderlich, the temperature below which homogeneous nucleation
takes place is about 0.8 times the absolute melting temperature of the sample (Wunderlich
1976) which, for PCL, would be about -5° C. When PCL:MePEG blends were melted and
left to crystallize in the freezer (-20° C) the resulting crystals did not show the spherulitic
pattern which had been observed on isothermal crystallization at room temperature (data not
shown). This supports the prediction of homogeneous nucleation, which would be characterized by stable nuclei forming throughout the sample (no pre-existing heterogeneous nucleation sites are needed). These nuclei would be in such close proximity to each other that no room would be available in which spherulites could grow (Wunderlich 1976).

Paclitaxel affected the crystallization kinetics of PCL. Thermal analysis scans showed a slight reduction of the ΔT necessary to initiate PCL crystallization suggesting that paclitaxel facilitated the crystallization of PCL by increasing the nucleation rate.

Analysis of the isothermal crystallization of PCL in the presence of paclitaxel using the Avrami equation supports this hypothesis. The crystallization of PCL from 20% paclitaxel loaded PCL:MePEG 80:20 at 37° C was characterized by an Avrami exponent of 4.2. This is indicative of spherulitic growth with sporadic, or thermal, nucleation (Wunderlich 1976; Cowie 1973). Such a situation could result if additional heterogeneous nucleation sites were provided which could develop into critical nuclei over time. The surfaces of paclitaxel crystals present in the sample have been proposed to act as these heterogeneous nucleation sites. This is supported by the melting point depression data and the crystallization rate data given in Table 6 and Figure 31. The additional reduction in Tm° for PCL in a PCL:MePEG 80:20 blend caused by the addition of 20% paclitaxel would result in a corresponding reduction in ΔT for the PCL crystallizing at 37° C in the presence of paclitaxel. If the nucleation mechanisms were identical in both systems, the PCL nucleation rate would be retarded when paclitaxel was present. In addition, paclitaxel raises the Tg of the melt, which would have the effect of increasing the viscosity and reducing the PCL crystallization rate. PCL nucleation rate was not retarded however, and in fact, the
crystallization rate increased in the presence of paclitaxel although this difference was not statistically significant. Microscopic evaluation of paclitaxel loaded PCL:MePEG 80:20 suggested that some PCL crystallization occurred on the surface of paclitaxel particles. It is felt that this is a likely explanation for the Avrami exponent taking on a value of 4 in the presence of paclitaxel (Wunderlich 1976).

6.2.6 Degradation and ageing of surgical paste

6.2.6.1 Molecular weight effects

GPC scans of PCL, MePEG, PCL:MePEG 80:20, and 20% paclitaxel in PCL:MePEG 80:20 (Figure 33) showed that MePEG and paclitaxel co-eluted when they were analyzed together because the MePEG molecular weight (relative to polystyrene) was about 680 g/mole which was not separated from the paclitaxel (molecular weight, 853 g/mole).

There was a statistically significant decrease in the molecular weight of PCL stored at 37°C in PBS buffer (Table 9). The largest decrease was observed in the PCL sample, followed by the PCL:MePEG 80:20 sample and the 20% paclitaxel in PCL:MePEG 80:20 sample. The main mechanism for PCL degradation is hydrolytic cleavage of the ester bond propagated through an autocatalytic mechanism (Pitt et al. 1981; Woodward et al. 1985; and Pitt 1990). This preferentially occurs in the amorphous phase since the PCL crystals are too tightly packed to allow water to penetrate. A possible explanation for the differences observed in the rates of molecular weight decreases of the PCL in the different formulations, is that the PCL had different Xc values in the different formulations. The presence of
MePEG or MePEG/paclitaxel served to increase the Xc of the PCL component which reduced the amount of water which could penetrate the PCL phase.

In the PCL:MePEG 80:20 sample stored in buffer, GPC analysis did not show the presence of a MePEG peak, indicating that the MePEG diffused out of the sample (Figure 33D). The MePEG is water soluble and can diffuse into the aqueous environment.

Gravimetric analysis of PCL:MePEG in an aqueous environment suggested that MePEG diffused from the paste into the aqueous environment. Swelling study results also showed that as the MePEG diffuses out, water is able to diffuse into the paste. PCL samples alone do not take up water and so the presence of MePEG had the effect of increasing the water uptake of the polymer paste samples. This is demonstrated in Figure 27, where SEM images of the interior of PCL:MePEG 80:20 and PCL paste samples which had been stored in PBS buffer for 15 days are shown. The water channels exposed by the MePEG lost from the paste are clearly visible in Figure 27B. The channels represent the interfibrillar spaces of the PCL spherulites. In the pure PCL sample, there was no evidence of channel formation.

There was no decrease in PCL molecular weight in any of the other samples. The apparent increase in molecular weight in the samples which had been stored dry is not understood. It is possible that the increase in PCL molecular weight over time was due to an error in determining the initial sample molecular weights. If this was the case, then the decrease in molecular weight found with the samples stored in buffer was underestimated.
6.2.6.2 Thermal properties

The Tm of PCL in the paste samples increased with time at all temperatures (Figure 34 A-C) due to the perfection process shown by PCL crystals over time as reported in the literature (Phillips et al. 1987; Phillips and Rensch 1989). In these studies the lamellar thickening that occurred in PCL samples stored at Tc was investigated and it was found that the lamellar thickness, and thus the Tm, of the PCL crystals continued to increase for at least 3 hours. The increase in the Tm of PCL was related to the storage temperature with the greatest increase occurring in the samples stored at 37° C in buffer. This was because when polymer crystals are allowed to anneal at a given temperature, the lamellar thickness increases to the thickness that they would have attained had the polymer crystallized at that temperature. The different annealing temperatures resulted in increases in lamellar thickness which were proportional to the storage temperature (Hearle 1982).

The small (but significant) increases in the Xc of the PCL in the samples stored over time was probably due to secondary crystallization (Wunderlich 1976). No statistical difference was found between the samples stored dry at 25° and 37° C. The reason for the smaller increase in Xc for the samples stored at 37° C in buffer than for those stored at 25° and 37° C is not understood. It was expected that with greater decreases in molecular weight shown by samples stored in buffer, the shorter PCL chains would have increased mobility and an increased tendency to crystallize. Studies have shown that PCL degradation leads to shorter, more mobile chains (decreased viscosity of sample), which initially are able to rearrange and crystallize, resulting in an increase in the Xc of the PCL (Pitt and Gu 1987; Pitt et al. 1981a).
Thermal analysis of the PCL:MePEG 80:20 samples stored at different temperatures over time revealed no change in the measured Tg of the blend nor the Tm of the MePEG up to 9 weeks of storage (Table 10). It is not known why at 13 weeks, a decrease in the Tg of the PCL:MePEG 80:20 blend stored at 37° C was detected as well as a decrease in the Tm of MePEG in the blends stored at 4° and 37° C. Since the samples were all stored above the melting point of MePEG there was no opportunity for the MePEG crystals to anneal.

The Tg of the 20% paclitaxel in PCL:MePEG 80:20 samples also remained relatively constant over time at all temperatures.

6.3 In vitro release studies

In the in vitro release studies, the solubility of paclitaxel was increased using albumin so that sink conditions could be maintained. The concentration of paclitaxel required to achieve a saturated solution would be higher, if only anhydrous paclitaxel were present compared to paclitaxel dihydrate. As a result, albumin may have inhibited the conversion of anhydrous paclitaxel to the dihydrate form, and allowed higher concentrations of paclitaxel to be achieved without approaching the saturation point. The sampling interval was selected so that sink conditions could be maintained for the paclitaxel being released into the release media. Sink conditions were assumed to be maintained if the concentration of paclitaxel in the release media did not exceed 15% of paclitaxel solubility (Carstensen 1977). In the initial rapid phase of release from the formulations, it is likely that sink conditions were exceeded despite sampling five times on the first day. This would have resulted in an underestimation of the amount of drug released on the first day of the study relative to the amount which may
have been released if sink conditions had been met, but would not be expected to alter the total amount of drug released after several days.

In all samples, the release of paclitaxel from PCL surgical paste (± MePEG) was characterized by an initial burst phase lasting between 1 and 2 days followed by a period of slow sustained release. The burst phase is due to the release of surface associated paclitaxel. Drug at the surface of the pellet does not have to diffuse through the polymer in order to be released and is limited only by the dissolution rate in the release media (Langer and Peppas 1981).

Following the initial burst phase, the release of paclitaxel from the surgical paste samples up to 3 months was diffusion controlled. This was shown by the linear dependence of the drug release rate with the square root of time (Figure 36). The release of paclitaxel from the PCL and PCL:MePEG paste samples followed the Higuchi model of drug release since it was diffusion controlled and the solubility of drug was exceeded in the polymer matrix. The solid particles of drug present in the matrix were uniformly distributed and small relative to the average diffusion distance (Higuchi 1961). The percentage of paclitaxel released from the surgical paste formulation was similar to that found for the release of paclitaxel from biodegradable polyanhydrides. A formulation consisting of 10% paclitaxel loaded polyanhydride, released 15% of its initial load after 77 days by a diffusion mechanism (Park et al. 1998). This slow release was attributed to the hydrophobic paclitaxel being encapsulated in a hydrophobic matrix.

The model of diffusion of drugs from the polymer matrix is the one most often encountered in the literature with PCL (Pitt et al. 1979b; Pitt et al. 1979a; Vandamme and
Mukendi 1996; Chang et al. 1986). This is thought to be due to the long degradation time encountered with PCL (Holland and Tighe 1986).

Table 11 shows that the cumulative amount of paclitaxel released after 2 weeks was greater from the PCL paste than from the PCL:MePEG 80:20 paste. This difference was observed at all paclitaxel loadings and was shown to be statistically significant at loadings of 1%, 20% and 30%. This reduction in cumulative release was independent of the amount of MePEG added between 5% and 30% MePEG loading (Figure 38). MePEG did not affect the terminal release rate ($V_{10}$, Table 11) and the difference in cumulative release observed at 14 days was due to a difference in the rate of release during the burst phase ($V_0$). The presence of MePEG appeared to retard the diffusion of surface associated paclitaxel into the release medium. The reason for this is not understood.

The observation that the percentage of drug release from the pellets was inversely proportional to drug loading (Figure 37) is consistent with the Higuchi model (Higuchi 1961) of drug release (Equation 25) which indicates that the release rate is dependent on the square root of both the solubility of the drug in the matrix and the initial drug loading level. Hence, increasing the drug loading should theoretically result in much smaller increases in drug release rate. Therefore the percentage of paclitaxel released over time from surgical paste decreased as the initial loading level was increased.

Over a 3 month release period, differences in release rates of paclitaxel from surgical paste became apparent which were not seen over 14 days. Figure 39 shows that the cumulative paclitaxel release from a PCL sample is significantly greater than from a PCL:MePEG 80:20 sample. This difference was due to paclitaxel release from PCL being
greater than from the PCL:MePEG 80:20, both in the burst phase and over the last 60 days of
the study.

When the PCL:MePEG paste was suspended in buffer, it has been shown that the
MePEG diffused out of the polymer, and the channels created in the polymer by the removal
of MePEG were filled with aqueous buffer. Evidence in the literature suggests that the
formation of water channels in a polymer matrix generally increases the release rate of drugs
from the polymer by providing a diffusional pathway through which drug may be released.
This mechanism of drug release has been described mathematically by Higuchi (Higuchi
1963). Release of bovine serum albumin from macroporous poly(ethylene-co-vinyl acetate)
(EVA) matrices was described to be via aqueous diffusion through water filled channels
(Siegel and Langer 1990). Polypeptides, including bovine serum albumin, β-lactoglobulin,
and lysozyme, released from EVA matrices were described using a similar model (Bawa et
al. 1985). The incorporation of water soluble polymers such as poly ethylene glycol, to
polymer matrices to increase drug release rates by acting as a porosigen have been described
(Baker 1987; and Korsmeyer et al. 1983). As the water soluble polymer diffused from the
polymer matrix, water channels were created and drug release rates were increased. When
glycerin was incorporated into a poly(D,L-lactide) formulation, the glycerin diffused from
the polymer in an aqueous environment, leaving pores which were clearly visible by SEM.
This resulted in a 100 fold increase in the flux of progesterone diffusing from the samples
(Pitt et al. 1979b).

The finding that, despite the loss of MePEG and the formation of water channels in
the PCL:MePEG pastes, the release rates of paclitaxel were decreased compared to PCL
pastes, was interesting and unexpected. Although the diffusional model of drug release applies to both systems, it is suggested that there are fundamental differences in the PCL and PCL:MePEG surgical pastes which may help explain the differences in the rates of paclitaxel release.

The paclitaxel incorporated in PCL paste is present as dissolved paclitaxel and as anhydrous paclitaxel crystals. Release of drug involves diffusion of the dissolved paclitaxel to the surface of the paste pellet, and partitioning into the bulk aqueous buffer. In paclitaxel loaded PCL:MePEG blends the polymer matrix is different from that of PCL surgical paste, because of the presence of water channels. Paclitaxel release may occur, not only through diffusion and partitioning at the surface of the pellet, but also via the internal water channels. These channels would likely become saturated with paclitaxel, in view of the very low aqueous solubility of paclitaxel. It has been shown that precipitation of paclitaxel dihydrate can occur within 24 hours from saturated aqueous solutions of paclitaxel (Liggins et al. 1997). If this occurred in the water channels then paclitaxel dihydrate crystals would form along the walls of the channels. The presence of the paclitaxel dihydrate crystals, which are a more stable form of paclitaxel than the anhydrous paclitaxel (Liggins et al. 1997), would reduce the concentration of paclitaxel in the water channels. It is proposed that this may oppose the enhancing effect of water channels on paclitaxel release rates.

Figure 40 shows that the sterilization method employed in this work did not affect the release profile of paclitaxel from either PCL or PCL:MePEG 80:20. This was probably due to the fact that paclitaxel release occurred primarily via a diffusion process and to the
negligible effect of γ-irradiation on the degradation lifetime of the polymer over the course of the drug release study.

Sterilization by gamma irradiation can have different effects on polymers and the drug release rates from these polymers. The effects include a reduction in the molecular weight of the polymer through the breaking of covalent bonds in the polymer backbone. The effects of irradiation can be instantaneous (breaking of bonds), or they may be delayed. The radiation may cause strains in bonds or result in the formation of free radicals which, over time, can alter the 3 dimensional structure of the polymer, or cause material breakdown (Lim et al. 1998; Martini et al. 1997; Sintzel et al. 1997).

In a study of the effects of sterilization by gamma irradiation on the release rates of captopril from poly(D,L-lactide-co-glycolide) (PLG) microspheres of different molecular weights, both increases and decreases in captopril release rates were found as a function of sterilization dose depending on the molecular weight of PLG used (Volland et al. 1994). In this study the authors noted that polymer degradation in response to irradiation was greater for high molecular weight PLG than for low molecular weight PLG.

The ideal formulation for the release of paclitaxel as a surgical paste would be one in which the release of drug was constant throughout the active life of the paste. For this to occur the concentration of dissolved drug in the paste and the surface area available for drug release must be constant (Higuchi 1961). The present surgical paste formulation fulfills the requirement for constant drug concentration in the matrix since it consists of paclitaxel crystals suspended in a polymer matrix which is saturated with dissolved paclitaxel. PCL has a long degradation lifetime and matrix erosion does not occur until the molecular weight of
the chains have been reduced to a low value, typically this takes months to years to occur. As a result, the surface area of a PCL matrix would be expected to remain relatively constant until polymer erosion occurred.

6.4 In vivo evaluation of formulation

6.4.1 CAM assay

The CAM assay has been shown to be a rapid method of testing agents for antiangiogenesis activity (Folkman 1985) and has been used to assess the antiangiogenic activity of other anticancer agents (Steiner 1992). Solid tumours require stroma in order to grow beyond 1 or 2 mm in size. Tumour stroma is composed of new blood vessels, inflammatory cells, and connective tissue. As a result, tumour growth beyond 2 mm is dependent on angiogenesis, which is stimulated by angiogenic factors released by tumour and inflammatory cells (Díaz-Flores et al. 1994).

CAM results, depicted in Figure 41, and summarized in Table 12, indicated that paclitaxel was released from formulations of PCL and PCL:MePEG 80:20 in an active form capable of inhibiting angiogenesis on the CAM. Paclitaxel loaded PCL paste at loadings of 0.1% (3 μg paclitaxel/pellet) showed some positive antiangiogenic activity while the 0.25% samples (7.5 μg paclitaxel/pellet) showed antiangiogenic activity in all samples tested. The effectiveness of paclitaxel in inhibiting angiogenesis is comparable to other potent antiangiogenic compounds reported in the literature including suramin and protamine (Takano et al. 1994).
The antiangiogenic activity of paclitaxel, although potentially beneficial for cancer therapy, is not likely to be an important factor in the application of surgical paste. This is because the concentration of paclitaxel at the active site would ideally be sufficient to cause tumour cell death before the development of tumours large enough to secrete angiogenic factors.

6.4.2 Pilot study for distribution of paclitaxel following surgical paste injection

Several reports in the literature provide analytical methods for paclitaxel detection in plasma or serum (Willey et al. 1993; Sparreboom et al. 1995; Andreeva et al. 1997). The detection limit in these HPLC assays was as low as 10 ng/mL.

It was felt that in vivo, the hydrophobicity of paclitaxel may result in its distribution into cell membranes, such as the membranes of red blood cells. As a result, whole blood was harvested in the biodistribution study conducted here, and an assay for paclitaxel in whole blood had to be developed. The developed HPLC assay for paclitaxel in whole blood was found to be precise down to 10 μg/g. This value represents a concentration of drug capable of completely inhibiting depolymerization of microtubules in cells but is still above paclitaxel concentrations which have been shown to be cytotoxic (Schiff et al. 1979; Roytta et al. 1987; Tishler et al. 1992).

The pilot study gave preliminary evidence that drug was being released from surgical paste (15% drug release after 17 days). Drug was detected in liver and muscle tissue but the measured amounts were not reliable due to the low sensitivity of the HPLC assay for paclitaxel. Variation was observed in the measured amounts of paclitaxel in muscle tissue at
the six hour time point and at 2 days (Table 18). This variation was thought to be due to the fact that the surgical paste pellet was in contact with only a portion of the muscle tissue, and therefore the levels of paclitaxel would vary depending on the distance which the selected tissue sample had been from the pellet during the course of the study. Since lower paclitaxel concentrations may still show some efficacy, greater assay sensitivity was needed for the \textit{in vivo} assay of paclitaxel in whole blood. For this reason the $^3$H-paclitaxel assay was developed and used in the biodistribution study.

6.4.3 Characterization of surgical paste samples following implantation in mice

Analysis of the PCL:MePEG 80:20 implants revealed that \textit{in vivo}, the MePEG was released from the polymeric implant. GPC chromatograms in Figure 43 of PCL:MePEG 80:20 following 2 and 17 days implantation did not show peaks corresponding to MePEG while the DSC traces in Figure 44 did not show melting peaks for the MePEG phase. This was consistent with the assumption that MePEG diffused from the pellet.

Calculation of the paclitaxel content of surgical paste pellets recovered from mice (Table 19) could be performed assuming that the loss of MePEG from the polymer was complete. These data show that by 17 days paclitaxel had been released from the pellet and that only about 75% of the original paclitaxel loading remained in the implants.
6.4.4 Biodistribution of $^3$H-paclitaxel in mice following implantation of surgical paste

6.4.4.1 Selection of animal model

A mouse model was chosen for these studies because mice have been used previously in biodistribution studies for paclitaxel (Eiseman et al. 1994) in which the distribution of drug following intravenous and extravascular routes were compared. The molten paste could be readily injected into the subcutaneous region in the back of the neck of the mice without the need for surgery. The location of the pellets made them easy to locate and harvest following sacrifice of the mice. Furthermore, the selection of mice, over rats or other larger animals, was advantageous for other practical reasons. The mice were relatively inexpensive, easy to handle, and housing facilities were available to conduct a study using radioactive label.

One of the drawbacks to this model was the relatively close contact of the paste pellet with the injection site. Paclitaxel is known to inhibit wound healing (Jampel et al. 1993), and therefore, when released at the injection site, it prevented the healing of the injection wound caused by the 18 gauge needle. This would not be anticipated to be a problem in clinical use since the intended application for surgical paste formulations would be at a tumour resection site distant from the surgical incision into the skin.

Another limitation of this model was that the geometry of the injected molten paste more closely resembled a spherical implant than a thin film. The intended clinical application of the surgical paste would involve spreading of the paste into a film to cover the
exposed areas in the resection site. The different geometries may translate to the pastes having different mechanical and drug release properties.

6.4.4.2 Biodistribution of $^3$H-paclitaxel

Validation of the radioassay for $^3$H-paclitaxel in various mouse tissues and the surgical paste (data given in Tables 20 - 28), indicated that the assay was sufficiently sensitive to measure $^3$H-paclitaxel at concentrations below 10 μg/g. The assay was able to detect the presence of radiolabel. This may be from paclitaxel or metabolites of paclitaxel. The assay was not able to differentiate between free drug and drug metabolites. In the following discussion it should be understood that levels of $^3$H-paclitaxel reported in the biodistribution study may be either $^3$H-paclitaxel or metabolites containing the label.

Paclitaxel inhibits wound healing to such an extent that it has been investigated as a way of reducing the scarring associated with glaucoma filtration surgery (Jampel et al. 1993). In the biodistribution study, paclitaxel released from the formulation inhibited the healing of the injection sites of the mice in the treatment group. In clinical use it is envisioned that the surgical paste would be applied distant from the incision and interference with the healing of the incision would not be expected. There is some preliminary evidence that inhibition of wound healing by paclitaxel at tissue sites other than directly adjacent to the incision, may not be a major problem. Paclitaxel released from polymer films applied perivascularly to rat carotid arteries at loadings higher than the PCL:MePEG 80:20 paste used in this study, did not show evidence of inhibition of vascular wound healing (personal communication). It should be noted that the three systems mentioned here: s.c. injection of paste pellet, application of surgical paste to tumour resection site, and perivascular films, involve different
tissues and wound healing environments. Therefore, direct comparisons of wound healing inhibition would not be appropriate. Surgical paste pellets could not be found in mice sacrificed at 30 days. It seems likely that between 15 and 30 days in the study the paste pellet fell out of the open wound. With the pellet removed the injection sites were able to heal over. The fact that wound healing is inhibited in these animals serves as an indication that the paclitaxel was being released and was active in preventing the proliferation of endothelial cells necessary for healing to occur.

Examination of the harvested paste pellets (Figure 46) revealed that 40% paclitaxel was released from the paste pellet after 15 days. These data were consistent with what was found in the pilot study. Although the variability in these data were high, the level of paclitaxel remaining in the pellet was still significantly different from the initial value. There are at least two possible reasons for this variability. The pellets were injected in the molten state and so, following injection, any pressure applied to the paste, could lead to a moulding of the pellet prior to solidification. This pressure could be due to either movement of the mouse or to external pressure as a result of handling of the animal. Moulding of the pellets would alter the geometry of each pellet individually and would result in differences in the surface areas available for paclitaxel release over the course of the study. Another possible source of variability is that paclitaxel exhibited variable release kinetics from surgical paste at elevated temperatures (between 50° and 37° C). If this was true, then differences in the temperatures of injected paste pellets, and the rates at which these pellets cooled to 37° C, could have been an additional source of variability in the release of paclitaxel from surgical paste in vivo.
In the present work, paclitaxel did not distribute to the heart, lung, kidney, or spleen following subcutaneous administration in the surgical paste formulation (Table 29). This agrees with the biodistribution studies in the literature. Eiseman et al. (1994) studied the distribution of drug following intraperitoneal, subcutaneous and oral administration of paclitaxel at 22.5 mg/kg. It was found that none of these extravascular routes of paclitaxel administration produced detectable levels of paclitaxel in either the mouse plasma or organs. This was in contrast to pharmacokinetic studies of paclitaxel given i.v. to mice, where it was found that paclitaxel distributed extensively to organs including muscle, lung, heart, spleen, liver and kidney (Lesser et al. 1995; Eiseman et al. 1994).

The levels of radiolabel found in whole blood rose quickly during the first day (10.5 μg/g detected at 6 hours) but had decreased substantially by day 2 and were not detected at the 15 day time point. This was thought to be due to a burst effect as drug was rapidly released from the surgical paste in the initial few hours following injection. Since levels detected in the liver at 6 hours were higher than those in blood it seems likely that the time required to achieve maximum blood concentration of \(^3\)H-paclitaxel occurred before 6 hours. Levels of paclitaxel were found to be higher in the liver at day two than at 6 hours, indicating that the levels were rising in the liver at 6 hours. Data describing the urinary excretion of paclitaxel from mice was not available. However, pharmacokinetic studies of paclitaxel in humans have shown that less than 5% of paclitaxel is excreted unchanged in the urine (Gianni et al. 1995). Hepatic metabolism and biliary excretion account for a great percentage of systemic clearance of paclitaxel (Kuhn 1994). Assuming that paclitaxel pharmacokinetics are similar in mice and humans, this explains the build up of radiolabel detected in the liver.
over the first two days. It seems likely that the levels found in the liver represent a combination of $^3$H-paclitaxel and its metabolites.

The levels of $^3$H-paclitaxel found in the muscle, which was immediately adjacent to the injected paste pellet, remained at a high level (above 10 μg/g) for the entire duration of the study. These levels were maintained on the muscle tissue adjacent to the pellet although the pellet had fallen out of the mice between 15 and 30 days. These levels are sufficient to stabilize microtubules in cells surrounding the paste and are above those needed for cytotoxicity (Schiff et al. 1979; Roytta et al. 1987; Tishler et al. 1992; and Derry et al. 1995).

The results demonstrate that the application of 20% paclitaxel loaded PCL:MePEG 80:20 paste will release paclitaxel into the local area resulting in tissue levels which remain above 10 μg/g for at least 1 month. There is no direct evidence that the paclitaxel label measured on the muscle tissue was from paclitaxel and not metabolites carrying radiolabel. However, the HPLC chromatogram of paclitaxel in muscle tissue from the pilot study (data not shown) showed no evidence of metabolite formation at 17 days. This observation serves as preliminary evidence that the radioactivity detected on the muscle tissue was from $^3$H-paclitaxel and not labelled metabolites.

6.4.4.3 Evaluation of formulation toxicity

In the course of the biodistribution study, the mice did not exhibit signs of toxicity on observation, following administration of the formulation, although the possibility of cellular toxicity was not investigated. All the animals gained weight (Figure 45) and appeared active and not distressed. Paclitaxel release was greatest in the first 6 hours of the study. Although
it was possible that toxic levels of paclitaxel were reached in the animals during this time, it is unlikely that this occurred since no toxic effects such as lethargy and loss of righting reflex were observed in the first several hours following treatment. Furthermore, release data from the paste pellet revealed that 1.2% of the drug had been released from the polymer at 14 hours. In the worst case, if all this drug had been released as a bolus dose, it would represent an amount of 300 μg of drug released into the subcutaneous space. The mice weighed 20 g and so, this release would therefore correspond to a dose of 15 mg/kg. This dose is not likely to cause toxicity since, an i.v. dose of 22.5 mg/kg was shown not to be toxic to mice (Eiseman et al. 1994). In the surgical paste formulation the dose was not only lower than that used in the Eiseman study, but also, it was in the subcutaneous space, from which paclitaxel was shown not to be bioavailable (Eiseman et al. 1994).
7. SUMMARY AND CONCLUSIONS

7.1 Characterization of surgical paste formulation

1. Blends of PCL and MePEG were homogeneous in the melt and underwent phase separation on cooling as the PCL crystallized below its melting point. PCL crystallized as spherulites in blends with MePEG. The MePEG was distributed within the PCL spherulites and was likely localized in the interfibrillar space of the spherulites.

2. The blending of MePEG with PCL was an effective means of optimizing the physicochemical properties of PCL. The blending of MePEG with PCL resulted in a reduced tensile strength, melt viscosity and depression in the equilibrium melting point of PCL crystals.

3. Incorporation of 20% paclitaxel in the PCL:MePEG 80:20 blend resulted in a surgical paste formulation in which paclitaxel crystals were present in the matrix. Dissolved paclitaxel was associated with the MePEG phase and resulted in an increased Tg for the formulation.

4. Ageing and degradation studies showed that on storage in the dry state at different temperatures over a 3 month period, some annealing of the PCL crystals occurred resulting in an increase in the Tm of the PCL crystals. Degradation of PCL chains was observed on storage in PBS buffer at 37° C with a PCL molecular weight reduction of about 20% at 3 months.
7.2 In vitro release of paclitaxel from surgical paste

1. Release of paclitaxel from surgical paste formulations was shown to occur via a burst phase, lasting about 1 day, followed by a period of slow sustained release which continued for at least 90 days. Cumulative release of paclitaxel was proportional to drug loading between 1% and 30% from PCL and PCL:MePEG blends. Release of paclitaxel from surgical paste appeared to be diffusion controlled and followed the Higuchi model of drug release from a solid matrix.

2. Both the rate and extent of paclitaxel release was greater from PCL paste than from PCL:MePEG blends. The amount of MePEG did not affect the release of paclitaxel between 1% and 30% MePEG. It was suggested that the presence of MePEG caused water channels in which dissolved paclitaxel could precipitate as the dihydrate form. This fundamental change in the properties of the surgical paste system, relative to PCL surgical paste, accounted for the reduction in the rate and extent of paclitaxel release from surgical paste in the presence of MePEG.

3. Sterilization of the surgical paste by $^{60}$Co gamma irradiation did not alter the time course of paclitaxel release from either 20% loaded PCL or PCL:MePEG 80:20 surgical paste.

7.3 In vivo evaluation of surgical paste formulation

1. Paclitaxel released from surgical paste was shown to be effective in inhibiting angiogenesis using a CAM bioassay.

2. Biodistribution studies of $^{3}$H-paclitaxel following subcutaneous implantation of 20% loaded PCL:MePEG 80:20 in a mouse model showed that the drug label could be detected in whole blood for 2 days following implantation, and in the liver for 30 days.
following implantation. The surgical paste pellets released approximately 40% of drug in 15 days.

3. Levels of $^3$H-paclitaxel recovered from the muscle tissue immediately adjacent to the implanted pellet were taken to be representative of the concentration of drug at the site of action. Levels of $^3$H-paclitaxel were above the M.E.C. of paclitaxel (10 μg/g) for the entire 30 day duration of the study.

4. Implantation of surgical paste in the mice did not produce signs of toxicity (with the exception of the inhibition of wound healing), and $^3$H-paclitaxel was present at the site of action at levels above the M.E.C., but did not reach high levels in the systemic circulation, nor did it accumulate in any organs except the liver. The surgical paste formulation appears to be a safe, and potentially effective formulation, for the long term, localized administration of paclitaxel.
8. FUTURE WORK

Future work could be conducted to optimize the surgical paste formulation. Polymers of
different molecular weight, and in different combinations can be employed to test the effect on
thermal, mechanical, and drug release properties.

The observation that MePEG caused a reduction in the extent and rate of paclitaxel
release from PCL surgical paste was interesting and unexpected. Further work could be
conducted to investigate the mechanism of drug release from PCL:MePEG surgical paste, and
the fate of paclitaxel initially present in the formulations. The fate of the surgical paste samples
as polymer erosion takes place must also be investigated.

Future work must also be conducted for preclinical evaluation of the formulation. More
extensive biodistribution studies need to be conducted which would evaluate toxicity and
distribution from different sites. The effectiveness of the formulation using a tumour model
should also be investigated.
9. REFERENCES


