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Amphiphilic dextran-graft-poly(ε-caprolactone) films for the controlled release of paclitaxel

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Abstract

Amphiphilic graft copolymers with dextrans as the main chains and poly(ε-caprolactone) as the side chains were synthesized by solution polymerization in dimethyl sulfoxide using stannous 2-ethylhexanoate as a catalyst. The copolymers were characterized with nuclear magnetic resonance spectroscopy and gel permeation chromatography. Paclitaxel loaded copolymer films were prepared using a solution cast method. In vitro release rates were influenced by both the initial paclitaxel loading and the morphology of the films. The films with paclitaxel loadings up to 10% (w/w) showed a single-phase morphology when dry. However, paclitaxel crystallization occurred in the films with high loadings (5 and 10%) upon incubation in phosphate buffered saline at 37 °C. The crystals had a spherulitic structure and were found to be paclitaxel dihydrate based on X-ray diffraction analysis. The formation of paclitaxel dihydrate in the films produced a marked effect on the in vitro release rates.

Keywords: Amphiphilic graft copolymers; Dextran; Poly(ε-caprolactone); Paclitaxel; Controlled release

1. Introduction

Paclitaxel is a natural alkaloid indicated for the treatment of ovarian (McGuire et al., 1989) and breast cancer (Rowinsky et al., 1990). It has also been shown to possess anti-inflammatory, anti-angiogenic and anti-proliferative properties (Burt et al., 1995; Belotti et al., 1996; Axel et al., 1997; Schiff and Horwitz, 1980). Studies conducted in our laboratory have demonstrated that paclitaxel is effective in the prevention of the formation of postsurgical adhesions (Jackson et al., 2002). Postsurgical adhesions are abnormal tissue attachments that may result from tissue abrasion during surgical procedures. They are a common and serious surgical problem resulting in considerable morbidity. Adhesion formation is initiated by inflammation and exacerbated by angiogenesis and fibroblast proliferation at the surfaces of damaged organs or tissues (Gomel et al., 1996). Biodegradable polymeric film formulations for the controlled delivery of paclitaxel are being developed by our group for potential application at surgical sites to prevent post-surgical adhesions. The films are intended to provide both a barrier effect to separate traumatized tissues and also to release paclitaxel to inhibit adhesion processes. The film formulations should be composed of biocompatible and biodegradable polymers which do not cause significant adverse tissue reactions at the placement sites and which can be absorbed by the body within approximately 1 month.

Dextran possesses excellent biocompatibility and has been used as a carrier for a variety of bioactive

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agents such as small molecule drugs, peptides, proteins and enzymes (Kumar and Banker, 1996; Park et al., 1993). However, hydrophobic drugs, such as paclitaxel, precipitate out when they are directly loaded into dextran matrices due to the highly hydrophilic nature of dextran. The synthesis of an amphiphilic copolymer of dextran with poly(ε-caprolactone) (PCL) as side chains should combine properties of the hydrophilic dextran with hydrophobic PCL to produce a biocompatible copolymer with an enhanced capacity for solubilizing paclitaxel and capable of forming suitable film formulations for potential application in preventing postsurgical adhesions.

The syntheses of amphiphilic graft copolymers with dextran as the backbone have been reported previously. Dextran-graft-poly(D,L-lactic acid) was synthesized using a photopolymerization method (Zhang et al., 1999), in which dextran and poly(D,L-lactic acid) (PDLLA) were modified firstly with unsaturated vinyl groups to form dextran-acrylate and PDLLA-diacrylate macromers, respectively. The macromers were then photocrosslinked by low-intensity ultraviolet (UV) light. The copolymer has been used as a carrier for the controlled release of albumin and indomethacin (Zhang and Chu, 2000, 2002). Dextran graft copolymers with both poly(L-lactic acid) and poly(D-lactic acid) as the side chains were also synthesized (de Jong et al., 2001). The hydrophilic dextran and hydrophobic poly(lactic acid) (PLA) were coupled using a chemical method instead of UV light. The hydroxyl groups of PLA were firstly activated with *N*,*N*′-carbonyldiimidazole in THF solution and then the coupling reaction was carried out in DMSO solution using 4-*N*,*N*-dimethylaminopyridine as the catalyst (de Jong et al., 2001). Dextran-graft-poly(ε-caprolactone) (Dx-g-PCL) was synthesized by solution polymerization in toluene (Ydens et al., 2000). The dextrans were modified by partial silylation by reacting with hexamethyldisilazane to make them soluble in toluene. The silylated dextrans were then reacted with ε-caprolactone to form the graft copolymers. After the formation of the graft copolymers, the trimethylsilyl groups were removed by hydrolysis using hydrochloric acid (1 M).

The objectives of this study were to synthesize graft copolymers of dextrans with short PCL side chains by ring-opening polymerization without silylation of the dextrans and to design and characterize paclitaxel loaded, controlled release film formulations of the copolymers for potential application in the prevention of postsurgical adhesions. Dextrans with two different molecular weights were used as the main chain polymers.

2. Materials and methods

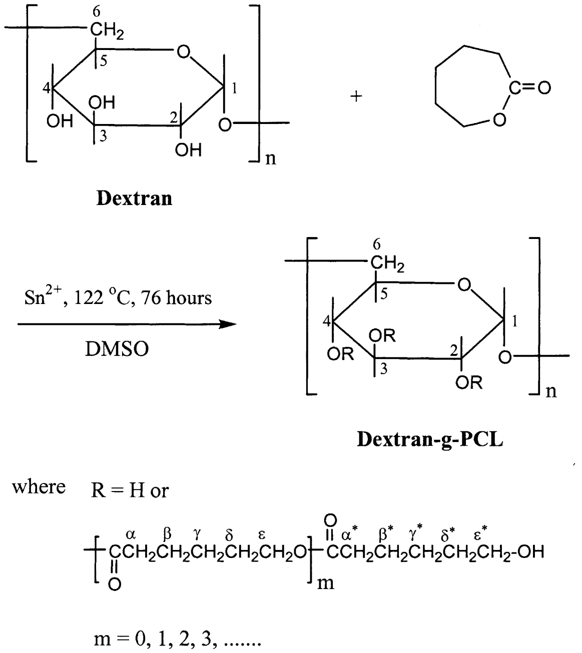
2.1. Materials

Dextran70 (Dx70) ($M_w = 69{,}000$, $M_w/M_n = 1.6$) and dextran500 (Dx500) ($M_w = 473{,}000$, $M_w/M_n = 2.4$) were purchased from Pharmacia (Sweden) and were dried under vacuum (0.3 mmHg) at 60 °C for 16 h before being used. Paclitaxel was purchased from Hauser Chemicals, Inc. (Boulder, CO). Paclitaxel dihydrate was prepared using a modified method of Liggins et al. (1997) as follows: commercial paclitaxel was dissolved in acetonitrile (ACN) to form a solution with a concentration of 1.5% w/v. The solution was added into an excess amount of water with stirring and a turbid solution resulted. The turbid solution was centrifuged at 14,000 rpm for 10 min. The precipitate was collected and dried at room temperature for 15 h in a vacuum oven (150 mmHg).

Dimethyl sulfoxide (DMSO, Fisher) was distilled over molecular sieves (4A type, Fisher) under vacuum with the first 20% of the distillate discarded. The distillate was sequentially dried with two batches of 4A molecular sieves for a total of 96 h (48 h per batch). The dried DMSO was stored over 4A molecular sieves. ε-Caprolactone was purchased from Fluka. It was dried over calcium hydride (CaH_2, from Fluka) for 24 h and distilled twice under a reduced pressure. Stannous 2-ethylhexanoate (95%, Sigma), tetrahydrofuran (THF) (HPLC grade, Fisher Scientific), hexanes (certified A.C.S, Fisher Scientific), methanol (HPLC grade, Fisher Scientific), acetonitrile (HPLC grade, Fisher Scientific), phosphorus pentoxide (P_2O_5) (Laboratory grade, Fisher Scientific), deuterated dimethyl sulfoxide (DMSO-d6) (D, 99.9%, Cambridge Isotope Laboratories, Inc.) and deuterated chloroform ($CDCl_3$) (D, 99.8%, Cambridge Isotope Laboratories, Inc.), were used as received.

2.2. Synthesis of the graft copolymers

Graft copolymers with Dx500 and Dx70 as main chains and PCL as side chains (Dx-g-PCLs) were



Scheme 1. Structure and synthesis of Dx-g-PCLs.

synthesized by solution polymerization using stannous 2-ethylhexanoate as the catalyst. The glass reactors were flamed and then cooled down to room temperature under vacuum and purged with nitrogen gas. Dextran and DMSO were added into the reactors at a ratio of 1:16 (w/v) and placed in a 60 °C oven until the dextran was dissolved. ε-Caprolactone (CL) and stannous 2-ethylhexanoate ($Sn(Oct)_2$) were then added into the dextran solution with agitation. The feed ratio of dextran:CL:$Sn(Oct)_2$ was 1:3:0.05 (w/w/w). The reaction mixtures were placed under vacuum (0.3 mmHg) at room temperature for 30 min, and then purged with nitrogen gas. The vacuuming/purging procedures were repeated three times, and then the tubes were sealed under a nitrogen blanket and placed in an oil bath at 122 °C for 76 h. The reaction process is shown in Scheme 1.

The unreacted ε-caprolactone was extracted from the original reaction product and assayed by HPLC. The reaction product (∼100 mg) was accurately weighed. THF (200 μl) was added to dilute the product. Distilled water (1 ml) was added to the solution to precipitate the graft copolymer and PCL homopolymer formed during the reaction. The mixture was centrifuged at 14,000 rpm for 20 min, and the supernatant was collected and analyzed using a Waters (Waters Corporate, Milford, MA) high performance liquid chromatography (HPLC) system, which was equipped with a model 600 controller and pump module, a model 486 UV detector, a model 717+ autosampler, and Millennium^{32} software for instrument control and data analysis. The column was an Aqua C18 (Phenomenex®), the mobile phase was methanol/water (35/65) at a flow rate of 0.5 ml/min. Pure ε-caprolactone was used as the standard to construct the calibration curve for the quantitation. The detection wavelength was 234 nm.

2.3. Purification of Dx-g-PCL

The reaction products were precipitated by an excess amount (10-fold volume) of water to remove unreacted dextrans (if present), unreacted ε-caprolactone and the solvent DMSO. The PCL homopolymer formed during the reaction was removed by fractional precipitation using THF as the solvent and hexanes as the precipitant. The reaction mixture precipitated by water was dissolved in THF at a concentration of 1% (w/v). Hexanes were added with agitation to precipitate the Dx-g-PCLs. The hexanes/THF ratio used in this study was 3/8 (v/v). The mixture was left at room temperature for 30 min to allow for complete precipitation. The dissolving/precipitating procedures were repeated 3 times.

2.4. Characterization of the graft copolymers

The purity, the molecular weight and molecular weight distribution were determined using a Waters gel permeation chromatography (GPC) system that was equipped with the same components as the HPLC instrument except that a model 2410 refractive index detector was used instead of the UV detector. The columns used were Styragel® HR3 and HR4 in series.

The structure of the graft copolymers was characterized using one- and two-dimensional NMR. The spectra of ^1H NMR, ^{13}C NMR and heteronuclear multiple quantum coherence (HMQC) of the graft copolymers were recorded in DMSO-d_6 at 95 °C using a Bruker AMX500 spectrometer. ^1H NMR spectra of PCL homopolymers formed during the reaction were acquired using a WH400 spectrometer in $CDCl_3$ operating at

25 °C. Chemical shifts are reported in parts per million (ppm) from tetramethylsilane with $CDCl_3$ and DMSO-d_6 as the internal references. For ^1H NMR spectra, residual $CHCl_3$ was taken as 7.24 ppm and residual DMSO as 2.49 ppm. For ^{13}C NMR, the central peak of DMSO-d6 was taken as 39.5 ppm. Sample concentrations were about 50 mg/ml.

Molar substitution of ε-caprolactone on dextrans (MS_{CL}), which is defined as the average number of ε-caprolactone molecules that have reacted with each anhydroglucose unit in dextran chains, was estimated using gravimetric method. The calculation for the gravimetric method is given as follows:

$$MS_{CL} = \frac{(W_{graft} - W_{Dx})/114}{W_{Dx}/162} \quad (1)$$

where W_{graft} is the weight of purified Dx-g-PCL; W_{Dx} is the initial weight of dextran; the number 114 is the formula weight of the repeating unit of PCL; and 162 is the formula weight of the repeating unit of dextran. In this calculation, dextran was assumed to be 100% converted to Dx-g-PCL.

MS_{CL} was also estimated using NMR spectroscopy method (Ho et al., 1972; Shi and Burt, 2003). A pulse angle of 36° and a relaxation delay of 2 s were used for the acquisition of the spectra. To ensure reliable peak intensities, spectra were also acquired at relaxation delays of 4 and 8 s, respectively. The spectra obtained using the three different relaxation delays gave identical peak intensities indicating that the peak intensities used in the following calculations were quantitatively reliable. The calculation is given as follows:

$$MS_{CL} = \frac{I_{H\alpha}}{2I_{H1}} \quad (2)$$

where $I_{H\alpha}$ is the peak intensity of H_α in PCL side chains and I_{H1} is the peak intensity of H1 in dextran.

2.5. Preparation of paclitaxel loaded Dx-g-PCL films

Paclitaxel loaded films of Dx500-g-PCL, Dx70-g-PCL, and PCL were prepared by the solution casting method. The films of the graft copolymers were cast in polytetrafluoroethylene dishes, which were made of Bytac® VF-81 PTFE film (Norton Performance Plastics Corporation, Wayne, NJ). THF solutions of the polymers with concentrations in the range of 20–30 mg/ml and THF solutions of paclitaxel with a concentration of 5 mg/ml were mixed at a given ratio with vigorous agitation. The mixtures were transferred to the dishes and dried in a fume hood at room temperature for 48 h, and then under reduced pressure in vacuum desiccators with P_2O_5 as a desiccant for 1 week. Films with 0, 1, 5, and 10% (w/w) paclitaxel loadings were prepared.

2.6. Characterization of the films

The swelling of the films of Dx500-g-PCL and Dx70-g-PCL with or without paclitaxel were conducted in 10 mM phosphate buffered saline (PBS, pH 7.4) at 37 °C. The dry films were accurately weighed and immersed into the PBS. At given time intervals, the swollen films were withdrawn from the buffer solution. The excess surface liquid was blotted using a filter paper and the films were weighed. The swelling was continued until a constant weight for each of the films was obtained. The degree of swelling was defined as:

$$\text{Degree of swelling (\%)} = \frac{W - W_o}{W_o} \times 100 \quad (3)$$

where W is the weight of the swollen film and W_o the weight of the dry film.

Differential scanning calorimetry (DSC) was conducted using a Pyris 1 differential scanning calorimeter (Perkin Elmer Corp., Norwalk, CT) equipped with a Perkin-Elmer Cryofill. The coolant was liquid nitrogen. The purge gas was prepurified helium at a flow rate of 30 ml/min. Three to 10 mg of samples were loaded in crimped, but not hermetically sealed, aluminum pans (Perkin-Elmer sample pans) and scanned at a heating rate of 10 °C/min. An empty pan was used as the reference. Results were analyzed using Perkin-Elmer Pyris Data Analysis Software.

X-ray diffraction patterns of the films were acquired using a Rigaku Rotaflex diffractometer equipped with a rotating target X-ray tube and a wide angle goniometer. The X-ray source was K_α radiation from a copper target with a graphite monochrometer. The X-ray tube was operated at a potential of 50 kV and a current of 150 mA. The range (2θ) of scans was from 3 to 40° and the scan speed was 2° per minute at increments of 0.02°.

2.7. In vitro paclitaxel release

The in vitro studies of paclitaxel release from the films of Dx500-g-PCL and Dx70-g-PCL were carried out at 37 °C in an Innova^{TM} 4000 Shaker (New Brunswick Scientific, Edison, NJ). Paclitaxel loaded films of the same size were accurately weighed and placed in Erlenmeyer flasks. Phosphate buffered saline (10 mM, pH 7.4) was used as the release medium. The volume of the release medium was adjusted to ensure sink conditions. Films of each of the polymers without paclitaxel were used as controls. The flasks were shaken at 100 rpm. The release medium was replaced by fresh PBS every 24 h. Paclitaxel in the release medium was extracted into 1 ml of DCM, dried under a stream of nitrogen, and reconstituted in 1 ml of 60% (v/v) ACN in water solution. The concentration of paciltaxel was analyzed by HPLC using a NovoPak C_{18} column. The mobile phase was acetonitrile/water/methanol (58:37:5), the flow rate was 1 ml/min, and the detection wavelength was 232 nm.

The morphological change of the films during the incubation in PBS at 37 °C was examined using optical microscopy and XRD. An Olympus BH-2 optical microscope was used and photographs were taken at a magnification of 150× using a Contax 167MT camera.

3. Results and discussion

3.1. Synthesis and purification of Dx-g-PCLs

Preliminary evidence for the formation of Dx-g-PCLs was the change in the solubility of the dextrans in THF. Before the grafting reaction, the dextrans were not soluble in THF. The reaction products were readily dissolved in THF and formed clear solutions indicating the dextrans were converted into graft copolymers. The results for the syntheses of Dx-g-PCLs are summarized in Table 1. For both Dx500-g-PCL and Dx70-g-PCL, the feed ratio of dextrans:CL:Sn(Oct)$_2$ was 1:3:0.05 (w/w/w) and the syntheses were carried out under the same conditions. The results represent averages of four batches for each copolymer. The reproducibility of the reaction was demonstrated by the small variance among the four batches in the conversion of CL, the yields, the molecular weights, and the polydispersity indices of Dx-g-PCLs, and MS_{CL}.

Table 1
Results for the syntheses of Dx-g-PCLs with a molar feed ratio of 1:3 (w/w) of dextran and CL[a]

Graft copolymer	Dx500-g-PCL	Dx70-g-PCL
M_w ($\times10^{-4}$)[b]	6.06 ± 0.47	3.60 ± 0.22
M_w/M_n[c]	2.2 ± 0.1	1.6 ± 0.0
CL conversion (%)[d]	74.5 ± 1.9	79.8 ± 2.8
Copolymer yield (%)[e]	44.4 ± 0.2	46.4 ± 1.1
MS_{CL}[f]	1.2 ± 0.0	1.3 ± 0.1

[a] The results represent mean ± S.D. ($n = 4$).

[b] Weight-average molecular weight, measured by GPC and calculated by Millennium$^{32®}$ software based on polystyrene standard curve. The mobile phase was THF at 1 ml/min. The columns used were Styragel® HR3 and HR4 in series.

[c] Polydispersity index, determined using the same method as for weight-average molecular weight.

[d] Calculated from the amount of reacted CL divided by the feed amount of CL.

[e] Calculated from the weights of the purified Dx-g-PCLs divided by the total weight of dextrans and CL initially added.

[f] Molar substitution of CL on dextran chains determined using the gravimetric method.

GPC chromatograms of the reaction product and purified Dx500-g-PCLs are shown in Fig. 1. Fig. 1a shows the chromatogram of the reaction product of Dx500-g-PCL, which includes peaks in two retention time regions. The bimodal peak (the component represented by the peak denoted as D1) in the range of 9.5–17.5 min corresponded to the graft copolymer Dx500-g-PCL. The multiple peaks (the component represented by the peaks denoted as P1) in the range of 17.5–21.5 min were due to PCL homopolymer with different molecular weights formed during the reaction. The fractional precipitation method was used to separate Dx500-g-PCL from the PCL homopolymer. The component represented by peaks P1 could no longer be detected after the purification process, as shown in Fig. 1b.

Similarly, GPC chromatograms of the reaction product of Dx70-g-PCL and purified Dx70-g-PCL are shown in Fig. 1c and d, respectively. In contrast to the bimodal peak (D1) in Fig. 1a, peak D2 in Fig. 1c was a single peak with a small shoulder at the left side. This was likely due to a difference in molecular weight distribution between Dx500 and Dx70.

Sn(Oct)$_2$ has been a catalyst for a variety of lactone polymerizations (Kricheldorf et al., 2000; Storey and Sherman, 2002; Zhang et al., 1994, 1996). A coordination-insertion mechanism of the catalysis

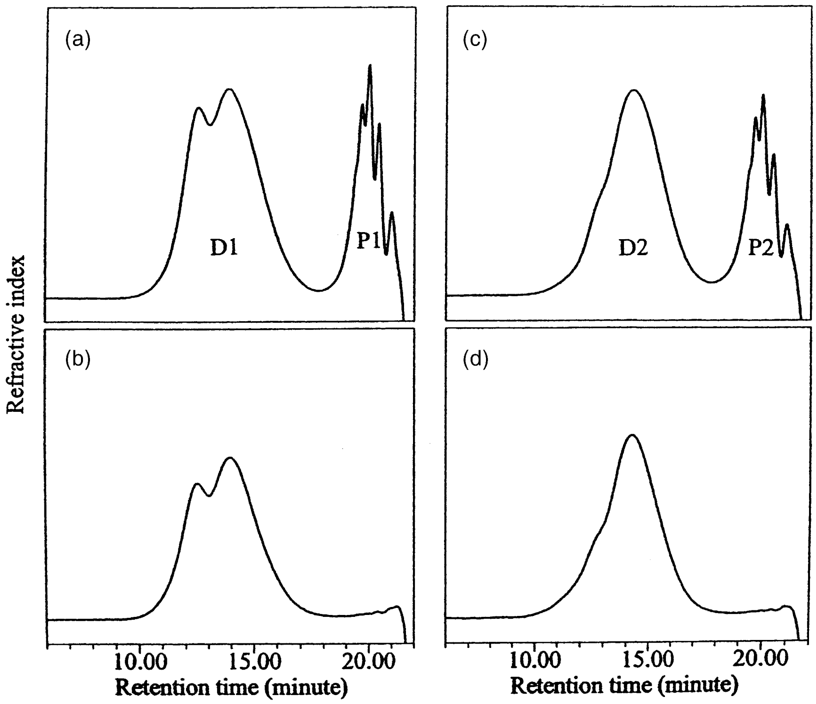


Fig. 1. GPC chromatograms of (a) the reaction product of Dx500-g-PCL, in which D1 and P1 represent the components of Dx500-g-PCL and the PCL homopolymer formed during the reaction, respectively; (b) purified Dx500-g-PCL; (c) the reaction product of Dx70-g-PCL, in which D2 and P2 represent the components of Dx70-g-PCL and the PCL homopolymer formed during the reaction, respectively; (d) purified Dx70-g-PCL.

has been well established, which includes two major steps: formation of stannous alkoxide initiation species with hydroxyl group-containing compounds, and coordination-insertion of CL monomer into the stannous alkoxide bond to initiate the polymerization (Kricheldorf et al., 2000; Kowalski et al., 2000; Storey and Sherman, 2002). In this study, the hydroxyl group-containing compounds were dextrans and traces of water in the reaction system. Graft copolymers would be formed if the stannous alkoxide initiation species were formed with dextrans. However, if the initiation species were formed with water, the polymerization would result in PCL homopolymer, which was considered to be a side product. To minimize the influence of water, vigorous drying procedures were performed in the syntheses as described in the experimental section. Nevertheless, PCL homopolymer formed with yields in the range of 35–40%. The higher yield of PCL homopolymer was a consequence of a higher water content in the system. The solution polymerization method involved using a large amount of DMSO as the solvent. Given the hygroscopic nature of DMSO, it was very difficult to completely eliminate water/moisture in the system.

Dextrans are soluble in only a few solvents including water, DMSO, formamide, ethylene glycol, and glycerol. DMSO was selected as the solvent for this reaction because the other four contained either hydroxyl groups or active hydrogens in their molecules that would result in only PCL homopolymer and dramatically suppress the formation of the graft copolymers.

3.2. Characterization of Dx-g-PCLs using NMR spectroscopy

A representative ^{1}H NMR spectrum of purified Dx-g-PCLs is shown in Fig. 2. The spectrum contained peaks from both the dextran and PCL indicating the formation of the graft copolymers Dx-g-PCLs. The peaks from side chain PCL could be readily assigned.

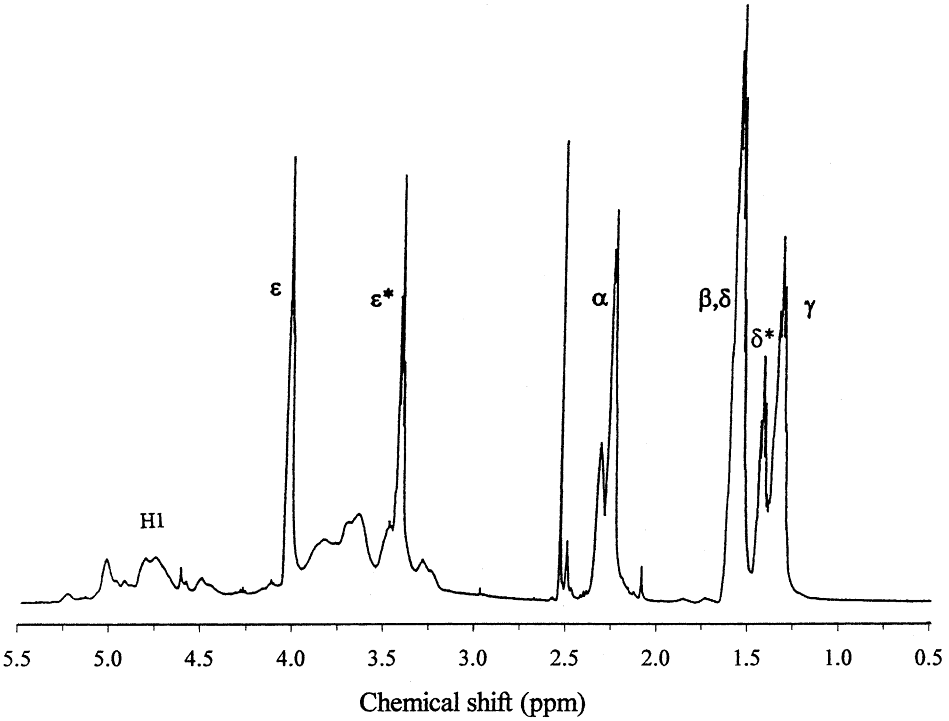


Fig. 2. Representative ^1H NMR spectrum of Dx-g-PCLs in DMSO-d_6 recorded at 95 °C using a Bruker AMX500 spectrometer.

The assignments, given in Table 2, are in agreement with those previously reported (Kricheldorf and Kreiser, 1987; Shi and Burt, 2003). The peaks corresponding to $H_{\varepsilon*}$ and $H_{\delta*}$ in the repeating units at the chain ends shifted to higher fields compared to the internal H_ε and H_δ. $H_{\alpha*}$ also had a different chemical shift from H_α, however, the peak shifted to a lower field. Most of the peaks from the main chain (dextrans) were highly overlapped, and therefore could not be assigned. The broad peak at 4.65–4.85 ppm in Fig. 2 was assigned to H1 based on the HMQC spectra (data not shown). The HMQC spectrum indicated that this broad peak contained three peaks corresponding to the protons at three different chemical shifts, and all of them connected to C1. The slightly different chemical shifts of H1 might originate from different substitution patterns on the anhydroglucose units, which resulted in different chemical environments for H1. Particularly, the grafting reaction could occur at three different hydroxyl groups (C2–OH, C3–OH, and C4–OH), and some of the anhydroglucose units might have no side chain grafted. The assignments

Table 2
Observed chemical shifts (δ) in the NMR spectra of purified Dx-g-PCL and PCL homopolymer formed during the reactions

Dx-g-PCL[a]				PCL[b]	
^{13}C	δ (ppm)	^1H	δ (ppm)	^1H	δ (ppm)
C1	98.21	H1	4.65–4.85	–	–
C_α	32.99	H_α	2.26	H_α	2.17
$C_{\alpha*}$	33.21	$H_{\alpha*}$	2.32	–	–
C_β	23.55	H_β	1.56–1.60	$H_{\beta,\delta}$	1.48–1.53
C_γ	24.43	H_γ	1.34	H_γ	1.26
C_δ	27.34	H_δ	1.56–1.60	–	–
$C_{\delta*}$	31.56	$H_{\delta*}$	1.44	–	–
C_ε	62.93	H_ε	4.01	H_ε	3.92
$C_{\varepsilon*}$	60.21	$H_{\varepsilon*}$	3.41	$H_{\varepsilon*}$	3.47
C=O	172.04				

[a] The spectra were recorded in DMSO-d_6 at 95 °C using a Bruker AMX500 spectrometer.

[b] The spectrum was recorded in $CDCl_3$ at 27 °C using a Bruker AV400 spectrometer.

are summarized in Table 2. The labels of the carbons and the protons are shown in Scheme 1.

The ^{1}H NMR spectra (not shown) of the components represented by the peaks P1 and P2 in Fig. 1 were identical to the spectrum of PCL indicating that the components were PCL homopolymer formed during the graft reaction. The peak assignments are given in Table 2.

3.3. Molar substitution (MS_{CL}) of the Dx-g-PCLs

The MS_{CL} values for Dx500-g-PCL and Dx70-g-PCL were determined to be 1.2 and 1.3 (given in Table 1), respectively, which means on average there were 12 and 13 CL monomers reacted with every 10 anhydroglucose units in Dx500 and Dx70, respectively. The MS_{CL} was also estimated using the ^{1}H NMR spectroscopy method (Shi and Burt, 2003), which is based on the fact that each different proton in different structural groups gives rise to peaks at a characteristic magnetic field strength, and the peak intensity is directly proportional to the concentration of the protons. As shown in the structure of Dx-g-PCLs in Scheme 1, each anhydroglucose unit of dextran has one proton (H1) connecting to C1, and each PCL repeating unit has two α protons. Therefore, MS_{CL} could be estimated using the peak intensities of these two types of protons in the ^{1}H NMR spectra of Dx-g-PCLs. Using Eq. (2), the MS_{CL} was calculated to be 1.4 for Dx500-g-PCL and 1.6 for Dx70-g-PCL. These values were in good agreement with those determined by the gravimetric method.

3.4. The length and the distribution of the side chains

The average side chain length ($\bar{L}$) was estimated on the basis of peak integration of the ^{1}H NMR spectra of the copolymers and the calculation was given as follows:

$$\bar{L} = \frac{I_{H\alpha}}{I_{H\alpha} - I_{H\varepsilon}} \quad (4)$$

where $I_{H\varepsilon}$ represents the peak intensities of the internal ε protons and $I_{H\alpha}$ the peak intensity of the α protons. The $\bar{L}$ obtained was 2.4 for Dx500-g-PCL, and 3.1 for Dx70-g-PCL. The average number of PCL side chains on each anhydroglucose unit of dextrans ($\bar{N}$) was then calculated by:

$$\bar{N} = \frac{MS_{CL}}{\bar{L}} \quad (5)$$

Based on the MS_{CL} estimated using the ^{1}H NMR method, $\bar{N}$ was estimated to be 0.6 for Dx500-g-PCL and 0.5 for Dx70-g-PCL. Hence, in both of the copolymers, approximately every two anhydroglucose units has one side chain, i.e. only half of the anhydroglucose units in dextran were grafted. The reactivity of the hydroxyl groups in dextrans was reported to be in an order of C2–OH > C4–OH > C3–OH (Arranz and Sanchez-Chaves, 1988; Zhang and Chu, 2000). Therefore, the PCL side chains were most likely grafted at C2.

3.5. Characterization of paclitaxel loaded Dx-g-PCL films

Both paclitaxel and the copolymers could be readily dissolved in the common solvent THF and they formed clear solutions. The films with or without paclitaxel were transparent when dry and no paclitaxel precipitation and crystallization was observed in the films with loadings up to 10% (w/w) indicating solubilization of paclitaxel by the amphiphilic copolymers. The dry films with or without paclitaxel were also examined using DSC. The thermograms (data not shown) for Dx500-g-PCL and Dx70-g-PCL films showed no thermal events in the temperature range of −100 to 130 °C. There was no evidence of either glass transitions or melting of PCL in the thermograms, indicating the absence of a PCL-rich phase formed in the films of both the copolymers. The films started to decompose after 130 °C.

The films were hygroscopic and swelled rapidly when immersed in aqueous media. The degree of swelling of the films loaded with 0, 1, 5, and 10% paclitaxel is shown in Fig. 3. The films were placed in the buffer solution for 12 h to ensure equilibrium was reached although the swelling reached a maximum extent within 1.5 h. For the films without paclitaxel, the degrees of swelling were 36.2 and 37.7% for the Dx500-g-PCL and Dx70-g-PCL films, respectively. An increase in paclitaxel loading resulted in decreased swelling for both the Dx500-g-PCL and Dx70-g-PCL films.

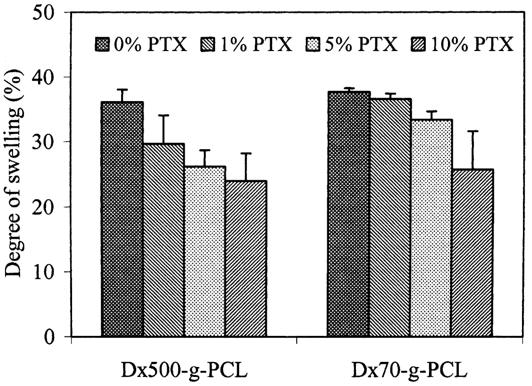


Fig. 3. Degree of swelling of the Dx-g-PCL films with different paclitaxel loadings. The films were incubated in 10 mM PBS (pH 7.4) at 37 °C for 12 h. The data represent mean ± S.D. ($n = 3$).

Morphological changes in the 1, 5 and 10% paclitaxel loaded films of both Dx500-g-PCL and Dx70-g-PCL following incubation in PBS at 37 °C were examined by optical microscopy, DSC and XRD. Before incubation, all the films were transparent at all paclitaxel loadings. No crystalline structure was detected in the films using either DSC or XRD. The 1% paclitaxel loaded films remained transparent, and no paclitaxel crystallization was observed throughout the period of the release study. However, crystals in the form of spherulites formed in both the 5 and 10% paclitaxel loaded films during the incubation, and both the size and number of the spherulites were found to grow with an increase in incubation time. The morphological changes were observed by optical microscopy, and representative micrographs are shown in Fig. 4 for the 5 and 10% paclitaxel loaded films, respectively. For 5% paclitaxel loaded films (Fig. 4A), the spherulites were small (approximately 20–30 μm) at 6 h and increased in size and number to a maximum size in about 5 days. For 10% paclitaxel loaded films (Fig. 4B), the size of the spherulites at 6 h was approximately 50–80 μm. The size and the number of the spherulites increased rapidly and the spherulites started to impinge on each other in less than 12 h. The growth of the spherulites reached a maximum within 48 h.

After being incubated for 5 days, the films were dried and examined using X-ray diffraction. Scans A and B in Fig. 5 show representative X-ray diffraction patterns of 10% paclitaxel loaded Dx500-g-PCL films and Dx70-g-PCL films, respectively, after incubation for 5 days. The peaks in the patterns are at the same 2θ angles as those in the pattern of paclitaxel dihydrate (Liggins et al., 1997), indicating that the spherulites formed in the films were crystals of paclitaxel dihydrate.

3.6. *In vitro paclitaxel release from the Dx-g-PCL films*

The cumulative paclitaxel release profiles for the films of Dx500-g-PCL and Dx70-g-PCL are shown in Figs. 6 and 7, respectively. In general, the release of paclitaxel from the films was characterized by an initial rapid release phase followed by a slower release phase. The initial paclitaxel loading had marked effects on the release rate, which increased when paclitaxel loading increased from 1 to 5%. However, the release rates at 10% loading were lower than those at 5% loading during the initial release phase (up to about 5 days for Dx500-g-PCL films and up to about 2–3 days for Dx70-g-PCL films). Subsequently, paclitaxel was released from the 10% paclitaxel loaded films at almost constant rates while the release rate of the 5% loaded films declined gradually.

The 1% paclitaxel loaded Dx-g-PCL films remained transparent throughout the release study. At this loading, the release profiles for paclitaxel from Dx500-g-PCL and Dx70-g-PCL films were very similar indicating that the difference in the molecular weights of the main chain polymer did not result in a significant difference in the paclitaxel release behaviour. However, films loaded with 5 and 10% paclitaxel showed evidence of paclitaxel crystallization during the release studies, indicating that the amounts of paclitaxel in the 5 and 10% loaded films exceeded the paclitaxel solubilization capacity of the copolymers following water uptake. It is apparent that a paclitaxel loading of 1% was lower than the paclitaxel solubilization capacity of the copolymers and that the paclitaxel molecules were likely associated via hydrophobic interactions with PCL side chains and solubilized by the copolymers following water uptake. However, when the loadings (5 and 10%) exceeded the solubilization capacity, it is speculated that sufficient concentrations of paclitaxel molecules were dispersed in aqueous "compartments" of the hydrated films, that crystallization of paclitaxel as the dihydrate occurred within

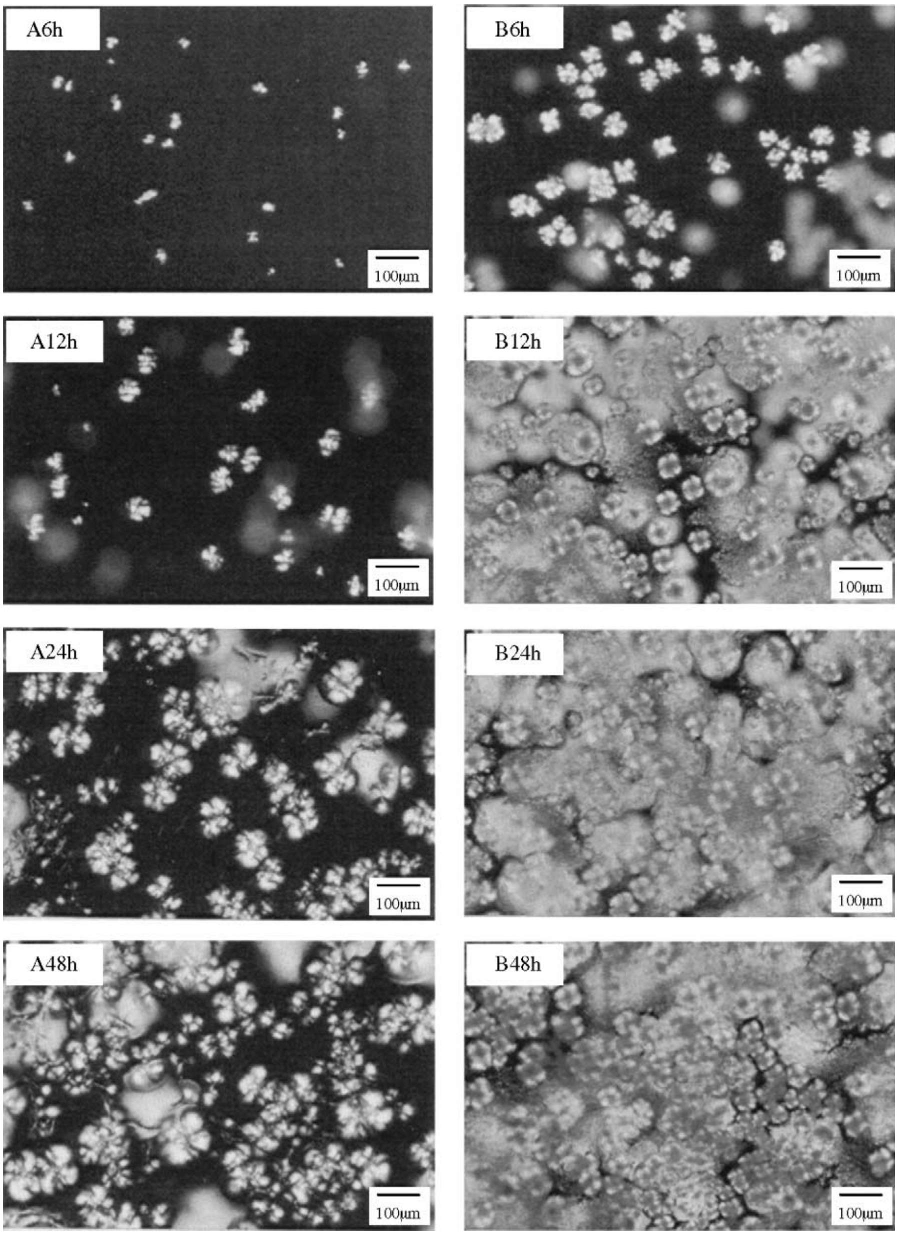


Fig. 4. Representative optical micrographs of the Dx-g-PCL films with paclitaxel loadings of (A) 5% and (B) 10% after incubation in 10 mM PBS (pH 7.4) at 37 °C for 6, 12, 24, and 48 h.

these “compartments”. More crystallization was evident as the loading was increased from 5 to 10%.

It is suggested that the crystallization events in the films of Dx-g-PCLs during the release study may provide an explanation for the unusual release profiles of 5 and 10% paclitaxel loaded films. The mechanism of paclitaxel release from the copolymer matrices would involve: (1) water diffusion into the matrices; (2) paclitaxel dissolution/partitioning; (3) paclitaxel diffusion through the matrices and release into the media.

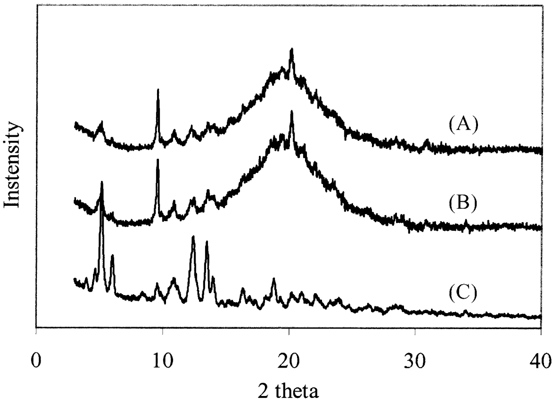


Fig. 5. Representative X-ray diffraction patterns of (A) 10% paclitaxel loaded Dx500-g-PCL films and (B) 10% paclitaxel loaded Dx70-g-PCL films after the incubation in 10 mM PBS (pH 7.4) at 37 °C for 5 days, followed by drying; and (C) paclitaxel dihydrate.

For 1% paclitaxel loaded Dx-g-PCL films, paclitaxel was completely solubilized by the copolymers and dispersed in the matrices at a molecular level throughout the period of the release study. Hence, the rate-limiting step for the release of paclitaxel from films at these low loadings was likely paclitaxel diffusion (step 3). However, in the 5 and 10% paclitaxel loaded Dx-g-PCL films, paclitaxel was dispersed at the molecular level (solubilized) and in the crystalline state. For solubilized paclitaxel, its release would be rate-limited by step 3 (paclitaxel diffusion). XRD data indicated that crystalline paclitaxel was present in the dihydrate form. It has been reported that the dissolution of paclitaxel dihydrate is a very slow process, and it takes 20 h to obtain a saturated solution (Liggins et al., 1997). Therefore, for crystalline paclitaxel, the rate-limiting step for its release from films at higher loadings was likely dissolution of paclitaxel within the film matrix. In the 10% loaded films, a large fraction of the loaded paclitaxel formed crystals during the first 12 h of the release study and the crystallization reached a maximum in 2 days. Hence, the rapid release phase corresponding to paclitaxel diffusion as rate limiting, only lasted for 2 days. Subsequently, the release rate was mainly controlled by the dissolution rate of paclitaxel. The crystallization in the 5% loaded films was slower than in the 10% loaded films, and did not reach a maximum until the fifth day. During the first 5 days, the release rate from the 5% loaded films was probably controlled by a mixed mechanism including paclitaxel diffusion (fast) and dissolution (slow), and overall release was therefore higher than from the 10% loaded films. A similar observation was reported for the release of diclofenac sodium (water insoluble) from poly(hydroxyethyl methacrylate) hydrogel, where diclofenac sodium precipitated out from the hydrogel at a high loading level and the release was then controlled by the slow dissolution of the drug (Lee, 1985).

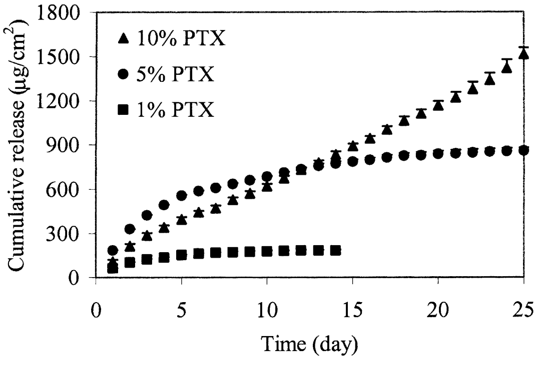


Fig. 6. In vitro paclitaxel release profiles from the Dx500-g-PCL films with different paclitaxel loadings: 1, 5, and 10%. The films were incubated in 10 mM PBS (pH 7.4) and shaken at 100 rpm at 37 °C. Data are shown as amount of paclitaxel released per unit area of film and represent the mean of four samples with error bars (standard deviation) shown only in the positive direction for clarity.

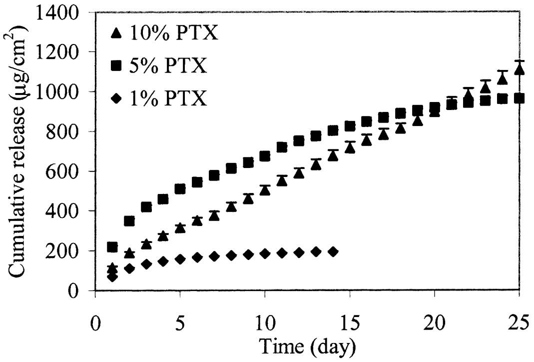


Fig. 7. In vitro paclitaxel release profiles from the Dx70-g-PCL films with different paclitaxel loadings: 1, 5, and 10%. The films were incubated in 10 mM PBS (pH 7.4) and shaken at 100 rpm at 37 °C. Data are shown as amount of paclitaxel released per unit area of film and represent the mean of four samples with error bars (standard deviation) shown only in the positive direction for clarity.

4. Conclusions

Amphiphilic graft copolymers of Dx500-g-PCL and Dx70-g-PCL were reproducibly synthesized by solution polymerization in DMSO using stannous 2-ethylhexanoate as the catalyst. Molar substitution was determined to be in the range of 1.2–1.4 for Dx500-g-PCL and 1.3–1.6 for Dx70-g-PCL using the gravimetric and ^{1}H NMR methods. In the dry state, Dx-g-PCL copolymers effectively solubilized paclitaxel, since the copolymer films with or without paclitaxel were transparent and showed a single-phase morphology. However, crystals in the form of spherulites formed in the 5 and 10% paclitaxel loaded films upon incubation in aqueous media. Paclitaxel dihydrate formed during incubation of 5 and 10% paclitaxel loaded films in PBS and the dihydrate formation influenced the in vitro release behavior of paclitaxel from the films. For 1% paclitaxel loaded films, paclitaxel was completely solubilized by the copolymers and dispersed in the matrices at a molecular level throughout the period of the release study.

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