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Copolymer Micelles and Nanospheres with Different In Vitro Stability Demonstrate Similar Paclitaxel Pharmacokinetics

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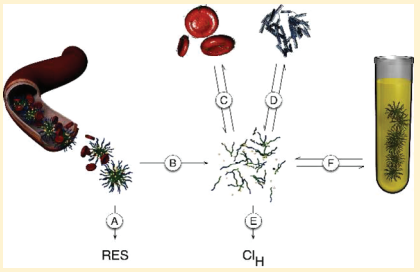
ABSTRACT: Paclitaxel loaded amphiphilic block copolymer nanoparticles have been demonstrated to enhance the aqueous solubility and improve the toxicity profile as compared to the commercially available product Taxol; however, in many cases long circulation of the drug is not achieved due to rapid partitioning of the drug from the carrier and/or carrier instability upon injection. In this work we investigated the effect of increasing the hydrophobic block length of methoxy poly(ethylene glycol)-*block*-poly(ε-caprolactone) (MePEG-*b*-PCL) copolymers on the physicochemical properties and in vitro stability of the formed nanoparticles as well as the pharmacokinetics and biodistribution of both the copolymer and solubilized drug. We hypothesized that copolymers composed of high molecular weight hydrophobic blocks ($MePEG_{114}$-*b*-PCL_{104}) that form nanoparticles with a kinetically "frozen core" (which we term nanospheres) would better retain their PTX payload as compared to micelles composed of shorter hydrophobic blocks ($MePEG_{114}$-*b*-PCL_{19}), thus leading to prolonged drug circulation. Nanospheres solubilized PTX more efficiently, released the drug in a more sustained fashion and were characterized by enhanced stability and drug retention in the presence of plasma proteins as compared to micelles. Using radiolabeled copolymers and PTX, it was found that, upon injection, $MePEG_{114}$-*b*-PCL_{104} circulated for longer than $MePEG_{114}$-*b*-PCL_{19}; however, the drug was rapidly eliminated from the blood regardless of the formulation. These results suggest that, despite formulation in more stable nanospheres, PTX was still rapidly extracted from these nanoparticles.



KEYWORDS: *amphiphilic block copolymer, micelle, nanoparticle, nanosphere, paclitaxel, pharmacokinetics, biodistribution*

INTRODUCTION

There is tremendous interest in the development of nanoparticulate formulations for the delivery of hydrophobic compounds in order to achieve various benefits, such as elimination of formulation excipients with undesirable toxicities,[1−3] enhancing drug solubilization properties,[1,2,4] controlled drug release,[5,6] as well as passive[7,8] and active targeting[9−12] to disease sites. Amphiphilic block copolymer (ABC) nanoparticles composed of hydrophilic blocks of poly(ethylene oxide) (PEO) or methoxy poly(ethylene glycol) (MePEG) and a variety of polyester hydrophobic blocks, including poly(D,L-lactide) (PDLLA),[1,13] poly(glycolic-*co*-lactic acid) (PLGA)[14,15] and poly(ε-caprolactone) (PCL),[5,16] have been extensively explored.

It is clear that a critical characteristic of these nanoparticulate formulations is their ability to alter pharmacokinetics and, in particular, to demonstrate long-circulating properties, in order to achieve passive drug targeting to disease sites characterized by leaky vasculature via the enhanced permeation and retention (EPR) effect.[17] Thus, following intravenous administration, ABC nanoparticles must retain good physical stability properties, maintaining reasonable structural integrity, providing a greater drug half-life and increasing drug exposure (AUC) compared to free drug.

Our group carried out some of the early work in which both the drug paclitaxel (PTX) and the ABC MePEG-*b*-PDLLA were both radiolabeled to enable the monitoring of the fate of the drug and polymer simultaneously following iv administration in rats. It was demonstrated that PTX rapidly dissociated from the micellar nanoparticles within a matter of minutes.[18] Other studies have also shown similar rapid loss of paclitaxel from the poly(lactide) core of ABC micelles following iv dosing in animals and no change in drug exposure or half-life.[19,20] On the other hand, pharmacokinetic studies in rats using paclitaxel loaded MePEG-*b*-PLA nanoparticles possessing a higher molecular weight poly(lactide) core showed increased half-life and AUC compared to Taxol.[21]

MePEG-*b*-PCL micelles with a semicrystalline and more hydrophobic core than MePEG-*b*-PDLLA micelles are likely to have greater kinetic stability and slower rates of disassembly at concentrations below the CMC.[22,23] Despite evidence that intact micelles of MePEG-*b*-PCL (M_n = 9800) were long-circulating and present in plasma of mice,[23] a study by Savic et al. showed that fluorogenic micelles of $MePEG_{45}$-*b*-PCL_{21} (M_n = 6960 with fluorogenic dye covalently attached to the PCL core-forming block) were rapidly disrupted and dissociated in the presence of serum.[24] Similarly, hydrophobic FRET probes loaded in PEG-*b*-PDLLA micelles dissociated rapidly following iv administration in mice.[25]

Interestingly, in spite of the tremendous research activity developing ABC nanoparticulate drug delivery systems with the

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goal of exploiting long-circulating pharmacokinetic and passive targeting advantages, it is perhaps surprising how little in vivo stability data are available. Furthermore, systematic studies are needed to investigate the in vivo integrity of block copolymer micelles of different block compositions and different core versus corona block lengths.[24,25] Gaucher et al. found that coating PDLLA nanoparticles with PVP-*b*-PDLLA decreased the blood circulation time compared to a PEG-*b*-PDLLA coating, but that both coated nanoparticle formulations were very rapidly cleared from the blood.[26]

Recently, we have shown that there are marked differences in the physicochemical properties of micelles and nanospheres composed of MePEG-*b*-PCL: the short hydrophobic block $MePEG_{114}$-*b*-PCL_{19} we have termed a "micelle-forming" copolymer, and a long hydrophobic block, $MePEG_{114}$-*b*-PCL_{104}, providing a kinetically frozen core, we have termed a "nanosphere-forming" copolymer.[16] Furthermore, we demonstrated that PTX solubilized in $MePEG_{114}$-*b*-PCL_{104} nanospheres resulted in the improved stability and retention of the drug with the carrier in vitro in the presence of human plasma compared to $MePEG_{114}$-*b*-PCL_{19} micelles, leading us to hypothesize that PTX loaded into the more kinetically stable $MePEG_{114}$-*b*-PCL_{104} nanospheres would result in greater in vivo integrity of the nanospheres and greater PTX blood circulation times compared to PTX loaded $MePEG_{114}$-*b*-PCL_{19} micelles. In this study, we used radiolabeled PTX and block copolymers in order to investigate the simultaneous distribution of PTX and the copolymer both in vitro in the presence of whole blood and the in vivo fate following iv administration in mice.

EXPERIMENTAL SECTION

Materials. Methoxy poly(ethylene glycol) (MePEG), ε-caprolactone and Cremophor EL were purchased from Fluka (Bucks SG, Switzerland). Stannous octoate and bovine serum albumin were obtained from Sigma Aldrich Canada Ltd. (Oakville, ON, Canada), paclitaxel from Polymed Therapeutics Inc. (Houston, TX, USA), and Paclitaxel for Injection from Biolyse Pharma Corp. (St. Catherines, ON, Canada) which contained 6 mg/mL paclitaxel formulated in 527 mg of Cremophor EL and 49% v/v dehydrated alcohol, which is the same concentration of drug and formulation components as Taxol. ^{3}H and ^{14}C paclitaxel was purchased from Moravek Biochemicals Inc. (Brea, CA, USA), and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate ($DiIC_{18}$) and 3,3′-dioctadecyloxacarbocyanine perchlorate ($DiOC_{18}$) were purchased from Invitrogen (Burlingon, ON, Canada). The solvents chloroform, *N*,*N*-dimethylformamide, anhydrous toluene, hexane, diethyl ether, acetonitrile, methanol and chloroform were all purchased from Fisher Scientific Co. (Ottawa, ON, Canada). Deuterated chloroform was obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

Synthesis and Characterization of Copolymers. Two MePEG-*b*-PCL copolymers with different PCL block lengths were synthesized by reacting MePEG (MW 5000 g/mol) with ε-caprolactone in weight ratios of either 30:70 or 70:30. Stannous octoate was used as a catalyst, and polymers were synthesized in anhydrous toluene under an inert nitrogen atmosphere at 95 °C for 20 h. Upon termination of the reaction, the toluene was removed under vacuum and the product was dissolved in chloroform followed by precipitation with a 70/30 mix of hexane and diethyl ether. Copolymers were dissolved in deuterated chloroform, and 1D ^{1}H NMR spectra were collected using a 400 MHz Bruker Advance II+ spectrometer (Bruker Corporation, Milton, ON, Canada). The NMR chemical shifts (spectra not shown) and peak assignments for the synthesized polymers are as follows: 4.07 ppm (2H CO−CH_2−CH_2−CH_2−CH_2−CH_2), 3.65 ppm (4H O−CH_2−CH_2−O), 3.40 ppm (3H CH_3−O), 2.3 ppm (2H CO−CH_2−CH_2−CH_2−CH_2−CH_2), 1.66 ppm (4H CO−CH_2−CH_2−CH_2−CH_2−CH_2), 1.4 ppm (2H CO−CH_2−CH_2−CH_2−CH_2−CH_2). The peaks situated around 1.4 ppm and 1.66 ppm from the caprolactone methylene protons and the peaks at 3.65 ppm from the MePEG methylene protons were used to calculate the degree of polymerization (DPn). The copolymer molecular weights were determined by gel permeation chromatography (GPC) against polyethylene glycol standards (Polymer Laboratories Inc., Amherst, MA, USA) in the range of 1900−22800 g/mol using chloroform as a mobile phase with a flow rate of 1 mL/min. Samples were injected using a Waters (Milford, MA, USA) model 717 plus autosampler, and separation was achieved through a Waters Styragel HR 3 column. Detection was through a Waters model 2410 refractive index detector with a cell temperature of 40 °C. To produce radioactively labeled copolymers, the hydroxyl terminus of PCL for both copolymers was end-capped with ^{3}H acetyl chloride (American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) as detailed by Liu et al.[23]

Formation, Drug Loading and Characterization of Nanoparticles. Nanoparticles were formed using a previously described nanoprecipitation technique.[16] Briefly, 0.5 mL solutions of copolymer and PTX were prepared in *N*,*N*-dimethylformamide (DMF) resulting in copolymer and PTX concentrations of 60 mg/mL and 600 μg/mL, respectively. This solution was added dropwise to 2 mL of rapidly stirring PBS (0.01M, pH 7.4). The DMF was removed from the solution by dialysis against PBS overnight using 3500 MWCO Spectra/Por dialysis membranes (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA). The resulting nanoparticle dispersions were diluted with PBS so that the final copolymer and PTX concentrations were 10 mg/mL and 100 μg/mL, respectively. The dispersions were centrifuged at 14000 rpm to remove any precipitate, and an aliquot of the nanoparticle solution was dried under a stream of nitrogen gas and reconstituted in 60/40 acetonitrile/water. This solution was analyzed for PTX content by HPLC as described elsewhere.[27] The loading efficiency of the nanoparticles was calculated as

$$\text{loading efficiency} = \frac{[\text{PTX}]_{\text{solubilized}}}{[\text{PTX}]_{\text{theoretical}}} \times 100$$

where $[PTX]_{solubilized}$ is the concentration of PTX found in solution after encapsulation in nanoparticles as determined by HPLC and $[PTX]_{theoretical}$ is the concentration of PTX added to solution during the formation of nanoparticles. The hydrodynamic diameter of PTX loaded nanoparticles was determined using a Malvern Zetasizer Nano ZS (Malvern, U.K.). PTX loaded nanoparticles prepared in distilled water were used for zeta potential analysis also using a Malvern Nano ZS particle size analyzer.

Nanoparticle Stability Studies Using FRET. The ability of the nanoparticles to retain a hydrophobic probe payload when dispersed in PBS or human plasma was investigated using a method developed by Chen et al.[25,28] Nanoparticles were prepared as detailed above with the exception that PTX was replaced with the FRET pair $DiIC_{18}$ and $DiOC_{18}$ at final

concentrations of 225 μg/mL. Nanoparticle dispersions were dialyzed against 4 L of PBS overnight to remove DMF and free $DiIC_{18}$ and $DiOC_{18}$ followed by dilution to 3 mL with PBS. To 2.5 mL of PBS, human plasma (Bioreclamation Inc., Hicksville, NY, USA), or DMF, 500 μL of the nanoparticle dispersions was added, and the dispersion was kept at 37 °C in an incubator with shaking at 50 rpm. At time points of 0, 5 min, 15 min, 30 min, 1 h, 2 h and 3 h the fluorescence spectrum was recorded on a Varian Cary Eclipse fluorescence spectrophotometer (Varian Inc., Palo Alto, CA, USA) with excitation wavelength of 484 nm and emission between 490 and 600 nm with slit widths of 5 nm. The FRET ratio was calculated as

$$\text{FRET ratio} = \frac{I_R}{(I_R + I_G)}$$

where I_R and I_G are the fluorescence intensity of the peaks 565 and 501 nm, respectively. The FRET ratio was plotted as a function of time.

In Vitro Release of Paclitaxel. Nanoparticles were prepared as described above with final polymer and paclitaxel concentrations of 10 mg/mL and 100 μg/mL, respectively. During the preparation of these nanoparticles, a small amount of ^{3}H PTX was added to the drug/copolymer solution prior to nanoprecipitation. Into 7000 MWCO Slide-A-Lyzer mini dialysis units (Thermo Scientific, Rockford, IL, USA), 20 μL of the nanoparticle dispersions, or a 1 μg/mL solution of free paclitaxel spiked with a small amount of ^{3}H PTX, was added, and the dialysis units were inserted into Franz diffusion cells and dialyzed with stirring against 9 mL of 10 mM PBS at pH 7.4 and 37 °C, containing 0.4 mg/mL bovine serum albumin in the receiving compartment. At predetermined time points, the entire contents of the receiving chamber of the diffusion cells was replaced with fresh media and the drug content in the release media analyzed by β-scintillation counting. The data were expressed as cumulative percentage of drug released as a function of time.

In Vitro Whole Blood Distribution. Nanoparticle solutions were prepared with a polymer concentration of 10 mg/mL and a loading of 100 μg/mL of cold PTX along with trace amounts of ^{3}H copolymer and ^{14}C PTX. The use of different labels for the copolymer and drug allowed us to assess the distribution of these individual components into the plasma and erythrocyte fractions. Similarly, Paclitaxel for Injection (PTX formulated in Cremophor EL (CrEL)) was prepared at 100 μg/mL PTX in PBS as well as a free PTX solution at 1 μg/mL in PBS, both spiked with a small amount of ^{3}H PTX. Fresh human blood was collected from three volunteers in BD Vacutainer collection tubes containing lithium heparin (BD, Franklin Lakes, NJ, USA) according to protocols approved by the UBC Clinical Ethics Board. To 250 μL of whole blood in 1.5 mL microcentrifuge tubes, 25 μL of the formulations were added and the mixtures were incubated at 37 °C with tumbling at 8 rpm. At 15 min, 30 min, 1 h, 3 h and 6 h, the samples were centrifuged at 2500 rpm to separate the plasma from erythrocytes and the plasma was carefully removed followed by addition of 200 μL of a 2% v/v solution of Triton X to lyse the red blood cells. Both the plasma and the lysed erythrocytes were transferred to scintillation vials, and 1 mL of Solvable (Perkin-Elmer, Waltham, MA, USA) was added followed by incubation at 60 °C overnight. The samples were allowed to cool followed by the addition of 100 μL of 200 mM EDTA and 400 μL of 30% hydrogen peroxide to decolorize the samples. Samples were dispersed in 15 mL of Ultima Gold (Perkin-Elmer, Waltham, MA, USA) and analyzed by β scintillation counting using a dual label program (^{3}H/^{14}C). The percentage of drug or copolymer in each blood fraction was determined by comparing the counts against the ^{3}H and/or ^{14}C counts of a 25 μL sample of the formulations as determined by β scintillation counting as mentioned above. In order to verify the ability of the dual label program to distinguish between isotopes and correct for any overlap in the ^{3}H and ^{14}C spectra, we conducted an experiment in which we prepared a series of ^{3}H paclitaxel standards ranging from 0.5 to 0.0078 μCi and a set of ^{14}C paclitaxel standards ranging from 0.05 to 0.00078 μCi. These standards were first analyzed using their respective single label scintillation counting programs. The standards were then combined in the same vial and read using the dual label program. The ^{3}H and ^{14}C counts obtained using the dual label program were then expressed as a percentage of the counts obtained for the corresponding isotope using the single label programs. A large deviation from 100% would indicate the inability of the dual label program to account and correct for significant spill of one isotope into the spectrum of the other. All samples were very close to 100% with no more than 5% difference from 100%, indicating sufficient correction for any overlap in the spectra and ensuring accuracy of the results (data not shown).

In Vitro Plasma Distribution of Paclitaxel. Nanoparticle solutions were prepared with a polymer concentration of 10 mg/mL and a PTX loading of 100 μg/mL of cold drug along with trace amounts of ^{3}H copolymer and ^{14}C PTX, as described above. Additionally, solutions containing free PTX at a concentration of 1 μg/mL and Paclitaxel for Injection at a drug concentration of 100 μg/mL, both with trace amounts of ^{3}H PTX, were prepared in PBS. To 3.33 mL of six different lots of human plasma (Bioreclamation Inc., Hicksville, NY, USA), 0.67 mL aliquots of the PTX formulations were added using a micropipet, and the samples were incubated for 1 h at 37 °C with shaking at 50 rpm. After the incubation period, 1 mL of plasma was removed to determine the total amount of radioactivity in the sample. Into the remaining 3 mL, NaBr was dissolved to adjust the density of the plasma to 1.25 g/mL, and the plasma was cooled at 4 °C for 2 h to prevent any further drug redistribution. After cooling, 2.8 mL of 1.21, 1.063, and 1.006 g/mL NaBr solutions were sequentially layered on top of the 1.25 g/mL fraction and the plasma was separated into its very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and lipoprotein deficient plasma (LPDP) fractions by density gradient ultracentrifugation as described elsewhere.[16] After centrifugation, each layer was carefully removed, their volumes were measured and the amount of radioactivity was determined by β scintillation counting using a dual label program and expressed as a percentage of the total radioactivity. Plasma-free controls were prepared similar to those described above, except the drug loaded nanoparticles, or Paclitaxel for Injection were incubated for 1 h in distilled water (in place of plasma) followed by adjustment to 1.25 g/mL with sodium bromide and layering of the density gradient as described above. The plasma distribution of free PTX in the presence of blank formulations was also investigated by adding an aliquot of PTX dissolved in ethanol doped with a small amount of ^{3}H PTX to human plasma followed by incubation at 37 °C for 30 min to allow the drug to distribute among the plasma components. To this plasma, blank formulations (micelles, nanospheres or CrEL)

Table 1. Physicochemical Properties and PTX Loading Efficiency of MePEG-*b*-PCL Copolymers and Nanoparticles

feed ratio[a]	DPn[b]	MW[c] (g/mol)	M_n[d] (g/mol)	M_w[d] (g/mol)	PDI[d]	hydrodynamic diam[e] (nm)	zeta potential (mV)	loading effic[f] (%)
70:30	$MePEG_{114}$-*b*-PCL_{19}	7166	7679	9009	1.17	27.9 ± 0.41 (0.092)	−4.9	82.7 ± 6.8
30:70	$MePEG_{114}$-*b*-PCL_{104}	16856	11730	17460	1.49	56.0 ± 0.38 (0.114)	−10.8	92.0 ± 3.8

[a]Reaction feed ratio of MePEG:PCL. [b]Degree of polymerization determined by ^{1}H NMR. [c]Molecular weight calculated through DPn. [d]Number average molecular weight (M_n), weight average molecular weight (M_w) and molecular weight polydispersity index (PDI) determined by GPC. [e]Average hydrodynamic diameter of nanoparticles ± SD (size polydispersity) as determined by dynamic light scattering. [f]Average PTX loading efficiency (± SD) of nanoparticles at a polymer concentration of 10 mg/mL and theoretical PTX loading of 1% w/w.

were added at the same concentrations described above followed by incubation at 37 °C and subsequent separation of the plasma frations by density gradient ultracentrifugation.

In Vivo Pharmacokinetics and Biodistribution of Copolymers and Paclitaxel. Animal studies were carried out according to protocols approved by the Animal Care Committee at the University of British Columbia and in accordance with the Canadian Council on Animal Care. ^{14}C PTX loaded ^{3}H labeled copolymer nanoparticles were formulated as described above. As a control, Paclitaxel for Injection was also prepared by adding a small aliquot of ^{3}H PTX to 330 μL of Paclitaxel for Injection and diluted to 20 mL in PBS, to produce a 100 μg/mL solution of PTX. Formulations were administered to CD1 mice via tail vein injection at doses of 100 mg/kg copolymer and 1 mg/kg PTX. Mice were terminated by CO_2 inhalation at 5, 15, 30, 45, 90 180 and 360 min, and blood was collected by cardiac puncture and plasma immediately separated by centrifugation at 2500 rpm for 15 min at 5 °C. Liver, spleen, kidneys, lungs and heart were harvested, rinsed with ice-cold saline, blotted dry and weighed. All organs and plasma were digested in 1 mL of Solvable overnight followed by addition of 50 μL of 200 mM EDTA and 200 μL of 30% hydrogen peroxide to decolorize the samples. Radioactive counts in plasma and organs were analyzed by β scintillation counting using a dual label program. A standard curve was prepared to calculate the radioactive counts and hence concentration of drug or copolymer with which each animal was dosed. These values were then used to calculate the percentage of the initial dose which was present in plasma or tissues after termination. The data were fit to a noncompartmental model using WinNonlin.

RESULTS

Synthesis and Characterization of Copolymers. Through the analysis of the NMR spectra, the DPn values for the copolymers were calculated as 19 and 104 caprolactone repeat units and the polymers were subsequently termed $MePEG_{114}$-*b*-PCL_{19} (micelle forming) and $MePEG_{114}$-*b*-PCL_{104} (nanosphere forming) with calculated molecular weights of 7166 g/mol and 16856 g/mol, respectively. Using GPC the M_n and M_w were found to be 7679 g/mol and 9009 g/mol for $MePEG_{114}$-*b*-PCL_{19} and 11730 g/mol and 17460 g/mol for $MePEG_{114}$-*b*-PCL_{104}. Molecular weight polydispersity indices were calculated as 1.17 and 1.49 for $MePEG_{114}$-*b*-PCL_{19} and $MePEG_{114}$-*b*-PCL_{104}, respectively (Table 1).

Formation, Drug Loading and Characterization of Nanoparticles. Nanoparticles were formed and loaded with PTX using a previously reported nanoprecipitation process.[16] Both nanoparticle dispersions were clear with the absence of any precipitate at the polymer and drug concentrations used; however, the $MePEG_{114}$-*b*-PCL_{104} nanosphere samples appeared slightly opalescent. The $MePEG_{114}$-*b*-PCL_{19} micellar nanoparticles were found to be considerably smaller, possessing a hydrodynamic diameter of 27.9 nm, whereas the nanospheres were 56 nm (Table 1). Additionally, as determined by zeta potential analysis, the nanospheres had a more negative surface charge (−10.8 mV), as compared to that of the micelles (−4.9 mV). At a 1% w/w theoretical PTX loading, the nanospheres loaded the drug with a 92% PTX loading efficiency as compared to the 82.7% efficiency of the micelles.

Nanoparticle Stability Studies Using FRET. The ability of the nanoparticles to retain a hydrophobic payload in the presence of PBS or human plasma was analyzed using the coloaded fluorescent probe FRET pair $DiIC_{18}$ and $DiOC_{18}$ (Figure 1). All samples, regardless of the media they were

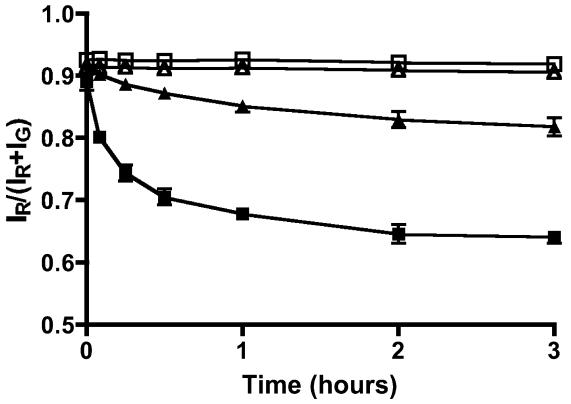

Figure 1. Release of $DiIC_{18}$/$DiOC_{18}$ FRET pair loaded in either $MePEG_{114}$-*b*-PCL_{19} micelles in PBS (□) or human plasma (■) or $MePEG_{114}$-*b*-PCL_{104} nanospheres in PBS (Δ) or human plasma (▲) at 37 °C. Each symbol represents the average of three samples ± SD.

dispersed in, had an initial FRET ratio of approximately 0.9. In PBS, both micelles and nanospheres retained a high FRET ratio with a very slight decrease over 3 h. When incubated in human plasma, the average FRET ratio for the micellar samples rapidly decreased to 0.64 over the 3 h incubation, whereas the average ratio for the nanosphere samples was considerably higher at 0.82 over the same time period. When nanoparticle dispersions were diluted in DMF, the FRET ratio immediately decreased to 0.35 for both formulations.

In Vitro Release of Paclitaxel. Using MWCO 7000 g/mol dialysis cassettes, the in vitro release of PTX from micelles and nanospheres was investigated (Figure 2). As a control, the release of free PTX (1 μg/mL) from the dialysis units was also conducted. Free PTX was rapidly released with 100% of the drug released within a day. The release rate from both nanoparticles was controlled and sustained with the nanospheres releasing 84% of the loaded drug over a 10-day period and the micelles releasing 95% of the loaded PTX over a 7-day period.

In Vitro Whole Blood Distribution. The distribution of PTX and the copolymers when incubated in whole human

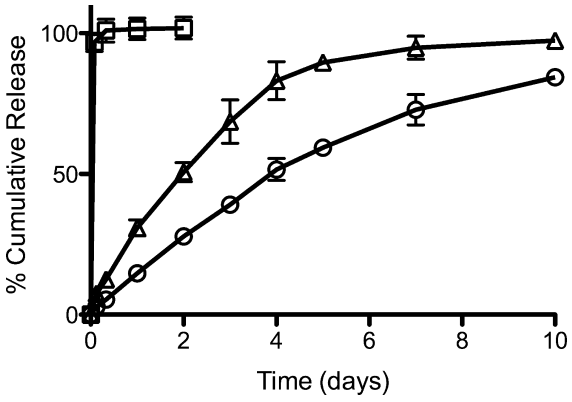

Figure 2. In vitro release of free PTX (□) or PTX solubilized in $MePEG_{114}$-*b*-PCL_{19} micelles (Δ) or $MePEG_{114}$-*b*-PCL_{104} nanospheres (○). Release medium was 10 mM PBS with 0.4 mg/mL bovine serum albumin at pH 7.4 and 37 °C. Each point is the average of three samples ± SD.

blood over a period of 6 h was investigated (Figures 3 and 4). PTX rapidly partitioned between the plasma and erythrocyte fractions with no significant change in the amount of drug

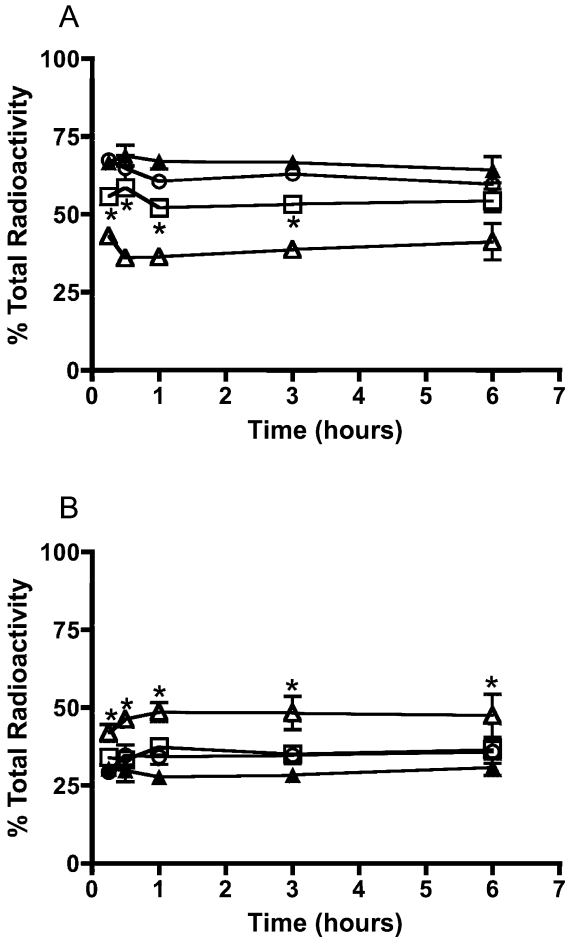

Figure 3. In vitro distribution of PTX into the (A) plasma and (B) erythrocyte fractions of whole human blood. Drug was formulated as Paclitaxel for Injection (□), free drug (Δ), $MePEG_{114}$-*b*-PCL_{19} micelles (○) or $MePEG_{114}$-*b*-PCL_{104} nanospheres (▲). Each point is the average of three samples ± SD. * $p < 0.05$ significantly different from other formulations as determined by two way ANOVA.

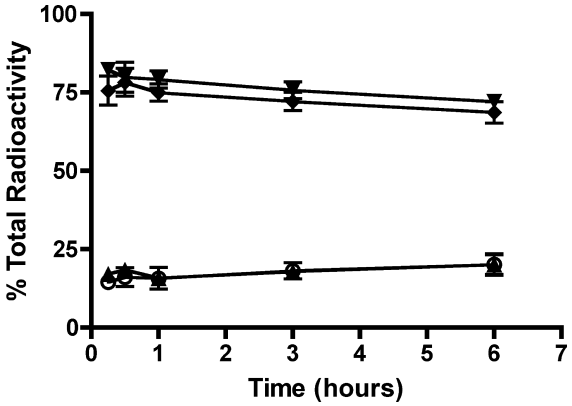

Figure 4. In vitro distribution of $MePEG_{114}$-*b*-PCL_{19} in plasma (▼) and erythrocytes (○) or $MePEG_{114}$-*b*-PCL_{104} in plasma (◆) or erythrocytes (▲). Each point is the average of three samples ± SD.

detected in the plasma or erythrocytes over time, regardless of the formulation. Approximately equal portions of the drug, formulated in its free form, were found split between the plasma and erythrocyte fractions. When PTX was formulated as Paclitaxel for Injection, copolymer micelles or nanospheres, significantly more drug was found in the plasma fraction with less PTX found in the erythrocyte fraction as compared to free PTX. With the exception of the 6 h time point, slightly more PTX was found in the plasma fraction when it was formulated in copolymer nanoparticles as compared to when it was formulated in CrEL; however, there was no significant difference between the two copolymer formulations. Analysis of the amount of copolymer present in the plasma and erythrocyte fractions demonstrated that between 75 and 80% of the copolymers was present in the plasma fraction with a slight decrease in polymer concentration over time.

In Vitro Plasma Distribution. The distribution of PTX and the copolymers in plasma fractions was investigated using density gradient ultracentrifugation (Figures 5 and 6). As a control, the distribution of the drug and copolymers in the density gradient, in the absence of plasma, was determined. As was previously found, after ultracentrifugation, a thin opalescent layer (which we termed the "NP fraction") was found at the interface between the 1.063 g/mL and 1.21 g/mL density fractions for the $MePEG_{114}$-*b*-PCL_{104} nanosphere group (Figure 5A inset); however, this layer was not visible in the other treatment groups.[16] Using the tubes from the $MePEG_{114}$-*b*-PCL_{104} nanosphere group as a template, the NP fraction layer was marked on the outside of the centrifuge tubes for the other treatment groups and separated from the other density layers for analysis of drug and copolymer content. In the controls, 88% and 96% of the PTX formulated in $MePEG_{114}$-*b*-PCL_{19} micelles and $MePEG_{114}$-*b*-PCL_{104} nanospheres was associated with the NP fraction, respectively (Figure 5A). Analysis of the copolymer in the density gradient revealed a similar profile as that for the drug, with 85% of the $MePEG_{114}$-*b*-PCL_{19} and over 90% of the $MePEG_{114}$-*b*-PCL_{104} found in the NP fraction (Figure 5B). When PTX was formulated as Paclitaxel for Injection, approximately 65% of the drug was associated with the 1.063 g/mL density fraction with the majority of the remaining drug in the NP fraction (Figure 5A). Incubation of the PTX loaded formulations in plasma resulted in a dramatic change in the distribution profiles for both the drug and copolymers (Figure 6A). For free PTX (i.e., not formulated in copolymer or CrEL), approximately 65% of the drug was found

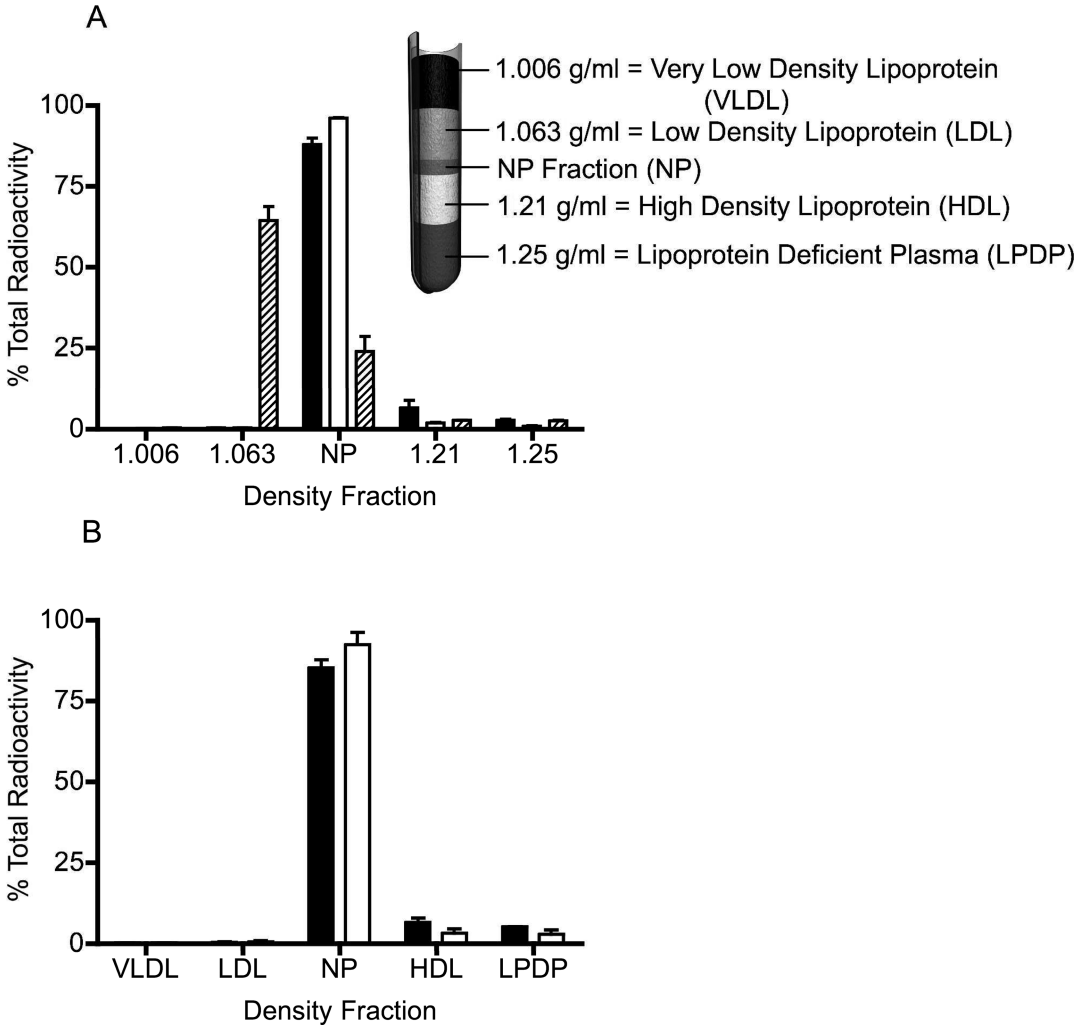

Figure 5. (A) Distribution of PTX in sodium bromide density gradient formulated as Paclitaxel for Injection (diagonal stripes), $MePEG_{114}$-*b*-PCL_{19} micelles (black) or $MePEG_{114}$-*b*-PCL_{104} nanospheres (white). Inset is a schematic of an ultracentrifugation tube with the relative postions of the density fractions and their corresponding lipoprotein fractions after centrifugation. (B) Distribution of $MePEG_{114}$-*b*-PCL_{19} (black) or $MePEG_{114}$-*b*-PCL_{104} (white) in sodium bromide density gradient. Each point is the average of three samples ± SD.

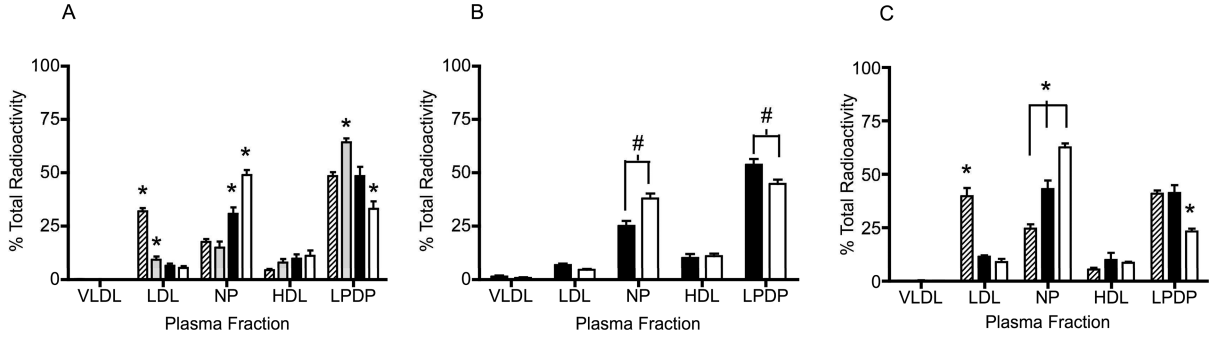

Figure 6. (A) Distribution of PTX in human plasma. Drug was solubilized in Paclitaxel for Injection (diagonal stripes), PBS (gray), $MePEG_{114}$-*b*-PCL_{19} micelles (black) or $MePEG_{114}$-*b*-PCL_{104} nanospheres (white). (B) Distribution of $MePEG_{114}$-*b*-PCL_{19} (black) or $MePEG_{114}$-*b*-PCL_{104} (white) in human plasma. (C) distribution of PTX in human plasma after incubation of free PTX followed by addition of Cremophor EL (diagonal stripes), blank $MePEG_{114}$-*b*-PCL_{19} micelles (black) or blank $MePEG_{114}$-*b*-PCL_{104} nanospheres (white) and an additional incubation of 1 h. Each point is the average of six samples ± SD. * $p < 0.05$ significantly different from other samples in plasma fraction as determined by one way ANOVA. # $p < 0.05$ significantly different from each other as determined by Student's *t* test.

to be associated with the LPDP (1.25 g/mL) fraction. Nearly 50% of the PTX was associated with the LPDP fraction when the drug was formulated as either Paclitaxel for Injection or $MePEG_{114}$-*b*-PCL_{19} micelles. The majority of the remaining drug was found in the NP fraction for the copolymer micelles or split between the LDL (1.063 g/mL) and NP fractions, for the Paclitaxel for Injection group. Approximately 50% of the PTX formulated in $MePEG_{114}$-*b*-PCL_{104} nanospheres was

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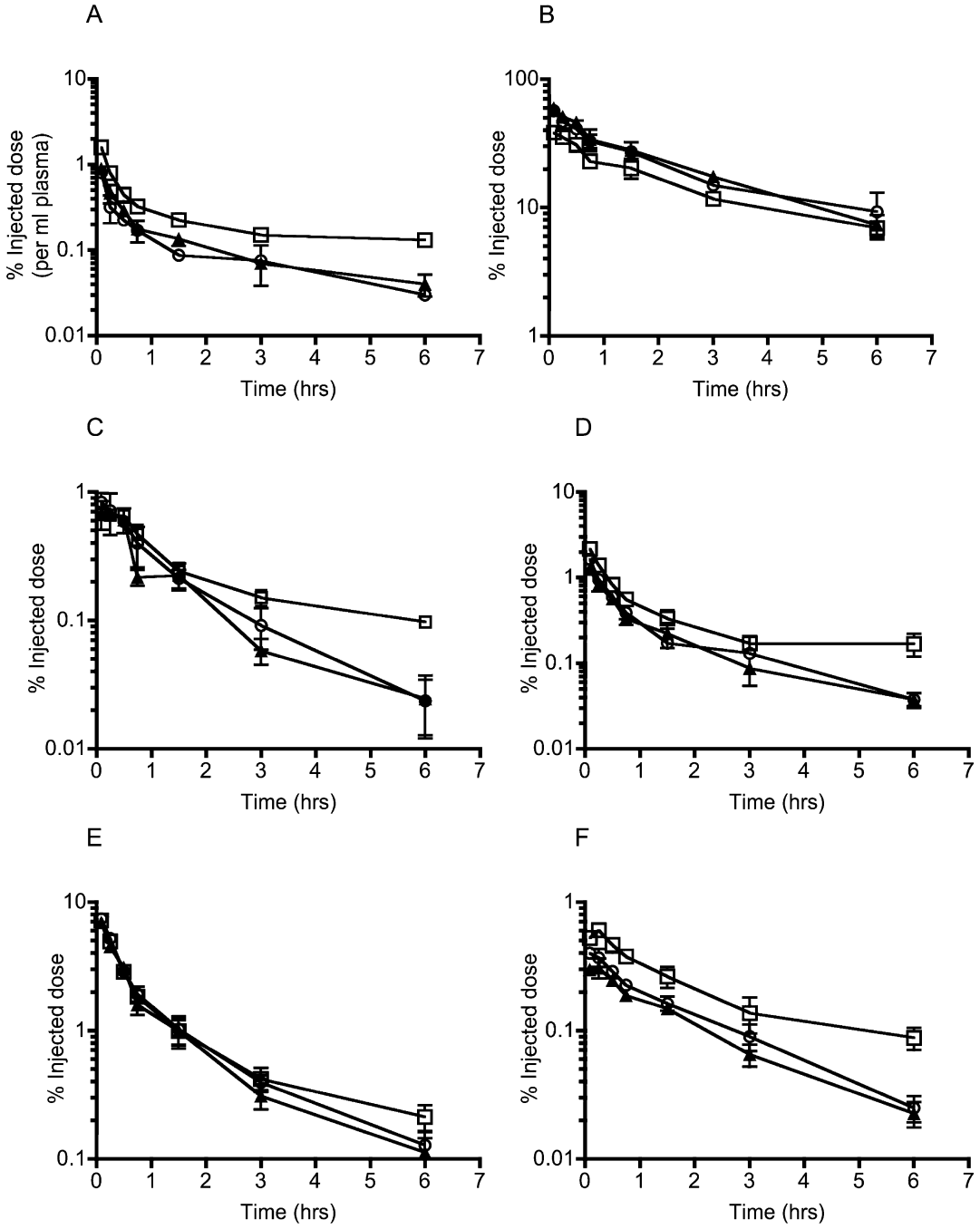

Figure 7. Pharmacokinetics and biodistribution of PTX formulated as $MePEG_{114}$-*b*-PCL_{19} micelles (○), $MePEG_{114}$-*b*-PCL_{104} nanospheres (▲) or Paclitaxel for Injection (□) in (A) plasma, (B) liver, (C) spleen, (D) lungs, (E) kidneys, and (F) heart.

found in the NP fraction with considerably less drug found associated with the LPDP fraction as compared to $MePEG_{114}$-*b*-PCL_{19} micelles or CrEL. Interestingly, for both the micelle and nanosphere groups, the majority of the copolymer was found in the LPDP fraction; however there was significantly more $MePEG_{114}$-*b*-PCL_{19} found in the LPDP fraction and less of this copolymer found in the NP fraction as compared to $MePEG_{114}$-*b*-PCL_{104} (Figure 6B). The addition of free PTX to plasma followed by incubation with blank formulations resulted in similar drug distribution patterns as those seen with drug loaded formulations (Figure 6C). For $MePEG_{114}$-*b*-PCL_{19} micelles and CrEL, approximately 40% of the drug was associated with the LPDP fraction and the remainder either found in the NP fraction, for the $MePEG_{114}$-*b*-PCL_{19} micelles, or split between the NP and LDL fractions in the case of CrEL. For $MePEG_{114}$-*b*-PCL_{104} nanospheres the majority of the drug (65%) was associated with the NP fraction with only 25% of the drug found in the LPDP fraction.

In Vivo Pharmacokinetics and Biodistribution. Upon injection of PTX loaded $MePEG_{114}$-*b*-PCL_{19} micelles, $MePEG_{114}$-*b*-PCL_{104} nanospheres or Paclitaxel for Injection, the drug was rapidly eliminated from the plasma with less than 1% of the injected dose per ml of plasma remaining 15 min postinjection, regardless of the administered formulation (Figure 7A). PTX formulated as Paclitaxel for Injection displayed a longer elimination half-life, longer mean residence

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time, lower clearance and higher area under the plasma concentration versus time curve as compared to the copolymer formulations (Table 2). No appreciable differences were found in the PTX pharmacokinetic parameters for the two copolymer

Table 2. Pharmacokinetic Parameters for Formulated PTX and MePEG-*b*-PCL Copolymers

formulation	$t_{1/2}$ (h)	AUC_{0-t} (h μg/mL)	CL (mL/h/kg)	V_{ss} (ml/kg)	MRT (h)
PTX in $MePEG_{114}$-*b*-PCL_{19}	2.75	0.20	4305.70	12024.33	2.85
PTX in $MePEG_{114}$-*b*-PCL_{104}	2.63	0.23	3719.76	10359.89	3.26
PTX in Paclitaxel for Injection	6.45	0.41	1334.76	10517.53	8.17
$MePEG_{114}$-*b*-PCL_{19}	7.97	1233.50	37.58	377.77	10.76
$MePEG_{114}$-*b*-PCL_{104}	8.85	2373.87	16.93	203.90	12.36

formulations. Interestingly, a significant amount of copolymer remained in the plasma for extended circulation times with $MePEG_{114}$-*b*-PCL_{104} displaying a longer elimination half-life, higher area under the curve, lower clearance and lower volume of distribution as compared to $MePEG_{114}$-*b*-PCL_{19} (Figure 8A and Table 2). Analysis of the biodistribution of PTX into the major organs indicated that the majority of the drug was rapidly distributed to the liver with nearly 60% and 40% of the dose recovered in this organ within 5 min for the copolymer nanoparticles and Paclitaxel for Injection, respectively (Figure 7B). Regardless of the formulation, similar PTX distribution profiles were found in the remainder of the organs, with little difference in the amount of drug depending on the administered formulation (Figure 7C–F). Other than in the plasma, there were no major differences in the amount of $MePEG_{114}$-*b*-PCL_{19} or $MePEG_{114}$-*b*-PCL_{104} found in the organs analyzed (Figures 8B–F).

DISCUSSION

Through a ring-opening polymerization of ε-caprolactone using MePEG as a macroinitiator and stannous octoate as a catalyst, two MePEG-*b*-PCL amphiphilic diblock copolymers were synthesized possessing two very different hydrophobic block lengths. As we have demonstrated previously, this reaction yielded copolymers of predictable composition, which was reliably controlled by variation in the feed ratio of MePEG to ε-caprolactone.[4,16] The synthesized copolymers possessed monodisperse molecular weight distributions with relatively low polydispersity indices indicating a narrow range of molecular weights. Due to the large hydrophobic block, $MePEG_{114}$-*b*-PCL_{104} was poorly water-soluble, and therefore a nanoprecipitation and dialysis method using DMF as the water miscible solvent was employed for the formation of nanoparticles from both copolymers. Micelles were considerably smaller in diameter than the nanospheres due to the larger hydrophobic block of the latter, leading to a larger hydrophobic core and hence a larger hydrodynamic diameter. The negative zeta potential of the particles is likely due to negatively charged carboxylic acid groups of the PCL blocks, and, as reported by Gref et al., the more negative zeta potential of the nanospheres is likely due to reduced PEG coverage on the surface of the nanoparticle due to a lower weight percentage of PEG in $MePEG_{114}$-*b*-PCL_{104} as compared to $MePEG_{114}$-*b*-PCL_{19}.[29] The nanospheres had a noticeably higher PTX loading efficiency compared to the micelles. As there was no evidence of precipitate after the loading of the nanoparticles, the loss of drug is assumed to have taken place during the dialysis step. Dialysis was used to remove the water miscible organic solvent from the dispersion; however, it is inevitable that there is some drug loss along with the removal of the solvent. This increase in PTX loading and loading efficiency with increased hydrophobic block length has been previously reported and is likely due to an increased interaction between PTX and PCL with the longer hydrophobic block of the $MePEG_{114}$-*b*-PCL_{104}.[4,10]

Using fluorescence spectroscopy, we investigated the ability of the nanoparticles to retain a hydrophobic FRET pair, $DiIC_{18}$ and $DiOC_{18}$, in the presence of PBS and human plasma. In PBS, micelles and nanospheres were characterized by a high FRET ratio of approximately 0.9 for the entire incubation time. This high FRET ratio indicated that these probes were in close proximity to one another, presumably in the nanoparticle core, with very little release over the experimental time period. As a control, the nanoparticle dispersions were diluted in DMF, which resulted in the immediate decrease of the FRET ratio, indicating release and separation of the FRET pair, due to disruption of the nanoparticle structure. When incubated in human plasma, the micelles were characterized by a decrease in the FRET ratio of approximately half of the original value over 3 h. This decrease provides evidence of the rapid release of a significant portion of the hydrophobic payload in the presence of plasma components. Other researchers have also demonstrated the lack of stability of polyester based copolymer micelles in vivo as well as in vitro in the presence of serum proteins. Chen et al. demonstrated the same rapid decrease in FRET ratio when MePEG-*b*-PDLLA micelles, also coloaded with $DiIC_{18}$ and $DiOC_{18}$, were administered to mice.[25] Further in vitro experimentation by these researchers indicated that the rapid probe release was likely due to an interaction with α and β globulins. Using a fluorogenic-based approach, Savic et al. demonstrated the instability of PEO_{45}-*b*-PCL_{21} micelles in the presence of serum proteins and made reference to the importance of investigating the effect of varying hydrophilic and hydrophobic block lengths on the in vitro and in vivo stability of block copolymer nanoparticles.[24] Our data demonstrate that, by increasing the hydrophobic block length from 19 to 104 repeat units, there was a significant reduction in the release rate of the FRET pair in the presence of human plasma. This result highlights the enhanced ability of the nanospheres to retain a hydrophobic payload in plasma in vitro as compared to the micelles. Since the experiments conducted in the present work were not able to determine whether the nanoparticles are still intact upon incubation with plasma proteins, it is unclear if the mechanism of release of the fluorescence probes is due to nanoparticle disruption or partitioning of the payload out of the hydrophobic core and reassociation with plasma proteins.

The in vitro PTX release from copolymer micelles and nanospheres also highlights the ability of the nanospheres to better retain their hydrophobic payload as compared to micelles. The release rate for both micelles and nanospheres was controlled and sustained for the duration of the experiment with release from micelles being noticibly faster than that of the nanospheres. The slower, more prolonged release of PTX from the nanospheres may be attributed to an increased interaction between the longer PCL chains and the drug, an effect that has been described by other investigators.[30,31]

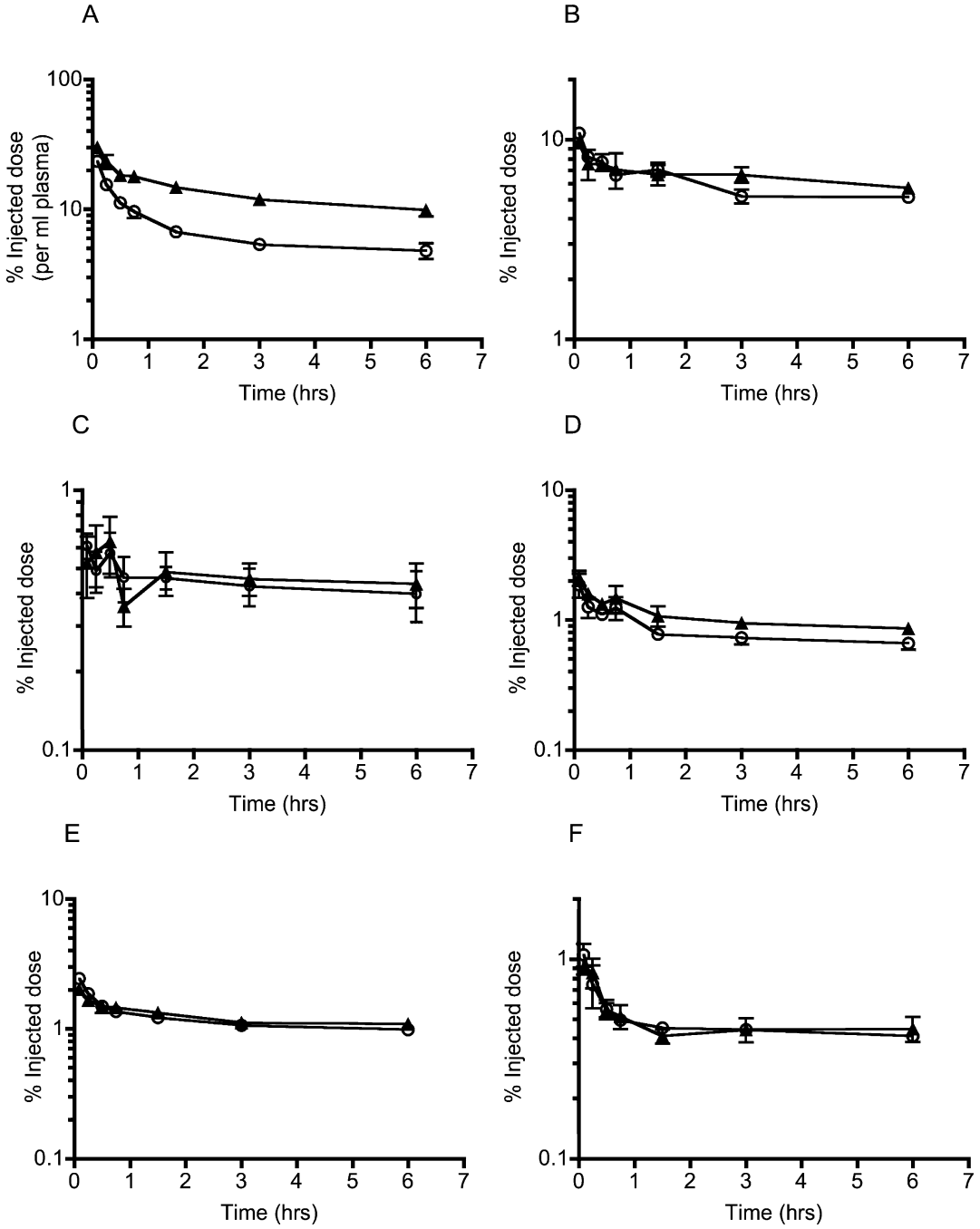


Figure 8. Copolymer biodistribution of $MePEG_{114}$-*b*-PCL_{19} (○) or $MePEG_{114}$-*b*-PCL_{104} (▲) in (A) plasma, (B) liver, (C) spleen, (D) lungs, (E) kidneys, and (F) heart.

When incubated in whole human blood, PTX rapidly partitioned between the plasma and erythrocyte fractions, with little change in the amount of drug found in these fractions over time, regardless of the formulation. When free PTX was incubated in whole blood, approximately 40% of the drug was found in the plasma fraction, with the remainder associated with the erythrocyte fraction. The rapid binding of PTX to plasma proteins has previously been shown to dramatically decrease the cellular accumulation in erythrocytes; however, it cannot be ignored that the erythrocyte fraction accounts for a significant portion of the drug and has been demonstrated to act as a secondary transport system for the drug, even in the presence of CrEL when administered to patients.[32] Sparreboom et al. demonstrated that, in the presence of increasing concentrations of CrEL, PTX drug partitioning into erythrocytes was significantly reduced in a dose dependent fashion.[32] The association of PTX with CrEL was attributed to the solubilization of the drug within the hydrophobic core of the CrEL micelles, primarily composed of polyoxyethylene-glycerol triricinoleate. Similar to these findings, we demonstrated that MePEG-*b*-PCL nanoparticles and CrEL significantly reduced the partitioning of PTX into erythrocytes, albeit to a slightly lesser extent for the CrEL, likely due to the low concentration of CrEL used in these studies. Considering free PTX readily binds to proteins in the plasma fraction, it is likely that, even in the presence of copolymer micelles, nanospheres or CrEL, a

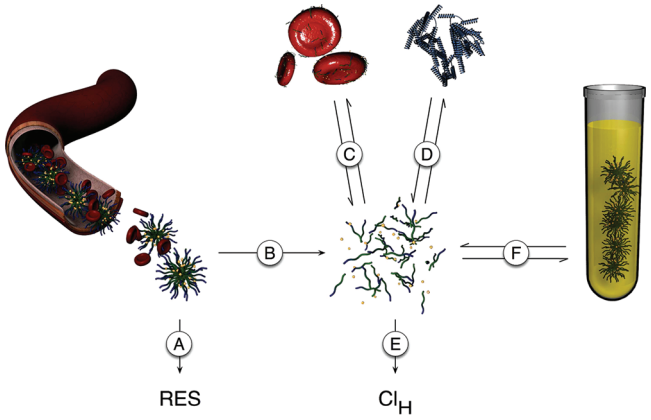
portion of the drug was associated not only with the carrier but also with the plasma proteins. When copolymer concentrations in plasma and erythrocytes were assayed, the vast majority (70−80%) of the copolymers were found to be associated with the plasma fraction. Since the remaining 20−30% of the copolymer that was associated with the erythrocytes is approximately equivalent to the fraction of PTX found associated with the red blood cells, it is difficult to conclude whether this fraction of drug is still associated with the copolymer or both the drug and copolymer are separately associated with the erythrocytes. Despite this copolymer association with the erythrocytes, there was no visual indication of hemolysis corroborating our previous hemocompatibility findings for these nanoparticles.[16]

Incubation of PTX formulated as Paclitaxel for Injection or copolymer nanoparticles in whole blood resulted in the majority of the drug being retained in the plasma fraction; however, it was unclear whether the drug still remained associated with the formulation or with the plasma proteins. In an attempt to address this question, we used a previously reported density gradient ultracentrifugation method to investigate the distribution of PTX and the copolymers in human plasma.[16,33] As a control, the distribution of the formulations in the density gradient was investigated to determine which density fraction the drug and copolymers would associate with, in the absence of plasma (Figure 5). As previously reported,[16] nearly all of the PTX solubilized in either copolymer micelles or nanospheres was associated with the NP fraction. Quantification of the copolymers also indicated that the majority of both copolymers were associated with the NP fraction, thus indicating that the drug was still associated with the nanoparticles in the absence of plasma. PTX formulated as Paclitaxel for Injection was found mainly associated with the 1.063 g/mL fraction with approximately 25% of the drug also found in the NP fraction. This split in PTX distribution is likely due to the density of the formulation lying between these two fractions.

When incubated in plasma, followed by density gradient ultracentrifugation, the distribution of PTX and copolymers dramatically changed as compared to the nonplasma controls (Figure 6). To determine which plasma components PTX would associate with in the absence of solubilizing excipients, free drug was incubated in the plasma. After centrifugation, approximately 65% of the free PTX was found associated with the LPDP fraction (1.25 g/mL density fraction). This result is in agreement with our previous findings[16] and is likely due to the fact that PTX reportedly associates with plasma proteins, including albumin and glycoproteins.[34] For both Paclitaxel for Injection and $MePEG_{114}$-*b*-PCL_{19} micelles, nearly 50% of the drug was associated with the LPDP fraction, significantly less than that for free drug. For $MePEG_{114}$-*b*-PCL_{19}, micelles, 30% of the remaining drug was associated with the NP fraction with the balance of the PTX distributed between the LDL and HDL fractions. For Paclitaxel for Injection, the distribution of the remaining 50% drug was split between the NP fraction and the LDL fraction, similar to the drug distribution profile in the absence of plasma. These results suggest that, for these formulations, half of the dose is associated with the plasma proteins found in the LPDP fraction and, in the case of Paclitaxel for Injection, the remaining drug is likely still associated with the carrier, whereas up to 30% of the PTX remained associated with the $MePEG_{114}$-*b*-PCL_{19} micelles. Similar to our previously reported findings, solubilization of PTX in $MePEG_{114}$-*b*-PCL_{104} nanospheres resulted in significantly more of the drug payload retained in the NP fraction with less of the PTX found in the LPDP fraction as compared to the copolymer micelle formulation. Previously, we could not confirm which plasma fraction the copolymer was associated with. In the present study, with the use of radiolabeled copolymers, we determined that aside from the NP fraction, a significant amount of the copolymer was also associated with the LPDP fraction. Due to this large amount of copolymer found in the LPDP, there still remains a question of the nature of the copolymer and PTX associated with this plasma fraction. As previously mentioned, Savic et al. demonstrated a rapid and significant loss of nanoparticle structure when PEO-*b*-PCL micelles were incubated in serum.[24] The cause of this disruption was not entirely clear; however, possible mechanisms suggested include the extraction of unimers from the structure, as has been shown for liposomes,[35] or the formation of mixed micelles with serum components, comparable to the mixing of small molecular weight surfactants with copolymer micelles.[36−38] Therefore, in light of this report along with our FRET and plasma distribution results, a likely possibility is that the accumulation of PTX and copolymer in the LPDP is due, in some part, to significant disruption of the micelles and to a lesser extent the nanospheres by plasma components. In an experiment designed to investigate the propensity of free PTX in plasma fractions to redistribute back into these formulations, free PTX was incubated with plasma followed by the addition of blank formulations. Interestingly, the drug distribution was similar to that of the drug loaded formulations (Figure 6C compared with Figure 6A). These findings imply that a large portion of the free drug is capable of preferentially reassociating with the blank formulations, a phenomenon that has previously been described for lipid nanocapsules in which docetaxel was found to reassociate with the plasma fraction containing the formulation.[39] Furthermore, it was demonstrated that these lipid nanocapsules were capable of sequestering the drug in vivo, leading to prolonged circulation of the drug. In the current studies, it is likely that, upon incubation of drug loaded formulations with plasma, some degree of disruption and drug release occurs and this released drug reassociates with intact nanoparticles (i.e., in the NP fraction for the copolymer micelles and nanospheres) resulting in the observed plasma distrubution profiles. Therefore, the majority of the drug in plasma consists of drug still solubilized in the core of the nanoparticles as well as free drug that reassociates with the nanoparticles but is in equilibrium with the plasma proteins (Scheme 1). It is likely that more PTX reassociated with the $MePEG_{114}$-*b*-PCL_{104} nanospheres as compared to the $MePEG_{114}$-*b*-PCL_{19} micelles due to the greater affinity of PTX for the nanoparticles with an increased hydrophobic block length.

Upon intravenous injection into mice, the PTX dose was rapidly cleared from the plasma, regardless of the formulation. Very little difference in the PTX pharmacokinetic parameters between the copolymer micelles and nanospheres was found; however, even at the low dose of CrEL used in these studies, the Paclitaxel for Injection displayed a larger AUC, longer $t_{1/2}$, reduced clearance and prolonged MRT as compared to the copolymer formulations. These differences may be due to the known ability of CrEL to influence the pharmacokinetics of PTX, reportedly due to its low volume of distribution and retention of PTX in CrEL micelles, resulting in significant confinement of the drug within the central blood compartment

Scheme 1. The In Vitro and In Vivo Fate of MePEG-*b*-PCL Nanoparticles and PTX[a]



[a]Upon iv injection, intact nanoparticles may undergo clearance by the RES (A) as well as disruption releasing loaded PTX (B). Released drug and copolymer may associate with erythrocytes (C) as well as with plasma proteins (D). In vivo, the drug undergoes rapid and extensive hepatic extraction and clearance (E) whereas in vitro the drug may reassociate with intact nanoparticles (F).

in a dose dependent fashion.[40] The fact that prolonged PTX circulation was not attained with MePEG$_{114}$-*b*-PCL$_{19}$ micelles was not surprising as it has been demonstrated by other researchers that PTX readily dissociates from micelles composed of polyester based copolymers.[18−20] The rapid dissociation of the drug from micelles is likely due to partitioning of the drug from the micellar core due to poor compatibility between the polyester hydrophobic core and PTX,[4,41] as well as structural instability upon injection.[18,24] In light of this, we hypothesized that, by increasing the hydrophobic block length of the copolymer and entrapping the drug in the more stable core of nanospheres, PTX would be retained with the carrier for longer than micelles. Our in vitro experiments demonstrated that more PTX was associated with the nanospheres as compared to the micelles, through either drug entrapment or reassociation. Furthermore, upon intravenous injection, MePEG$_{114}$-*b*-PCL$_{104}$ circulated for longer than MePEG$_{114}$-*b*-PCL$_{19}$ with a larger AUC, longer $t_{1/2}$, and MRT and decreased CL and V_{ss}, suggesting improved stability of nanospheres as compared to micelles. However, despite this improved stability and in vitro drug association, prolonged in vivo PTX circulation was not achieved as demonstrated by similar pharmacokinetic parameters for PTX delivered by copolymer micelles or nanospheres. Rijcken et al. described a similar phenomenon in their study of the pharmacokinetics and biodistribution of PTX loaded, core cross-linked micelles.[42] In their investigation they demonstrated that, despite the prolonged circulation and evasion of the RES by the carrier, the drug was rapidly eliminated from the blood within minutes. Clearly, in their case, the drug elimination was due not to carrier instability but, rather, to rapid partitioning of the PTX from the nanoparticles. Several studies have shown that trapping the drug in a nanosphere core composed of high molecular weight polymer can reduce partitioning of PTX out of the core of nanoparticles. Using high molecular weight PDLLA (22,000 g/mol) stabilized by a surface coating of MePEG-*b*-PDLLA, Gaucher et al. were able to prolong the circulation of the drug in vivo.[26] Likewise, using diblock copolymers composed of MePEG or D-α-tocopheryl polyethylene glycol 1,000 succinate and high molecular weight PDLLA or PLGA hydrophobic blocks, the circulation of PTX was dramatically increased.[21,43] However, in all three of these studies, the nanoparticles formed were relatively large (200−400 nm), leading to substantial uptake by the RES, likely due to the large size and insufficient PEG surface coverage. Furthermore, efficacy of these large nanoparticles may be further hampered by poor vascular permeability and diffusion through the interstitial matrix of the tumor. These transport processes are highly size dependent and significantly decrease with increased nanoparticle size.[44] In contrast, our data indicates that there was not substantial uptake of the nanoparticles by organs of the RES as indicated by relatively low concentration of copolymer found in the liver, spleen and lungs (Figure 8B−D). This is possibly due to the high PEG content of the particles and relatively small hydrodynamic diameter. However, it is likely that the molecular weight of the core of the nanospheres was not high enough to prevent rapid partitioning of the drug out of the carrier in vivo. We suggest that, upon iv injection of PTX loaded micelles or nanospheres, the combination of blood flow and high shear stress leads to rapid mixing of nanoparticles likely resulting in some disruption of the nanoparticle structure[24] and partitioning and redistribution of PTX in different blood components including plasma proteins and red blood cells. Subsequent passage of PTX through the liver (reported to have a high hepatic extraction ratio[45]) then results in rapid and extensive extraction of PTX from various blood fractions and PTX bound to nanoparticulate "fragments". Hence, PTX is rapidly eliminated with high levels of PTX found in the liver (Scheme 1).

■ CONCLUSION

The goal of this study was to determine whether the solubilization of PTX in nanospheres composed of MePEG-*b*-PCL would result in a more stable formulation in the presence of plasma and whole blood as compared to their micellar counterpart, ultimately resulting in a nanoparticulate delivery system that prolongs the circulation of PTX in vivo. As compared to MePEG$_{114}$-*b*-PCL$_{19}$ micelles, MePEG$_{114}$-*b*-PCL$_{104}$ nanospheres demonstrated better stability, as assessed by FRET probe release in the presence plasma, a more prolonged PTX in vitro release and better retention of PTX in plasma. Upon intravenous injection of PTX loaded micelles and nanospheres, the copolymer circulation was prolonged; however, the drug was rapidly eliminated from plasma. Very little difference in the PTX pharmacokinetic parameters between the systems was found with a lower AUC, shorter $t_{1/2}$, increased clearance and shorter MRT than the commercially available formulation, Paclitaxel for Injection. This difference in circulation time between copolymer and PTX indicates that the drug rapidly partitioned out of the nanoparticles and, therefore, these copolymer nanoparticles were incapable of retaining their drug payload in vivo. These in vivo results are surprising given the enhanced in vitro stability of the nanospheres and serve to highlight the fact that in vitro testing is often a poor predictor of the in vivo performance of a drug delivery system. Reports in the literature have demonstrated that the use of high molecular weight polyester-based nanoparticles are capable of retaining PTX in their core and thus increasing the circulation time of the drug, however, often at the cost of increased uptake by the RES and poor tumor penetration. Additionally, some researchers are demonstrating that increased compatibility between the core forming block and PTX can result in

improved PTX retention and in some cases prolonged circulation.

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