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The characterization of novel polymeric paste formulations for intratumoral delivery

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Abstract

The objective of this work was to characterize a polymeric paste formulation of the anticancer drug paclitaxel that was injectable through a narrow gauge needle at room temperature and set to a solid implant in vivo for the intratumoral treatment of localized cancer. Pastes were manufactured from a triblock copolymer composed of poly(D,L-lactide-co-caprolactone)-block–polyethylene glycol-block–poly(D,L-lactide-co-caprolactone) (PLC–PEG–PLC) or triblock blended with a low molecular weight polymer methoxypolyethylene glycol (MePEG). Characterization of pastes was performed using differential scanning calorimetry (DSC), gel permeation chromatography (GPC) and drug release studies. Paste integrity in water was measured by determining the degree of fragmentation under initial agitation. MePEG was found to be miscible with the triblock polymer and paclitaxel dissolved in various blends of these polymers up to 15% drug loading. Pastes composed of 40:60 triblock:MePEG blends and 10% paclitaxel were found to inject through a 23-gauge needle and set to a solid pellet in phosphate-buffered saline at 37 °C. Such pellets released paclitaxel in a controlled manner over 7 weeks. Pastes composed of 40:60 triblock:MePEG blends containing 10% paclitaxel are proposed as suitable injectable formulations of the drug for intratumoral therapy.

Keywords: Polymeric paste; Paclitaxel; Intratumoral

1. Introduction

The unique physiology of tumors, which comprises a highly disordered vasculature and zones of rapidly proliferating cells, offers an appropriate site for the use of controlled release formulations of anticancer drugs. Systemic delivery of drugs to tumors has the disadvantage of providing relatively low concentrations of the drug at proliferating cell boundaries which may be located far from the abnormal capillary networks in the tumor (Brown and Giaccia, 1998; Jain, 1994). Many common solid tumors, including breast, brain and prostate tumors do not respond well to conventional systemic chemotherapy. If the tumor is operable, surgical removal is the preferred therapy, but local recurrence of the malignancy, usually within a 2 cm margin of the excision site, poses a significant clinical problem (Winternitz et al., 1996; Hunter et al., 1997; Fung and Saltzman, 1997). Polymer-based anticancer drug loaded implants, pastes and microparticu-

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lates provide an opportunity to deliver high, localized doses of drug for a prolonged period directly into a tumor or at the site of tumor resection.

Solid implantable, polymeric discs or pellets composed of polyanhydrides and loaded with anticancer drugs have been evaluated for interstitial chemotherapy of brain tumors (Brem et al., 1991; Walter et al., 1994; Fung and Saltzman, 1997). Paclitaxel loaded polymeric pastes, termed "surgical pastes", based on either low melting point blends of poly(caprolactone) (PCL) with methoxypolyethylene glycol (MePEG) or triblock copolymers of poly(D,L-lactic acid) and polyethylene glycol (PEG) were developed for local application during tumor resection surgery to prevent recurrence (Zhang et al., 1996; Dordunoo et al., 1997; Winternitz et al., 1996).

For tumors that are not resectable, there is a great deal of interest in the development of injectable polymeric delivery systems for relatively non-invasive, direct intratumoral delivery of high concentrations of chemotherapeutics. Anticancer drugs and cytokines have been formulated as microspheres and injected intratumorally into various tumor models, including sarcoma, hepatic, breast, brain and non-small cell lung tumors (Fung and Saltzman, 1997; Hanes et al., 2001; Egilmez et al., 2000; Harper et al., 1999). An alternative approach is the development of injectable formulations that form a semisolid implant in the tumor tissues. There are numerous reports of injectable polymer hydrogel-based delivery systems that have been developed for a variety of drugs, based on the polymeric materials possessing thermosensitivity and biodegradability. The polymers exhibiting properties of reversible thermal gelation are triblock copolymers consisting of A-blocks and B-blocks, arranged as BAB or ABA, where A is poly(D,L-lactic-co-glycolic acid) (PLGA) and B is PEG. Aqueous solutions of these polymers undergo a reversible gel-sol transition and form a free-flowing sol at room temperature, becoming a transparent gel at body temperature (Jeong et al., 1997, 2000; Kim et al., 2001; Zentner et al., 2001). Paclitaxel has been dissolved at high concentrations in aqueous solutions of ABA-type copolymers and following intratumoral injection into mice bearing human breast carcinoma xenografts, there was a slow clearance of paclitaxel from the injection site (about 6 weeks) with minimal distribution into any organ (Zentner et al., 2001). Other injectable, biodegradable implant delivery systems utilize a miscible blend of a water insoluble polymer (such as PLGA) and a water miscible biocompatible solvent (such as *N*-methyl-2-pyrrolidone or glycofurol) (Shively et al., 1995; Eliaz and Kost, 2000). Upon injection into an aqueous tissue environment, the water soluble solvent diffuses out of the polymer, which then precipitates, resulting in a solid implant in vivo. Chenite et al. (2000) developed an injectable, thermally sensitive, pH-dependent hydrogel of chitosan with polyol salts. These formulations are liquid at room temperature and form gels at body temperature.

We have been exploring the development of injectable pastes based on blends of a triblock copolymer with low molecular weight MePEG, which are viscous liquids at room temperature, can be injected through a 22-gauge needle and solidify to a semisolid implant in vivo (Jackson et al., 2000). A biodegradable ABA-type triblock copolymer, where A is a random copolymer of D,L-lactide and caprolactone (PLC) and B is PEG (abbreviated as PLC–PEG–PLC) was blended in a 40:60 ratio with MePEG (molecular weight 350). Intratumoral injection of the viscous liquid, polyblend formulation loaded with paclitaxel was effective in treating a human prostate cancer cell line tumor model in mice (Jackson et al., 2000). It was hypothesized that, following injection into an aqueous tissue environment, MePEG would diffuse out of the formulation and the residual PLC–PEG–PLC/paclitaxel components would solidify due to increased melting point of the residual polymer and precipitation of the paclitaxel (Jackson et al., 2000). In this work, we have characterized the novel paste formulation loaded with paclitaxel and other drugs and provided evidence to support the mechanism of solidification of the polyblend matrix implant.

2. Materials and methods

2.1. Materials

Phosphate-buffered saline (PBS) pH 7.4 was manufactured using sodium chloride and sodium phosphate salts obtained from Fisher Scientific. The molarity of the phosphate component was 10 mM. Methoxypolyethylene glycol (MePEG 350) molecular weight 350, was obtained from Union Carbide, New York,

NY. Paclitaxel was obtained from Hauser Chemical Company, Boulder, CO. Amphotericin, nystatin and tretinoin were a kind gift from Dr. K. Wasan (University of British Columbia, Vancouver, BC, Canada). All other drugs were obtained from Sigma. Triblock copolymer with the following composition was obtained from Angiotech Pharmaceuticals Inc.: poly(D,L-lactide-co-caprolactone)-block–polyethylene glycol-block–poly(D,L-lactide-co-caprolactone) (PLC–PEG–PLC). The molecular weight of PEG was 4600 and the weight percentages of D,L-lactide, -caprolactone, and PEG, respectively, used in preparing the triblock copolymer (TB) were 35, 35 and 30%. The TB copolymer was synthesized through ring opening polymerization as previously described (Burt et al., 1999; Liggins and Burt, 2002).

Some TB samples were sterilized using gamma radiation from a cobalt-60 source and exposed to 2.5 Mrad (over 8 h) of radiation.

2.2. Manufacture of pastes

Pastes were manufactured using triblock copolymer (TB), MePEG and drug by warming the three components to 50 °C in the specified ratios in a 20 ml glass scintillation vial (Fisher Scientific) and levigating the mixture for 5 min to form a solution or suspension of drug in polymer. Pastes were sealed in 20 ml scintillation vials, stored at 4 °C and used within 2 months of manufacture.

2.3. Differential scanning calorimetry (DSC)

DSC was performed using a Perkin-Elmer Pyris 1 calorimeter. Approximately 10 mg of paste was placed in crimped aluminum DSC pans and quench cooled by heating to 80 °C followed by rapid cooling at 500 °C/min. Samples were initially heated to 80 °C to ensure all components were melted. This procedure removed any thermal history from the sample and allowed for uniform surface contact of the paste with the pan. Quenched samples were heated at a rate of 10 or 40 °C/min.

2.4. Weight loss determinations

Quantities (300 mg) of molten paste (50 °C) were pipetted onto the base of 16 ml glass tubes with caps and the total weights of all tubes were recorded. After cooling, 15 ml of distilled water were added, the tubes were capped and placed in an orbital shaker at 90 rpm at 37 °C (Innova 4000 incubator, New Brunswick Scientific). At designated times, each set of tubes ($n = 3$) were taken from the incubator, the water was removed and the polymer blends were dried in a vacuum oven set at a negative pressure of 25 mmHg and a temperature of 50 °C. After 2 days, the tubes were removed from the oven and reweighed. The weight loss was then determined by subtracting the final weight from the original weight of each tube.

2.5. Gel permeation chromatography (GPC)

Quantitative GPC was performed on the paste samples used in the weight loss experiments at ambient temperature using a Shimadzu LC-10 AD HPLC pump, a Shimadzu RID-6A refractive index detector coupled to a 50 Å Hewlett Packard Plgel column. The mobile phase was chloroform with a flow rate of 1 ml/min. The injection volume of the polymer sample was 50 μl at a polymer concentration of approximately 0.25% (w/v).

For degradation studies a calibration graph of log molecular weight versus retention time was established for the 50 Å Plgel column using polystyrene standards (Polymer Laboratories, Church Stretton, Salop, England) in the 1000–50,000 molecular weight range. To fifty-milligram samples of a 40:60 (TB:MePEG) paste blend (±paclitaxel at 10% loading, w/w) in 16 ml glass tubes, were added 15 ml of PBS (pH 7.4) and capped. The tubes were incubated at in an oven at 37 °C. In all tubes, the buffer was changed every few days to remove any drug or polymer that had been released from the pellet. At designated times, tubes were removed from the oven, the supernatant was discarded, the pellets were washed once in water and dried under nitrogen. The dried pellets were then dissolved in chloroform for GPC analysis of the molecular weight of the TB.

2.6. Paste solidification

In order to assess whether the paste formulations solidified in aqueous media to form an intact solid/semi solid, the various formulations were incubated in water for 1 h and then the paste pellets

were vigorously but reproducibly disturbed. This experiment was performed by allowing 15 mg pellets to form in the base of a 20 ml flat bottomed glass vial set on a 45° angle containing 5 ml of PBS. After one hour, a small magnetic stir bar (8 mm × 2 mm) was added and the mixture was stirred at a constant speed. By measuring the time for the pellets to break up under such conditions, a qualitative determination of the "intactness" of the matrix was possible. Pastes containing 10% (w/w) drugs (methotrexate, colchicine, curcumin, genistein, tretinoin, nystatin, amphoterecin, camptothecin or paclitaxel) were manufactured using a 40:60 (TB:MePEG) composition. Fifteen milligram weights of each composition (molten at 50 °C) were pipetted into 20 ml glass scintillation vials held at 4 °C (on ice) to form uniform solid pellets. Five milliliters of ice-cold PBS pH 7.4 containing 0.2% bovine serum albumin (Fraction 4 Boehringer Mannheim) was placed on top of the pellet and the vial was placed in a stationary position in a 37 °C oven for 1 h. At this time a 5 mm × 1 mm stir bar was placed in the vial and stirring was continued at 300 rpm and the time for the paste pellet to break up into more than three pieces was recorded.

2.7. Drug release profiles

Pastes containing 10% (w/w) drugs (methotrexate, colchicine, curcumin, genistein, tretinoin, nystatin, amphotericin, camptothecin or paclitaxel) were manufactured using a 40:60 (TB:MePEG) composition. Pastes containing 2.5, 5, 10 and 15% paclitaxel were manufactured in ratios of TB:MePEG ranging from 30:70 to 90:10. Fifteen milligram weights of each composition were placed in 20 ml glass scintillation vials and cooled to 4 °C to form uniform solid pellets. Five milliliters of ice-cold PBS pH 7.4 containing 0.2% bovine serum albumin (Fraction 4 Boehringer Mannheim) was placed on top of the pellet followed by 5 ml of *n*-octanol (Fisher Scientific). The octanol formed an upper immiscible phase on top of the PBS so that any drug released into the PBS would partition into the octanol phase. The vials were capped and incubated at 37 °C.

The concentration of the drug in the octanol phase was analyzed by either UV-Vis absorbance methods or HPLC methods (for paclitaxel only). This octanol phase was replaced back into the vial. UV-Vis analysis was performed by determining the absorbance at the specified wavelength using a photo-diode array spectrometer (Hewlett Packard, Richmond, BC, Canada). Calibration graphs of the drugs in octanol were established by measuring the absorbance of a set of standards of each drug in octanol in the 0–50 μg/ml concentration range. HPLC analysis of paclitaxel was performed using a Waters HPLC system (Mobile phase 58:37:5. Acetonitrile:water:methanol, 1 ml/min, 20 μl injection, C18 Novapak Waters column with detection at 232 nm) as previously described (Jackson et al., 2000). The solubility of paclitaxel in PBS was approximately 1–2 μg/ml, whereas in *n*-octanol was greater than 10 mg/ml. This substantial difference in solubility ensured rapid and complete partitioning of released drug into *n*-octanol. This was confirmed by drying amounts of paclitaxel equivalent to those used in these studies onto the base of glass vials and adding 5 ml of PBS and 5 ml of *n*-octanol. All drug partitioned into the *n*-octanol within 3 h. For other hydrophobic drugs, similar studies showed very small amounts of drug in the aqueous phase, compared to the octanol phase.

3. Results

3.1. Paste manufacture and general characteristics

All concentrations of paclitaxel (2.5, 5, 10 and 15%) in all blends of TB:MePEG (30:70 to 90:10) were completely miscible in the molten polymer matrix during blending at 50 °C. Also, when the blends were allowed to cool to room temperature, there was no evidence of paclitaxel crystallization using optical microscopy. The presence of increasing concentrations of MePEG in paclitaxel loaded pastes led to increasingly less viscous formulations. Pastes containing 30% (or greater) MePEG could be injected through a 23-gauge needle at room temperature. Pastes containing either 20 or 10% MePEG were more viscous but could be injected through 22- and 20-gauge needles, respectively.

At 10% drug loading all other drugs formed homogeneous dispersions or solutions of the drugs in the molten polymer at 50 °C. All the compositions were free flowing molten fluids during blending at 50 °C using the 40:60 (TB:MePEG) polymer blend and could readily be injected through a 23-gauge needle at room

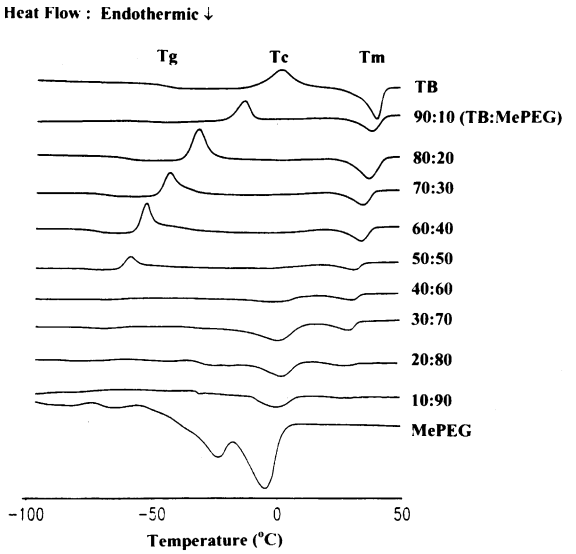

Fig. 1. DSC thermograms of TB:MePEG blends showing glass transition (T_g), crystallization (T_c) and melting point (T_m) peaks following heating at 40 °C/min. All blends had been heated to 80 °C and quenched cooled prior to heating.

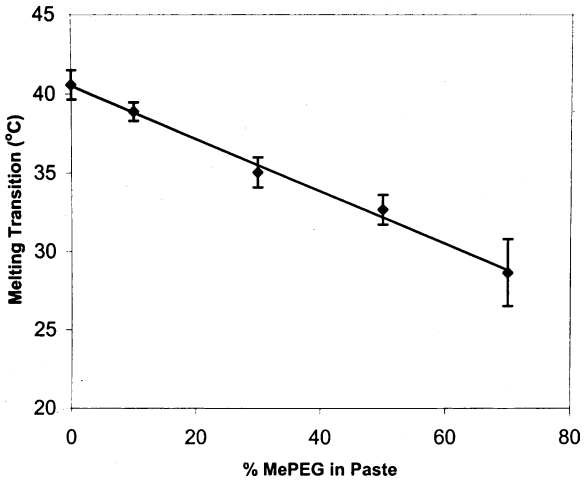

Fig. 2. The effect of increasing concentrations of MePEG in 10% paclitaxel loaded TB:MePEG pastes on the melting transition temperature (T_m) using a heating rate of 10 °C/min. Values are the mean of T_m values determined from three separate paste samples at each MePEG concentration.

temperature. There was no evidence of drug particle aggregation in the polymer blends.

3.2. Differential scanning calorimetry

DSC thermograms for 100% TB, MePEG and pastes composed of different blend ratios of TB:MePEG are shown in Fig. 1. The TB showed three thermal events corresponding to a glass transition (T_g) at −44 °C, a recrystallization exotherm (T_c) at 2 °C and a melting transition (T_m) at 40.5 °C. Thermograms of MePEG showed a T_g at −98 °C and a broad melting transition between −50 and 5 °C with a double endothermic peak at −24 and −4 °C. As the concentration of MePEG in the blend was increased to 70%, the thermograms of the blends showed a single T_g intermediate between the T_gs of the pure TB and MePEG as shown in the summary of the thermal data given in Table 1.

As the concentration of MePEG in the blend was increased to 50%, the thermograms of the blends showed a single T_m intermediate between the T_ms of the pure TB and the MePEG. The dependence of melting point depression on the blend composition is shown in Fig. 2. At MePEG concentrations in the blend between 60 and 90%, two melting transitions were evident. Recrystallization peaks (T_c) were present in paste blends in which 50% or more of the composition was TB. The TB crystallized at 1.3 °C and increasing MePEG concentrations (up to 50%) reduced T_c. Sterilizing doses of gamma radiation had no effect on the thermal properties of the triblock copolymer (data not shown).

3.3. Weight loss determinations

Following incubation in water at 37 °C, all blends showed rapid weight loss. The weight loss was assumed to be almost exclusively the loss of MePEG from the blend into the water. Weight loss was therefore expressed as the percent weight loss of the total MePEG in the blend and the results are shown in Fig. 3. The 100% TB was used as a control and did not lose any weight during the experiment. The weight changes for all blends were characterized by a rapid weight loss in the initial 5 h followed by a slower more sustained phase. Between 80 and 100% of the MePEG had dissolved out of all of the blends by 48 h. To determine the effect of including paclitaxel in a polymer blend on the weight loss in water the experiment was repeated for a 40:60 (TB:MePEG) com-

Table 1
Summary of the thermal data obtained from the DSC thermograms of quenched PLC-PEG-PLC triblock copolymer (TB) with MePEG blends using a heating rate of 40 °C/min, as shown in Fig. 1

TB: MePEG	T_g (°C)	T_c (°C)	T_m (°C)	
100:0	−44.5	1.3	40.5	
90:10	−51.7	−12.9	38.2	
80:20	−60.4	−32.2	37.5	
70:30	−67.1	−42.7	34.8	
60:40	−71.0	−51.9	34.2	
50:50	−74.5	−57.9	31.5	
40:60	−74.9	ND	−1.0	30.2
30:70	−75.6	ND	1.0	28.9
20:80	ND	ND	2.3	27.7
10:90	ND	ND	0.3	ND
0:100	−98	−70	−24.3	−4.7[a]

ND: Not detected.
[a] Broad endothermic peak between −24 and −4.7 °C. Largest peak at −4.7 °C.

position containing different amounts of paclitaxel using triplicate samples. The inclusion of paclitaxel had little effect on the rate of MePEG loss from the 40:60 blend during the first 24 h. However, after 24 h, the MePEG dissolved out of the 5 and 10% paclitaxel loaded pastes at a slower rate than pastes containing 2.5% or no paclitaxel.

3.4. Weight loss determinations: gel permeation chromatography

Dried samples from the weight loss determination experiments were used for GPC analysis. Both TB and MePEG gave characteristic peaks with retention times of 7.5 and 9.3 min, respectively. It was determined that these peaks could be used for the quantitative determination of each polymer remaining in the blend. Using standard weights of TB or MePEG, it was found that plots of peak areas against weight gave linear calibration curves with correlation coefficients of 0.98 or higher. These calibration graphs were used to determine the relative amounts of TB and MePEG in a blend following incubation in water. The relative peaks areas of MePEG would decrease and the relative peak areas of TB would increase following incubation in water. A representative set of data for a 30:70

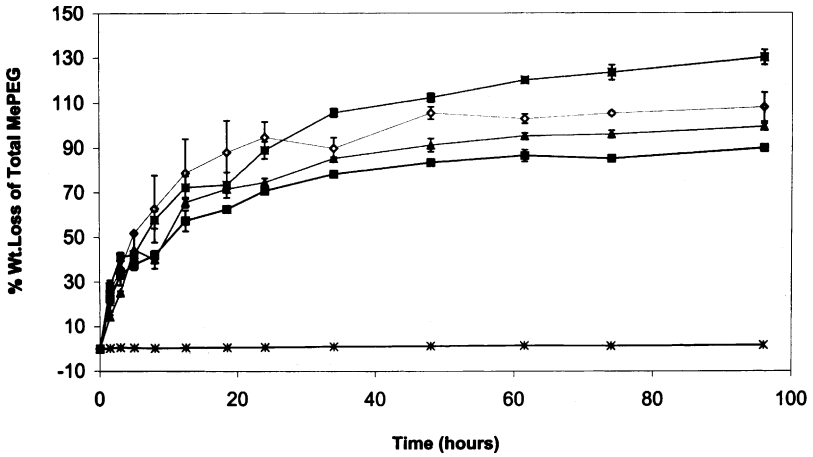

Fig. 3. The time course of the loss of MePEG from various TB:MePEG blends (no paclitaxel). The weight loss of MePEG from 300 mg pellets of polymer blends into distilled water at 37 °C is expressed as percent weight loss of the total original amount of MePEG in the blend ($n = 3$, mean ± S.D.). The ratio of TB:MePEG in each blend was (×) 100:0, (■) 90:10, (▲) 70:30, (△) 50:50 and (◇) 30:70.

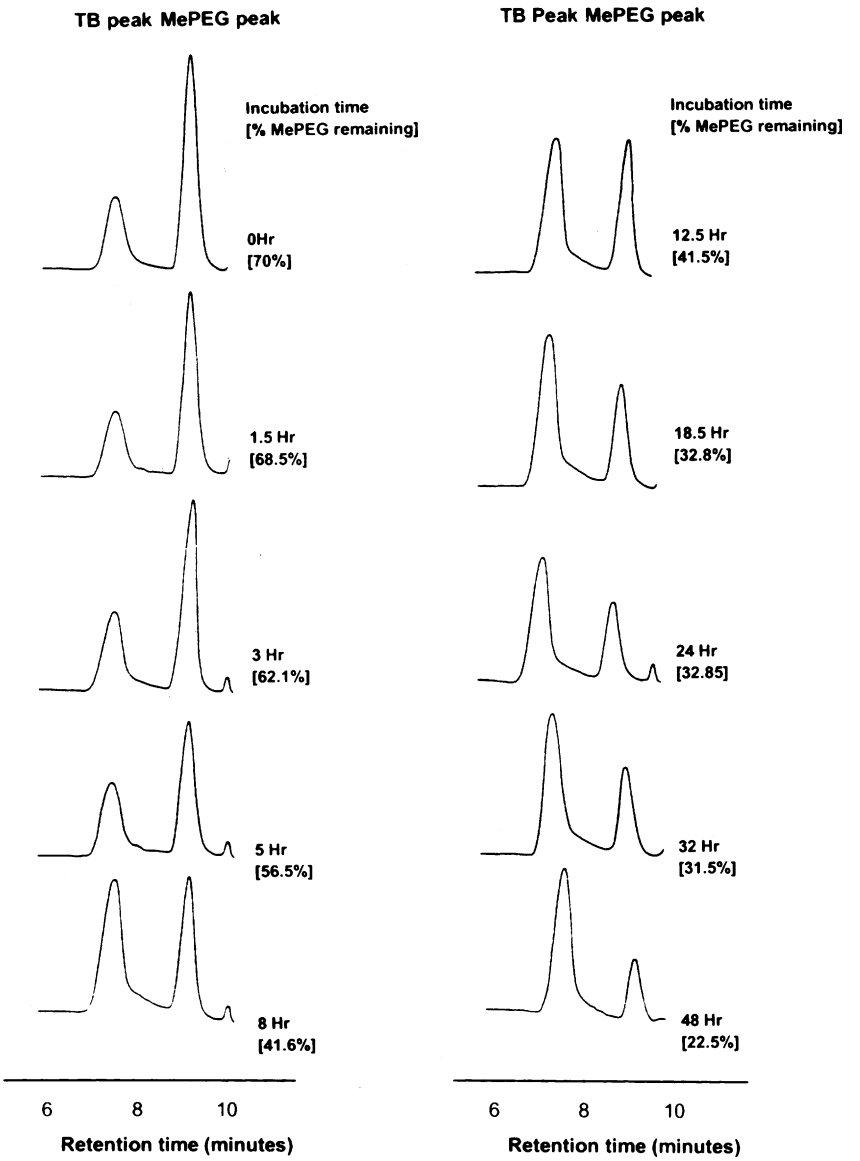

Fig. 4. Gel permeation chromatograms (GPC) of a 30:70 (TB:MePEG) blend showing the TB and MePEG peaks at 7.5 and 9.3 min, respectively. GPC runs of the blend following incubation in water for 0, 1.5, 3, 5, 8, 12.5, 18.5, 24, 32 and 48 h are shown. The values in brackets show the percent of MePEG in the blend at each time point calculated from the peak area using a standard curve.

(TB:MePEG) blend are shown in Fig. 4. Fig. 5 shows the percent MePEG remaining in the paste blends following incubation in water for specified times as determined using GPC. All blends lost MePEG in a rapid manner over the first 12–20 h in water followed by a slower loss of the polymer from the blend over the next 30–38 h.

3.5. Degradation studies: gel permeation chromatography

Using GPC and a calibration graph of log molecular weight versus retention time for polystyrene, the molecular weight of the TB copolymer was determined to be 16,700. The calculated molecular

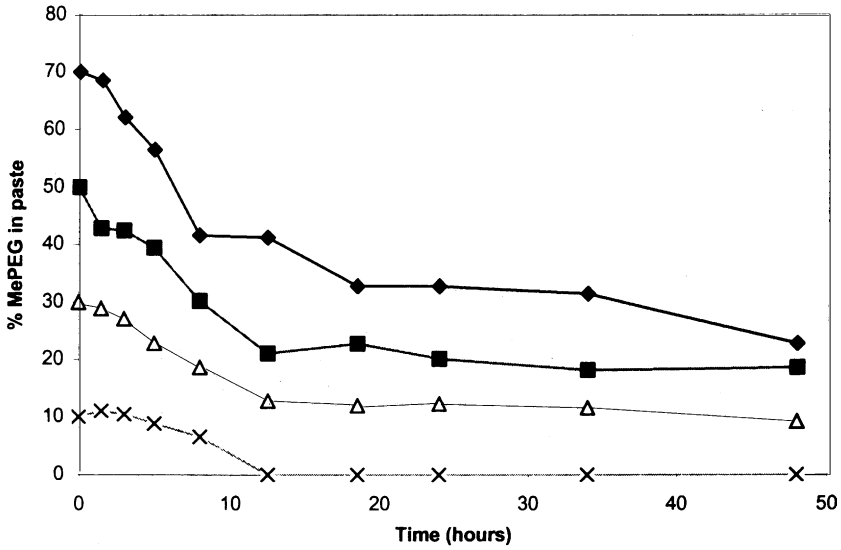

Fig. 5. The time course of MePEG loss from various TB:MePEG blends using quantitative GPC. The initial concentrations of MePEG in the blends were (◆) 70%, (■) 50%, (△) 30% and (×) 10%.

weight of the TB was determined to be 15,333. The time-dependent changes in the molecular weight of small TB pellets placed in water are shown in Fig. 6. The polymer degraded from a molecular weight of 16,700 to approximately 12,000 over a period of 30 days. The inclusion of paclitaxel or MePEG in the composition had no significant effect on the degradation rate of the TB. After 30 days there was no measurable change in the molecular weight of the polymer. However, the sizes of the polymer pellets

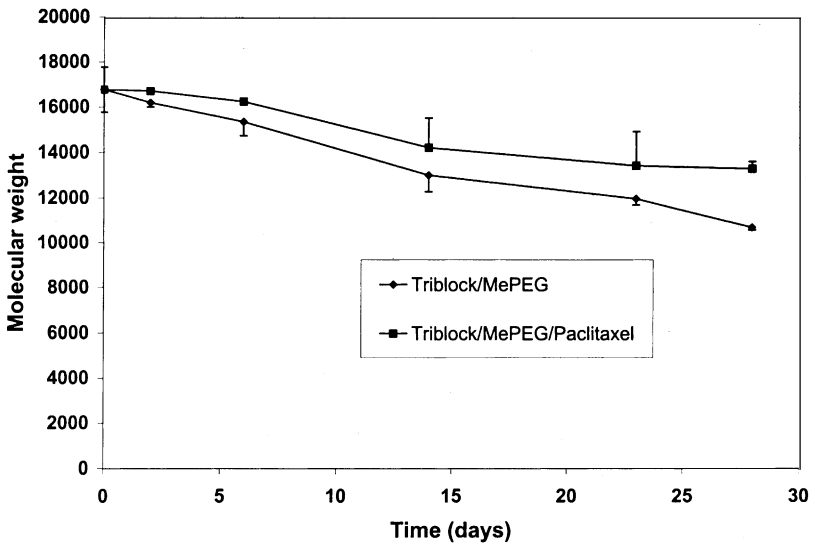

Fig. 6. Time course of the degradation of the triblock copolymer in water as determined by the change in the molecular weight of TB in 40:60 (TB:MePEG) paste blends containing either no paclitaxel or 10% (w/w) paclitaxel. Molecular weight determined by GPC ($n = 3$, mean ± S.D.).

were observed to diminish rapidly in the sample tubes. Similarly, the size of the peaks on the chromatograms reduced rapidly so that accurate determinations of the retention time were not possible (data not shown). In a separate experiment, 200 mg samples of TB blended with 10% MePEG were incubated in PBS at 37 °C for periods up to 41 days and then dried and reweighed to determine the mass loss. The mass loss at 7, 14, 22, 28 and 41 days was 40% (±3), 57% (±1), 75% (±2) 87% (±2) and 96% (±1) ($n = 3$), respectively. The GPC molecular weight of the TB in these samples decreased from 16,981 to 11,945 over the 41-day period at a rate similar to those shown in Fig. 6 for the 60% MePEG blends.

Gamma radiation had no effect on the molecular weight of the TB copolymer determined by GPC. Paclitaxel was included in some irradiated TB samples at concentrations up to 10%. In all cases there was no degradation of paclitaxel as determined by HPLC analysis of drug recovered from the irradiated TB.

3.6. Solidification determinations

When placed in quiescent aqueous media at 37 °C, all blends showed evidence of solidification, becoming a semi-solid paste at high concentrations of MePEG (70%) or a waxy solid at high concentrations of TB (90%) within one hour in PBS. However, when paste blends (no drug) were subsequently stirred, they broke up into small pieces within 30 s, indicating that the paste had failed to solidify to a form with good structural integrity in water at 37 °C (Fig. 7). Including the hydrophobic drug paclitaxel in a 40:60 (TB:MePEG) blend had a pronounced effect on the structural integrity of the polymer blend in PBS so that the pellet remained intact under vigorous stirring for 25 min. Furthermore, paste compositions containing the highly hydrophobic and water insoluble drugs curcumin, genistein, tretinoin, nystatin, amphotericin or camptothecin stayed intact for over 4 min under conditions of rapid stirring, indicating that these compositions had solidified to pellets with structural integrity in aqueous media. The paste compositions containing the more water soluble drugs colchicine and methotrexate disintegrated rapidly when stirred (2.5 min and 30 s, respectively; Fig. 7) and the pellets possessed little to no structural integrity.

In separate experiments paste pellets containing paclitaxel and more than 60% TB did not disintegrate significantly over 30 days when left stationary in PBS at 37 °C. Increased paclitaxel loading into pastes with less than 60% TB led to a reduction in the rate of disintegration of the pellets.

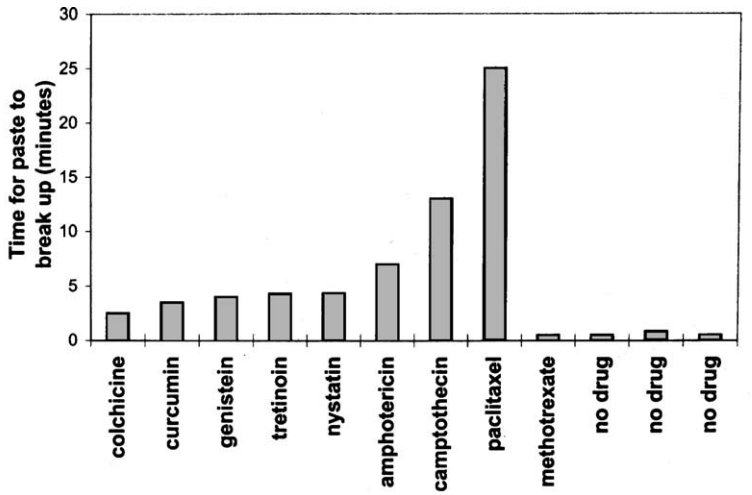

Fig. 7. Disintegration of paste pellets of 40:60 (TB:MePEG) blends containing 10% (w/w) loadings of drug as determined by the time for a paste pellet to break up under conditions of rapid stirring. Paste pellets were allowed to solidify in water for 1 h at 37 °C without agitation. Pellets were then stirred at 300 rpm and the time for fragmentation into three or more pieces was measured.

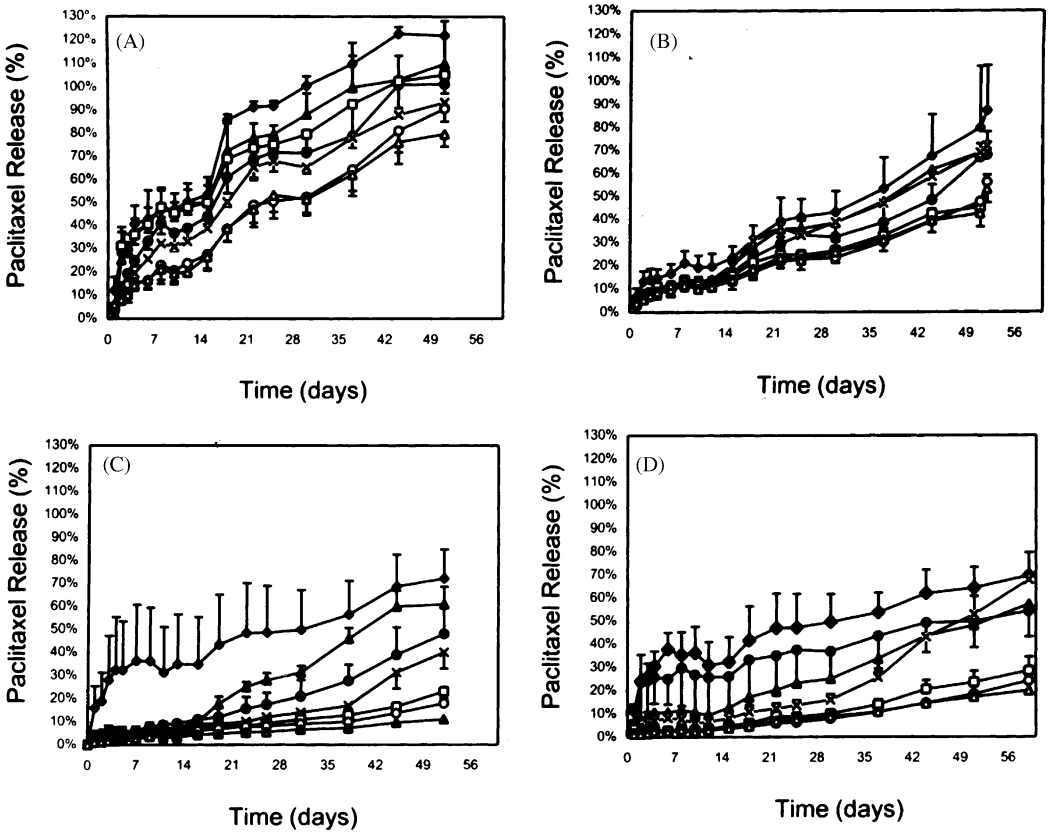

Fig. 8. The time course of paclitaxel release from various blends of TB:MePEG for (A) 2.5% paclitaxel; (B) 5% paclitaxel; (C) 10% paclitaxel; and (D) 15% paclitaxel loaded pastes. The ratio of TB to MePEG in each blend was (◆) 30:70, (▲) 40:60, (●) 50:50, (×) 60:40, (□) 40:30, (○) 80:20 and (Δ) 90:10 ($n = 4$, mean ± S.D.).

3.7. Drug release experiments

Fig. 8A–D show the release profiles for 2.5% (Fig. 8A), 5% (Fig. 8B), 10% (Fig. 8C) and 15% (Fig. 8D) paclitaxel loaded pastes composed of TB:MePEG blends in the range 30:70 to 90:10. All formulations released paclitaxel with a short burst in the first 3 days followed by a slower phase of release over the following 40 days. In general, blends containing higher concentrations of MePEG released drug more quickly than blends with lower concentrations of MePEG. Blends that contained higher concentrations of paclitaxel released drug more slowly than blends that contained lower concentrations. The 2.5% paclitaxel loaded pastes released the largest percentage of encapsulated drug of all the drug loadings, so that after 30 days between 50 and 100% of encapsulated drug had been released. The 5, 10 and 15% paclitaxel loaded pastes had a wide range of drug release profiles which depended on the TB:MePEG ratios as shown in Fig. 8B–D.

Fig. 9 shows the drug release profiles for 10% drug loaded TB:MePEG (40:60) paste. Pastes containing the moderately water soluble drugs methotrexate and colchicine released almost all the encapsulated drug in the first 24 h of the drug release experiment. However, pastes containing all the hydrophobic, water insoluble, drugs released the encapsulated drug much more slowly. Consistent with the solidification experiments described in this study (Fig. 7), which used stir bar agitation, the paste pellets containing the hydrophobic drugs formed distinct solid pellets in PBS which did not disintegrate in the drug release vials. On the other hand, the pellets containing the water soluble drugs

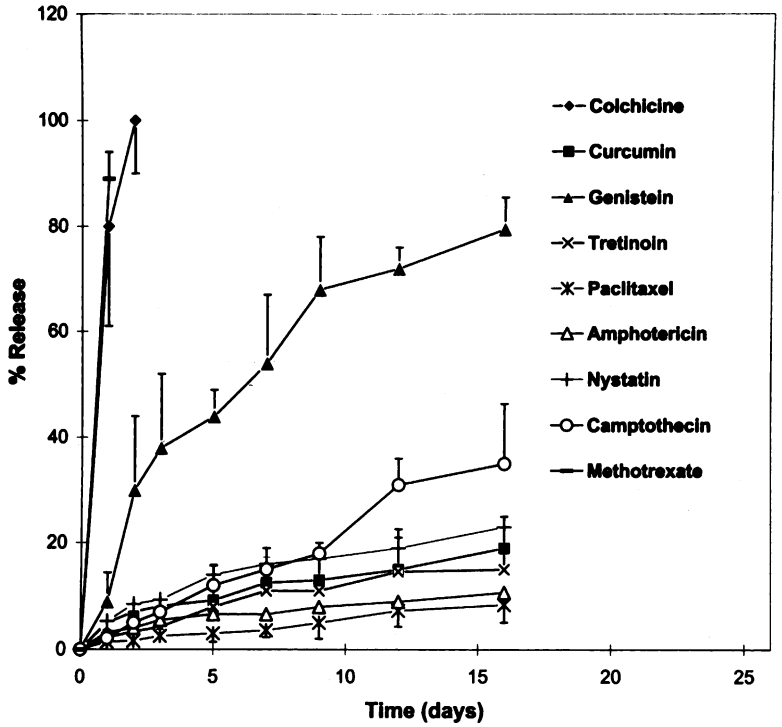

Fig. 9. The time course of drug release from 10% drug loaded pastes composed of a 40:60 blend of TB:MePEG ($n = 4$, mean ± S.D.).

colchicine and methotrexate disintegrated extensively after 2 days of drug release in quiescent media.

4. Discussion

The triblock copolymer of PLC–PEG–PLC is composed of PEG with a molecular weight of 4600 and a random copolymer of D,L-lactic acid and caprolactone with a calculated molecular weight of 15,333. It is a waxy solid at ambient temperature. The addition of increasing amounts of the low molecular weight (350) MePEG component to the TB did not lead to any observable phase separation and a miscible blend resulted. As the proportion of MePEG in the blend increased, the viscosity decreased and the blend became free-flowing.

The melting transition for MePEG (Fig. 1) was a broad double endothermic event between about −55 and 5 °C, with the peak temperature of the second endotherm occurring at −5 °C. 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 crystallites. The melt from the metastable crystallites then underwent recrystallization to more stable crystallites which subsequently melted (Nichols and Richardson, 1992). After quench cooling, there was a very small recrystallization peak at −70 °C just prior to the melting endotherm of MePEG. MePEG (350 g/mol molecular weight) is made up of small molecules, which gives them greater mobility by reducing the likelihood of chain entanglements. As a result, MePEG crystallization likely occurred following quench cooling. Quench cooled samples of TB showed a recrystallization event at 1 °C and melting at 40 °C. The melting of TB and MePEG blends occurred at temperatures that were dependent on blend composition. There was a single melting transition for TB:MePEG blends containing MePEG up to a concentration of about 50%. Values of T_m were highest for 100% TB and decreased as the proportion of MePEG in the blend increased (Table 1). Melting point depression data also confirmed that TB and MePEG were completely miscible up to 50% MePEG

content (Nishi and Wang, 1975). At a TB:MePEG ratio of 40:60, a very small additional melting endotherm appeared at approximately −1 °C and this endotherm gradually increased in size for blends with greater proportions of MePEG (TB:MePEG of 30:70, 20:80 and 90:10). This endotherm appeared to correspond to the second (higher temperature) melting peak observed for 100% MePEG samples. At all proportions of TB and MePEG, DSC scans showed that the blends possessed only one T_g with a value intermediate between the T_g values of the individual polymers (−44 and −98 °C for TB and MePEG, respectively). This would indicate that the polymers were miscible at the concentrations present in the amorphous phase (Rosen, 1993). Both the T_g and T_c decreased as the MePEG concentration increased in the blends (Table 1). As the T_g is lowered, the mobility of the polymer chains is increased at any given temperature above T_g and recrystallization becomes possible at lower temperatures.

The addition of MePEG to TB resulted in blends possessing decreased viscosity, making it possible to inject the compositions through a needle and syringe. Following the addition of water or PBS to TB:MePEG blends, the materials became increasingly viscous and eventually semi-solid or solid at 37 °C. The studies involving weight loss and MePEG loss were all carried out by the addition of water to paste samples of constant volume and geometry. The weight loss and GPC studies demonstrated that in the presence of aqueous media, the MePEG component diffused and partitioned out of the blends rapidly (Figs. 3–5) leading to an increase in the melting temperature, so that the material began to solidify and eventually formed a semi-solid mass at 37 °C. However, these semi-solid masses were very fragile and did not retain structural integrity on stirring of the aqueous incubation medium (see Fig. 7 for pastes with no drug loading). When paclitaxel was added to the TB:MePEG blends, the drug dispersed as a homogeneous solution in the matrix. The addition of water or PBS to these paclitaxel loaded blends resulted in the formation of solid masses with significantly greater structural integrity, as measured by the simple, yet reproducible method of determining the time taken to break the masses into more than 3 pieces upon vigorous stirring (Fig. 7). It was observed that incubation of the TB:MePEG blends in aqueous media led to precipitation of the paclitaxel in the matrix. Hence, the loss of MePEG from the matrix in the presence of water probably reduced the solubility of paclitaxel in the remaining TB-rich phase and also caused greater water uptake into the matrix leading to precipitation of paclitaxel. The greater structural integrity of the paclitaxel loaded matrix was likely due to the presence of solid drug dispersed throughout the waxy TB-rich phase. To determine whether precipitation of a drug dispersed in the TB-rich matrix was an important component of improving the mechanical strength of the mass in water, different drugs with a range of hydrophobicities were dispersed in 40:60 TB:MePEG blends. Similar to paclitaxel, camptothecin and amphotericin B are highly hydrophobic compounds with a very low water solubility and these drugs also precipitated within the TB-rich phase giving greater mechanical strength to the masses (Fig. 7). More water soluble drugs, such as colchicine or methotrexate did not solidify extensively in TB-rich matrices in water and correspondingly showed less structural integrity (Fig. 7).

GPC analysis showed the molecular weight of the TB copolymer to be 16,700 which was close to the calculated value of 15,333. The MePEG component was lost from the TB:MePEG blends rapidly and extensively in water within 100 h (about 4 days) (Figs. 3 and 5). There may also have been a small contribution of hydrolytic degradation of TB and erosion to the total weight loss shown in Fig. 3. The molecular weight of the TB decreased to about 13,000–14,000 over the first 2 weeks and then degradation was slower over the next 2 weeks. After about 1 month, there appeared to be negligible further changes in molecular weight of the TB in the residual paste. However, after 4 weeks, the size and mass of paste samples continued to decrease rapidly and a substantial weight loss of 96% after 41 days was found, for paste samples of TB blended with 10% MePEG. We speculate that the degradation products of TB with a molecular weight of around 12,000 were water soluble leading to dissolution, erosion and weight loss. It is possible that the end blocks of PLC on the TB degraded preferentially over this time period, leaving a more water soluble copolymer which dissolved in the aqueous medium.

Blends of TB:MePEG containing MePEG at concentrations ranging from 10 to 70% and paclitaxel at concentrations from 2.5 to 15% formed semisolid masses or “pellets” in PBS at 37 °C. These pellets all released paclitaxel in a controlled manner (Fig.

8). Compositions of pastes containing lower paclitaxel loadings or higher MePEG concentrations released drug faster than other paste compositions. This effect may be related, at least in part, to different rates of break-up of the pellets into smaller fragments. Paste pellets containing low paclitaxel concentrations and high MePEG concentrations were observed to break up rapidly into small fragments over 7 days. Hence, the surface areas of these masses exposed to water would increase, leading to greater water uptake, more rapid loss of MePEG, faster degradation of the TB and increased rates of drug release.

We determined that paste blends containing 60% MePEG, 40% TB and 10% paclitaxel possessed optimal injectability characteristics. The paste could be injected through a 23-gauge needle at room temperature and formed a semisolid implant in water at 37 °C. This was also the case for 40:60 TB:MePEG blends containing the hydrophobic drugs camptothecin and amphotericin B and these pellets released drug with a similar profile to paclitaxel (Fig. 9). Structural integrity of the pellet formed in vivo is considered desirable, since drug release would likely be more predictable and controlled. The 40:60 TB:MePEG paste loaded with 10% paclitaxel was injected intratumorally into mice bearing human prostate LNCaP tumors grown subcutaneously (Jackson et al., 2000). The paste solidified in vivo within 3 h and the tumors regressed dramatically by 8 weeks. There was evidence of some residual paste at week 8 in the mice. On-going studies are evaluating combination therapy of the paste with radiation in the treatment of localized prostate tumors.

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