Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 596–606
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

The effect of core composition in biodegradable oligomeric micelles as taxane formulations

Myrra G. Carstens [a,b], Pascal H.J.L.F. de Jong [a], Cornelus F. van Nostrum [a], Johan Kemmink [c], Ruud Verrijk [b], Leo G.J. de Leede [b], Daan J.A. Crommelin [a], Wim E. Hennink [a,*]

[a] *Department of Pharmaceutics, Utrecht University, Utrecht, The Netherlands*
[b] *OctoPlus N.V., Leiden, The Netherlands*
[c] *Department of Medicinal Chemistry, Utrecht University, Utrecht, The Netherlands*

Received 25 June 2007; accepted in revised form 29 August 2007
Available online 12 September 2007

Abstract

Docetaxel (DCTX) and paclitaxel (PTX) are very potent anti-cancer drugs, but the currently marketed formulations, Taxotere® and Taxol®, respectively, are associated with vehicle-related toxicity. An attractive alternative to formulate these hydrophobic cytotoxic agents are polymeric micelles. In this study, the loading of taxanes into oligomeric micelles composed of mPEG750-*b*-oligo(ε-caprolactone)$_5$ (mPEG750-*b*-OCL$_5$) with a hydroxyl (OH), benzoyl (Bz) or naphthoyl (Np) end group was investigated. Next, the release characteristics and cytotoxicity of the loaded micelles were studied. MPEG750-*b*-OCL$_5$-OH micelles loaded with taxanes formed unstable particles with rapid leakage of the drug. In contrast, the presence of an aromatic end group (Bz or Np) resulted in the formation of small (10 nm), almost monodisperse micelles with stable encapsulation of 10% (w/w) of PTX or DCTX. This was ascribed to a better compatibility between the micellar core and the drug as compared to the oligomers with the hydroxyl end group. ^{1}H NMR studies showed that the micellar core was liquid, and that PTX was molecularly dissolved in the core. The *in vitro* stability was studied in PBS at 37 °C, which showed that leakage of PTX from 10% and 5% (w/w) loaded mPEG750-*b*-OCL$_5$-Bz micelles started after 8 and 24 h, respectively. The presence of albumin did not affect the stability, suggesting that the micelles are not destabilised and the drug was not extracted from the micellar core by this protein. The *in vitro* cytotoxic effect of the taxane-loaded micelles on C26 carcinoma cells was comparable to that of the commercial formulations, but the empty micelles were far less toxic than the Cremophor EL® vehicle. The results show that mPEG-*b*-oligo(ε-caprolactone) micelles hold good promise for the formulation of taxanes.

Keywords: Formulation; Polymeric drug carrier; Micelle; Poly(ethylene glycol)-oligo(ε-caprolactone); Paclitaxel; Docetaxel; Particle size; Particle stability; Drug solubilisation; Core composition

1. Introduction

Paclitaxel (PTX) and docetaxel (DCTX) (Fig. 1) belong to the taxane family of anti-neoplastic agents, and have been demonstrated to have good clinical efficacy among others in breast, ovarian, non-small cell lung and prostate cancer [1,2]. Their cytotoxic effect is caused by interference with the microtubule function in the cell, which results in disrupted mitosis and cell death [1,3]. Both PTX and DCTX are very potent anti-cancer agents, but they are highly hydrophobic and therefore poorly soluble in water. To overcome this solubility issue, for parenteral administration PTX is currently formulated in a mixture of Cremophor EL® (CrEL®), a polyoxyethylated castor oil vehicle, and ethanol (EtOH) (1/1 v/v). DCTX is slightly less hydrophobic than PTX, and is solubilised using Tween 80 (Tw80,

* Corresponding author. Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Pharmaceutical Sciences, Utrecht University, P.O. Box 80.082, 3508 TB Utrecht, The Netherlands. Tel.: +31 30 2536964; fax: +31 30 2517839.
E-mail address: W.E.Hennink@uu.nl (W.E. Hennink).

0939-6411/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2007.08.014

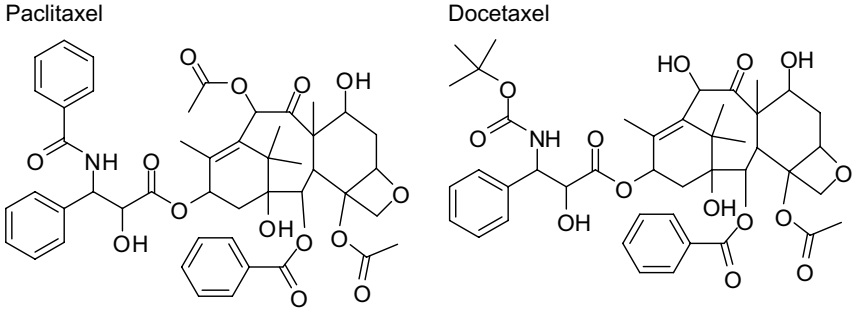

Fig. 1. Chemical structures of the taxanes paclitaxel (PTX, left) and docetaxel (DCTX, right).

polysorbate 80) [2,4]. The commercially available formulations of PTX and DCTX are called Taxol® and Taxotere®, respectively. Both CrEL® and Tw80 are not inert and have been associated with a number of adverse effects, such as hypersensitivity reactions and neurotoxicity [2,4]. The occurrence of vehicle associated toxicities has led to the development of alternative methods to formulate the poorly soluble taxanes [2,5], such as drug-polymer conjugates [6], and nanoparticles composed of albumin-bound PTX (Abraxane®) [7]. Because of the high hydrophobicity of taxanes, polymeric micelles are of particular interest for the formulation of these compounds. Polymeric micelles are core-shell structures with a size of 10-100 nm, formed by the self assembly of amphiphilic block copolymers [8–14]. Their hydrophobic core can accommodate hydrophobic drugs such as taxanes, and their hydrophilic shell, which usually consists of poly(ethylene glycol) (PEG), confers the micelles long circulating behaviour. In combination with their small size, this enables extravasation from the leaky vasculature at the tumour site, resulting in passive targeting and specific accumulation of the drug-loaded micelles in the tumour (i.e., the so-called enhanced permeability and retention (EPR) effect) [8–14], thus making polymeric micelles highly suitable for the (targeted) delivery of hydrophobic drugs to tumour tissue.

Both PTX and DCTX have been loaded into a variety of polymeric micelles, composed of, e.g., PEG-*b*-poly(D,L-lactide) (PLA) [15–20], PEG-*b*-poly(benzylaspartate) [21], poly(*N*-vinylpyrrolidone) (PVP)-*b*-PLA [22], PEG-*b*-poly(*N*-2-hydroxypropyl methacrylamide dilactate) (PHPMAmDL) [23], poly(2-ethyl-2-oxazoline)-*b*-poly(ε-caprolactone) (PEOx-*b*-PCL) [24], PEG-*b*-poly(styreneoxide) (PSO) [25] and core crosslinked PEG-*b*-PCL [26]. PTX-loaded PEG-*b*-PLA micelles have even reached clinical trials [16]. Promising results were obtained with micellar PTX with regard to drug accumulation in the tumour and the anti-tumour effect *in vivo* compared to Taxol® [19–22]. However, these were mostly related to a lower toxicity and a higher maximum tolerated dose (MTD), allowing higher dosing, rather than a different biodistribution profile of the (carrier loaded with) drug [15,19,22]. Likely, premature loss of the drug occurred, due to micelle destabilisation and/or extraction of the drug from the micelles by plasma proteins [27–29]. This was illustrated by biodistribution studies of a dual labelled system of PTX in PEG-*b*-PLA micelles, in which rapid dissociation of the PTX from the micelle-forming block copolymers was observed after intravenous injection [30]. Several papers have demonstrated that the retention (and loading capacity) of a drug in the micelles can be improved by optimising the compatibility between the drug and the micellar core, i.e., 'matching' the drug and the core-forming polymer [31–35].

We recently developed biodegradable oligomeric micelles, based on PEG-*b*-oligo(ε-caprolactone) [36]. Because of their small size (sub-20 nm), these micelles may have better properties than the larger micelles used so far, in terms of biodistribution and tumour penetration. The PEG-*b*-oligo(ε-caprolactone) micelles can be easily prepared by the film-hydration method. Furthermore, the end group of the PEG-*b*-oligo(ε-caprolactone)s was modified with an aromatic group, which may serve as a tool to improve the compatibility between the drug and the core. Here, we investigated novel taxane formulations, composed of PEG-*b*-oligo(ε-caprolactone)-based micelles. The aim of this study was to explore the effect of the composition of the hydrophobic block on the encapsulation and retention of PTX and DCTX, and to investigate their *in vitro* stability and cytotoxicity.

2. Materials and methods

2.1. Materials

Chloroform-*d* ($CDCl_3$, 99.8%D), deuterium oxide (D_2O, 99.9%), docetaxel (DCTX, >97.0%), Cremophor EL® (CrEL®), Tw80, and albumin immobilised on crosslinked 4% beaded agarose (BSA-SepCL-4B, 14 mg BSA per mL packed gel) were purchased from Sigma-Aldrich Chemie BV (Zwijndrecht, The Netherlands). Dichloromethane (DCM, peptide synthesis grade) and acetonitrile (ACN, HPLC gradient grade) were obtained from Biosolve Ltd. (Valkenswaard, The Netherlands). Paclitaxel (PTX) was from MP Biomedicals Inc. (Illkirch, France), and EtOH (absolute) from Mallinckrodt Baker BV (Deventer, The Netherlands). Methoxy-poly(ethylene glycol)-*b*-oligo(ε-caprolactone) (mPEG-*b*-OCL) block oligomers with monodisperse hydrophobic blocks (4 or 5 CL-units) and different end groups (Fig. 2) were prepared as described previously

[36]. In brief, mPEG-*b*-OCL was synthesised by ring-opening polymerisation of ε-caprolactone (20 g, 175 mmol), initiated by mPEG750 (26 g, 35 mmol) and catalysed by $SnOct_2$ (0.71 g, 1.8 mmol) overnight at a temperature of 130 °C. Benzoylated and naphthoylated mPEG750-*b*-OCL (mPEG750-*b*-OCL-Bz, mPEG750-*b*-OCL-Np, respectively) (Fig. 2b and c) were obtained by reacting the hydroxyl end group with a fivefold excess of benzoyl- or 2-naphthoyl-chloride in the presence of an equimolar amount of triethyl amine as HCl scavenger. To obtain monodisperse hydrophobic blocks, the polydisperse block oligomers were fractionated by preparative reversed phase HPLC (RP-HPLC), as reported elsewhere [36]. In brief, a system composed of a Waters 600EF Quaternary gradient pump and a Waters 2700 sample manager was used, and a solution of polydisperse block oligomer in ACN/1 M ammonium acetate buffer pH 5 was injected onto the column (Waters Xterra Prep MS C18 10 μm, 19 × 250 mm, including a guard column) followed by elution using a water/ACN gradient. Phosphate-buffered saline (PBS, pH 7.4) was obtained from Braun Melsungen AG (Melsungen, Germany), and was filtered through a 20 nm filter (Anotop®, Whatmann, Breda, The Netherlands) prior to use. Taxol® (6 mg/mL PTX, 16.7 mL) and Taxotere® (10 mg/mL DCTX in 2.0 mL premix solution) were purchased from Mayne Pharma BV (Brussels, Belgium), and Aventis Pharma S.A. (Antony Cedex, France), respectively.

2.2. Formation of taxane-loaded mPEG-b-OCL micelles

Micelles were formed by the film-hydration method, as described previously [36]. Typically, a film of block oligomer (10 mg) was formed by solvent evaporation from a 10 mg/mL solution of oligomer in dichloromethane in a 10 mL round-bottomed flask. The film was dried for 30 min under an N_2 stream, followed by hydration with 1.0 mL PBS at room temperature. The resulting dispersion was filtered through a 200 nm filter (Anotop®, Whatmann, Breda, The Netherlands) [36]. Drug was loaded into the micelles at different feed ratios by addition of 0.10, 0.20, 0.40 or 1.0 mL of a 5.0 mg/mL solution of the taxane (PTX or DCTX) in EtOH to the oligomer solution, prior to evaporation of the solvent, representing feed ratios of 5%, 10%, 20% or 50% (w/w) taxane/oligomer, respectively. As a control this procedure was performed without the addition of a block oligomer solution.

2.3. Analysis of the taxane-loaded mPEG-b-OCL micelles

2.3.1. Dynamic light scattering

The particle size and size distribution of loaded and unloaded mPEG-*b*-OCL-based micelles were measured by dynamic light scattering (DLS) using a Malvern CGS-3 multi-angle goniometer (Malvern Ltd., Malvern, UK), consisting of a HeNe laser source ($\lambda = 632.8$ nm, 22 mW output power), temperature controller (Julabo Waterbath) and a digital Correlator ALV-5000/EPP. Time correlation functions were analysed using the ALV-60X0 Software V.3.X provided by Malvern, to obtain the hydrodynamic diameter of the particles (Z_{ave}) and the particle size distribution (polydispersity index, PDI). The samples were measured at a scattering angle of 90°, at 25 or 37 °C, directly (at 10 min) after preparation, and after incubation for 30 min, 1, 2, 4, 8, and 24 h.

2.3.2. Loading efficiency

The amount of PTX or DCTX loaded into the micelles was determined according to an adapted literature method [23,37], using a Waters Acquity UPLC® system, consisting of a binary solvent manager, a sample manager and a TUV detector. An Acquity® BEH C8 1.7 μm column (2.1 × 50 mm) was used, and a gradient was run from 60% A (5/95 (w/w) ACN/H_2O) to 90% B (ACN) in 120 s, followed by isocratic elution at 100% B for 30 s, at a flow rate of 0.5 mL/min, and a column temperature of 50 °C. The detection wavelength was 200 nm (40 datapoints per second), which enabled simultaneous detection of benzoylated block oligomer (mPEG-*b*-OCL-Bz). Samples were taken directly after micelle preparation and after storage at room temperature for 24 h and diluted 30-60 times in 50/50 (w/w) ACN/H_2O, and 5 μL was injected. The chromatograms were

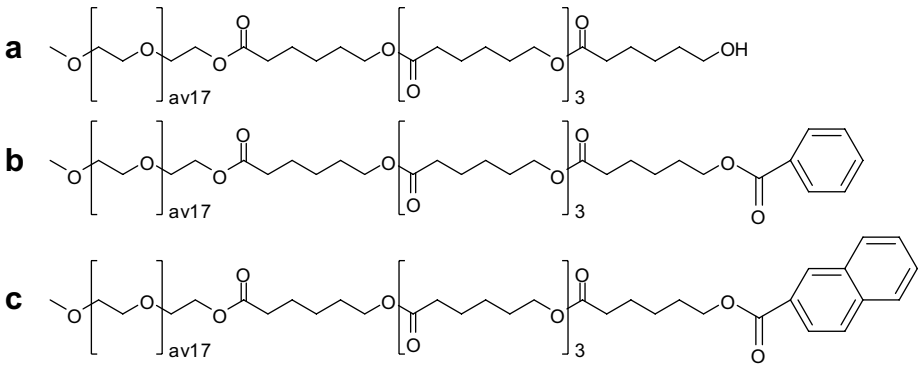

Fig. 2. Chemical structures of mPEG750-*b*-oligo(ε-caprolactone)$_5$ (mPEG750-*b*-OCL$_5$-OH) (a), benzoylated mPEG750-*b*-OCL$_5$ (-Bz) (b) and naphthoylated mPEG750-*b*-OCL$_5$ (-Np) (c). The average degree of polymerisation of mPEG750 is 17 ($_{av17}$).

analysed using Empower software (Empower Pro, Waters Chromatography BV, Etten-Leur, The Netherlands). The amount of taxane (PTX or DCTX) present was calculated using a calibration curve, prepared in 50/50 (w/w) ACN/H_2O. The loading efficiency was calculated using:

$$\text{Loading Efficiency} = \frac{\text{mass of drug loaded}}{\text{mass of drug fed}} \times 100\% \qquad (1)$$

2.3.3. ^1H NMR spectroscopy

^{1}H NMR spectra were recorded using a Varian INOVA-500 spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA) operating at 500 MHz with $CDCl_3$ or D_2O as a solvent. As reference lines the chloroform peak at 7.24 ppm and methyl peak of the mPEG-block at 3.40 ppm were used, respectively. Spatial information was derived from NOESY experiments ($\tau_m = 300$ ms). A spectral width of 5 kHz was used for all spectra. Samples of 10 mg/mL mPEG750-*b*-OLC_5-Bz with 10% (w/w) PTX dissolved in $CDCl_3$ or dispersed in D_2O were measured at 20 °C. Data were processed using Varian VNMR 6.1C (1D spectra) or NMRPipe (NOE spectra) software [38].

2.3.4. In vitro stability

The *in vitro* stability of 5 and 10% (w/w) PTX- and DCTX-loaded mPEG750-*b*-OCL_5-Bz micelles was studied in PBS at 37 °C. Due to their low aqueous solubility, the released taxanes crystallised and subsequently precipitated. In detail, mPEG750-*b*-OCL_5-Bz micelles loaded with 5% or 10% (w/w) taxane were prepared as described before, and incubated at 37 °C. The Z_{ave} and PDI were monitored by DLS. In addition, samples were taken from the dispersion at regular time points (10 min, 30 min, 1, 2, 4, 8, and 24 h), centrifuged at 10,000*g* for 1 min to remove the precipitate, and after dilution of the supernatant in 50/50 (w/w) ACN/H_2O, the concentrations of taxane and block oligomer were determined by UPLC®-UV, according to the method described above. The stability of the loaded micelles was also studied in the presence of bovine serum albumin (BSA). BSA immobilised on crosslinked 4% beaded agarose (BSA-SepCL-4B) was used, which has been applied by others to remove albumin-binding compounds from plasma, such as bilirubin and digitoxin [39]. In this study, 200 μL of a micelle dispersion in PBS (10 mg/mL mPEG750-*b*-OCL_5-Bz, containing 5% (w/w) PTX), was added to 1.8 mL of PBS, or a 50% (v/v) BSA-SepCL-4B-gel in PBS, corresponding to 12.6 mg BSA. The BSA concentration in the resulting dispersion was 6.3 mg/mL, and the molar ratio of PTX/BSA was 1/1.6, which means that a molar excess of BSA was present. Samples were taken at regular time points, and centrifuged for 1 min at 10,000*g* to separate the micelles (supernatant) from the BSA-SepCL-4B and/or precipitated PTX, which was confirmed by dynamic light scattering. The supernatant was diluted in 50/50 (w/w) ACN/H_2O and both the PTX- and oligomer-concentration were determined by UPLC®-UV.

2.3.5. In vitro cytotoxicity studies

The *in vitro* cytotoxic effect of mPEG750-*b*-OCL_5-Bz micelles, empty or loaded with 10% (w/w) PTX or DCTX on C26 murine colon carcinoma cells, was determined, and compared to Taxol®, Taxotere®, and the empty vehicles, CrEL®/EtOH and Tw80/13% (w/w) EtOH. Loaded and empty micelles were prepared as described in the previous section of this manuscript. Taxol® (6 mg/mL PTX) was diluted sixfold with PBS, and the corresponding control formulation without PTX was prepared according to Lee et al. [24]. In detail, 1.0 mL of CrEL® and 1.0 mL of EtOH were mixed, sonicated for 30 min, and subsequently diluted sixfold with PBS. Taxotere® was prepared according to the instructions on the package leaflet [40], i.e., the Tw80-solution containing DCTX was mixed gently with the 13% (w/w) EtOH in water for injections, resulting in a 10 mg/mL DCTX premix solution, which was subsequently diluted 10-fold with PBS. The control formulation without DCTX was prepared in the same manner, using Tw80. All stock solutions were diluted further with PBS to obtain taxane concentrations of 0.0001-10 μg/mL, and the empty vehicles were treated equally.

C26 murine colon carcinoma cells were cultured at 37 °C in a 5% CO_2-containing humidified atmosphere in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) heat-inactivated foetal calf serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Gibco, Breda, The Netherlands). Cells were seeded in a 96-well plate at a density of 5×10^3 cells per well. After 24 h, the culture medium was refreshed, and 100 μL of the different taxane and control formulations was added. As a reference 100 μL of PBS was added. The cells were incubated for 72 h at 37 °C in a humidified atmosphere with 5% CO_2. After incubation, the cell viability was determined by an XTT colorimetric assay [41]. The experiments were repeated twice.

3. Results and discussion

3.1. Loading of the micelles

Empty and taxane loaded micelles were prepared by hydration of an mPEG750-*b*-OCL_5-OH, -Bz, or -Np (see Fig. 2) block oligomer film without or with different amounts of drug with PBS. Block oligomers with 5 CL units were used, because these oligomers (with an OH, Bz, or Np end group) had an acceptable temperature sensitivity profile, meaning a Krafft point below 4 °C and a cloud point above 37 °C, and were able to form sub 20-nm micelles, as reported elsewhere [36]. The *Z*-average hydrodynamic diameter (Z_{ave}) of the formed particles and the loading efficiency of taxanes at different feed ratios into the micelles are presented in Fig. 3 and Table 1, respectively. In the control samples, formed by hydration of a taxane film, without the presence of block oligomers, taxanes were not detected by UPLC-UV®, not even after relatively long hydration times. After hydration of a film

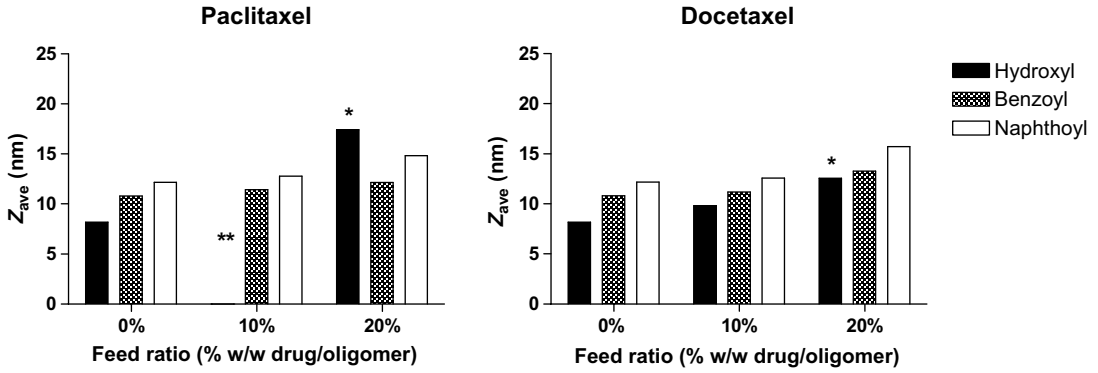

Fig. 3. Particle size of (loaded) mPEG750-*b*-OCL_5 micelles, with a hydroxyl, benzoyl or naphthoyl end group. The variation in the values was 5% ($n = 2$–3). The polydispersity index (PDI) of the particles was <0.1, except for the loaded mPEG750-*b*-OCL_5-OH micelles (*, >0.2). ** The particle size was out of scale (>500 nm).

Table 1
Loading efficiency (%) of paclitaxel and docetaxel in oligomeric micelles at different feed ratios[a]

End group	Paclitaxel				Docetaxel	
	5% w/w	10% w/w[b]	20% w/w[b]	50% w/w	10% w/w[b]	20% w/w[b]
Hydroxyl	ND[c]	87	8.5	6.8[e]	91	99
Benzoyl	80[d]	89	97	94[e]	89	96
Naphthoyl	ND[b]	89	70	65[e]	96	100

The variation in the values is 5–10% (n=2–3).
[a] Feed ratios were 5, 10, 20, and 50 weight percentage (% w/w) of taxane relative to the oligmer.
[b] The Z_{ave} and PDI are presented in Fig. 3.
[c] ND, not determined.
[d] The Z_{ave} was 12 nm, with a PDI of 0.06.
[e] The Z_{ave} was 23-200 nm with a PDI > 0.2.

composed of mPEG750-*b*-OCL_5 with a hydroxyl end group and 10% (w/w) PTX, and subsequent filtration, around 90% of the initially added drug was recovered (Table 1), but DLS measurements demonstrated that very large aggregates ($Z_{ave} > 500$ nm) rather than small micelles were formed (Fig. 3). At a feed ratio of 20% (w/w) PTX in mPEG750-*b*-OCL_5-OH, less than 10% of the drug was present in the final preparation, indicating a low loading capacity. Again, relatively large and polydisperse particles were detected by DLS (Fig. 3, Z_{ave} 20 nm, PDI 0.4). DCTX-loading of mPEG750-*b*-OCL_5-OH micelles resulted in almost full recovery of the added drug (Table 1), but also with this taxane, the mPEG750-*b*-OCL_5-OH nanoparticles were rather polydisperse (PDI of 0.2 and 0.3 for 10% and 20% DCTX (w/w) feed ratio, respectively). Interestingly, when mPEG750-*b*-OCL_5 with an aromatic end group (Bz or Np) was used, almost the entire amount of added drug was encapsulated at a feed ratio of both 10% and 20% (w/w) of either PTX or DCTX (Table 1). The taxane concentrations were nearly 1 and 2 mg/mL, respectively, which is markedly higher than the aqueous solubility of PTX and DCTX (<1 μg/mL). Moreover, DLS measurements demonstrated that, in contrast to loaded mPEG750-*b*-OCL_5-OH particles, these particles were small (Z_{ave} 10-16 nm) and nearly monodisperse (PDI < 0.1) (Fig. 3), indicating that the aromatic end group is essential for the formation of well-defined taxane-loaded mPEG750-*b*-OCL_5 micelles. Even a feed ratio of 50% (w/w) PTX resulted in much higher PTX recovery in end group modified mPEG750-*b*-OCL_5-micelles than that in unmodified ones, but at such high loading quite large and polydisperse particles were formed (Table 1). The highest loading capacity (approx. 20% w/w) observed in the small, monodisperse micelles is considerably higher than those reported for both taxanes in micelles composed of PVP-*b*-PLA (max. 5% w/w) [22], PEOx-*b*-PCL (8%) [24], PEG-*b*-PSO (4%) [25], or core crosslinked PEG-*b*-PCL (5%) [26], and comparable to those reported for PTX in PEG-*b*-PHPMAmDL (22% w/w) [23], or PEG-*b*-PLA micelles (25% w/w) [18].

At room temperature, the Z_{ave} and PDI of the micelles composed of mPEG750-*b*-OCL_5 with an aromatic end group (Bz or Np), loaded with 10% PTX or DCTX, remained stable for at least 24 h. Moreover, more than 75% of the initially loaded amount of taxane were retained in these micelles during this time period, as determined by UPLC®-UV analysis (Table 2). At 20% (w/w) loading, the mPEG750-*b*-OCL_5-Bz or -Np micelles retained their small size and low polydispersity for 2–8 h, and after 24 h the taxane concentration in the formulation was 25-50% of the initially loaded drug (Table 2). The lower micellar stability upon higher drug loading was also observed in PTX-loaded PEG-polyester micelles [30]. It may be related to exceeding the solubilisation capacity of the micelles, resulting in 'super-saturation' of the micellar core, which causes release in the continuous phase, followed by crystallisation and precipitation of the drug [13]. In contrast to the end group modified mPEG750-*b*-OCL_5, it was not possible to form small and stable micelles composed of mPEG750-*b*-OCL_5-OH at a feed ratio of 10% or 20% (w/w) PTX or 20% DCTX. At 10% (w/w) DCTX loading, the Z_{ave} and PDI remained stable for 8 h at room temperature, but at 24 h around 60% of the loaded DCTX dose was released (Table 2). These data further confirm that an aromatic end group at the chain end of mPEG750-*b*-OCL_5 improves the solubilisation capacity of mPEG750-*b*-OCL_5-based

Table 2
Stability of PTX- and DCTX-loaded oligomeric micelles at room temperature

End group	10% w/w PTX		20% w/w PTX		10% w/w DCTX		20% w/w DCTX	
	Stability (h)[a]	PTX at $t = 24$ h (%)[b]	Stability (h)[a]	PTX at $t = 24$ h (%)[b]	Stability (h)[a]	DCTX at $t = 24$ h (%)[b]	Stability (h)[a]	DCTX at $t = 24$ h (%)[b]
Hydroxyl	<0.15	12	<0.15	8	8	43	<0.15	11
Benzoyl	>24	88	4	23	>24	74	8	42
Naphthoyl	>24	77	4	19	>24	84	2	52

[a] Time (hours) until $Z_{ave} > 20$ nm or POI >0.3.
[b] Amount of PTX or DCTX in the micellar dispersion at 24 h after preparation, presented as % of the amount of taxane fed; the variation in the values is 5–10% ($n = 2$–3).

micelles, and that it is necessary for the formation of stable taxane-loaded micelles.

The observed differences clearly demonstrate that the drug loading and stability of these micelles depends on both the micellar core and the taxane structure. This may be partly explained by the differences in hydrophobicity of both taxanes and the three micellar cores. The log P values of the OCL_5-OH, -Bz and -Np cores of the micelles, and of PTX and DCTX were calculated with the ClogP program (Table 3), which is based on the additive, constitutive character of partition coefficients, but also considers the interactions between neighbouring groups [42]. Likely, the higher hydrophobicity (reflected by the higher log *P* value) of the micellar core composed of OCL_5 with an aromatic end group (Bz or Np), when compared to OCL_5-OH, is responsible for the better encapsulation of both taxanes. Similarly, Lin et al. demonstrated differences in indomethacin loading in a series of PEG-*b*-polyester micelles, which was ascribed to differences in core hydrophobicity [43]. The slightly lower hydrophobicity of DCTX (as reflected by its lower log *P* value) when compared to PTX may be the reason for the better encapsulation and retention of DCTX in mPEG750-*b*-OCL_5-OH micelles. It may be anticipated that besides differences in hydrophobicity, specific interactions between the drugs and the micellar core play a role. Both taxanes contain several aromatic rings, which may form π-π-interactions with the aromatic rings in the micellar core of the micelles composed of mPEG750-*b*-OCL_5-Bz or -Np. These type of interactions have been suggested to be involved in the encapsulation of another highly aromatic drug, camptothecin, into micelles composed of PEG-*b*-PBLA based block copolymers with different aromatic content. High contents of benzyl or methylnaphthyl in the core-forming polymers resulted in the encapsulation of camptothecin in high yields and high stability, which was ascribed to π–π interactions between the drug and the core-forming polymer [32].

Table 3
Log *P* values of both the taxanes and the three different micellar cores

Drug/micellar core	Log *P*[a]
Paclitaxel (PTX)	4.7
Docetaxel (DCTX)	4.1
Oligo(ε-caprolactone)$_5$ (OCL_5-OH)	5.6
Benzoylated oligo(ε-caprolactone)$_5$ (OCL_5-Bz)	8.5
Naphthoylated oligo(ε-caprolactone)$_5$ (OCL_5-Np)	9.7

[a] Calculated with the ClogP method, based on the additive–constitutive character of partition coefficients [42].

In addition to the nature of the micellar core, its size may affect the solubilisation capacity. Therefore the drug loading efficiency and stability of mPEG750-*b*-OCL_5-Bz micelles as reported above was compared to that of micelles composed of mPEG750-*b*-OCL_4-Bz, i.e., one unit of CL less. At a feed ratio of 10% (w/w) of PTX, it was found that mPEG750-*b*-OCL_4-Bz formed small and almost monodisperse micelles (Z_{ave} 10 nm, PDI 0.02) with nearly complete encapsulation efficiency (85%), similar to the block oligomer with 5 CL units (Fig. 3, Table 1). However, the size of the 10% (w/w) PTX loaded mPEG750-*b*-OCL_4-Bz micelles started to increase at 8 h after preparation, and after 24 h around 60% of the loaded drug was released. In contrast, the micelles containing a block oligomer with 5 CL units retained their small size for more than 24 h, and after 24 h hardly any PTX was released. This suggests that indeed a larger core size has a positive influence on the solubilisation capacity of the micelles.

One of the formulations, mPEG750-*b*-OCL_5-Bz loaded with 10% (w/w) PTX, was investigated by ^{1}H NMR, both as micelles in D_2O and dissolved in $CDCl_3$. The ^{1}H NMR spectrum of mPEG750-*b*-OCL_5-Bz with 10% (w/w) PTX in D_2O is presented in Fig. 4. Interestingly, the D_2O spectrum revealed signals from both the OCL_5-Bz block and the PTX, besides the signals of the PEG-block. This is in contrast to ^{1}H NMR studies with PEG-*b*-PLA, core cross-linked PEG-*b*-PCL, and PEG-*b*-PHPMAmDL micelles in D_2O, where only the PEG signals were visible in the ^{1}H NMR spectrum [26,44–47]. The absence of the signals of the hydrophobic blocks was related to the impaired mobility of these chains, as a result of the formation of a 'solid-like' core [26,44–47]. Our data suggest that the core of PTX-loaded mPEG750-*b*-OCL_5-Bz micelles does not have a 'solid-like', but a viscous nature. This can be explained by the low melting temperature (5 °C) of this block oligomer [36], causing a molten rather than a crystalline state at room temperature. Expansion of the aromatic region (7.0 to 8.4 ppm) of the spectra of mPEG750-*b*-OCL_5-Bz with 10% (w/w) PTX (inset Fig. 4) clearly shows the aromatic signals of the block oligomer end group (7.40, 7.53 and 7.95 ppm) and of PTX. Comparison of the D_2O to the $CDCl_3$ spectra shows a shift of the PTX signals at

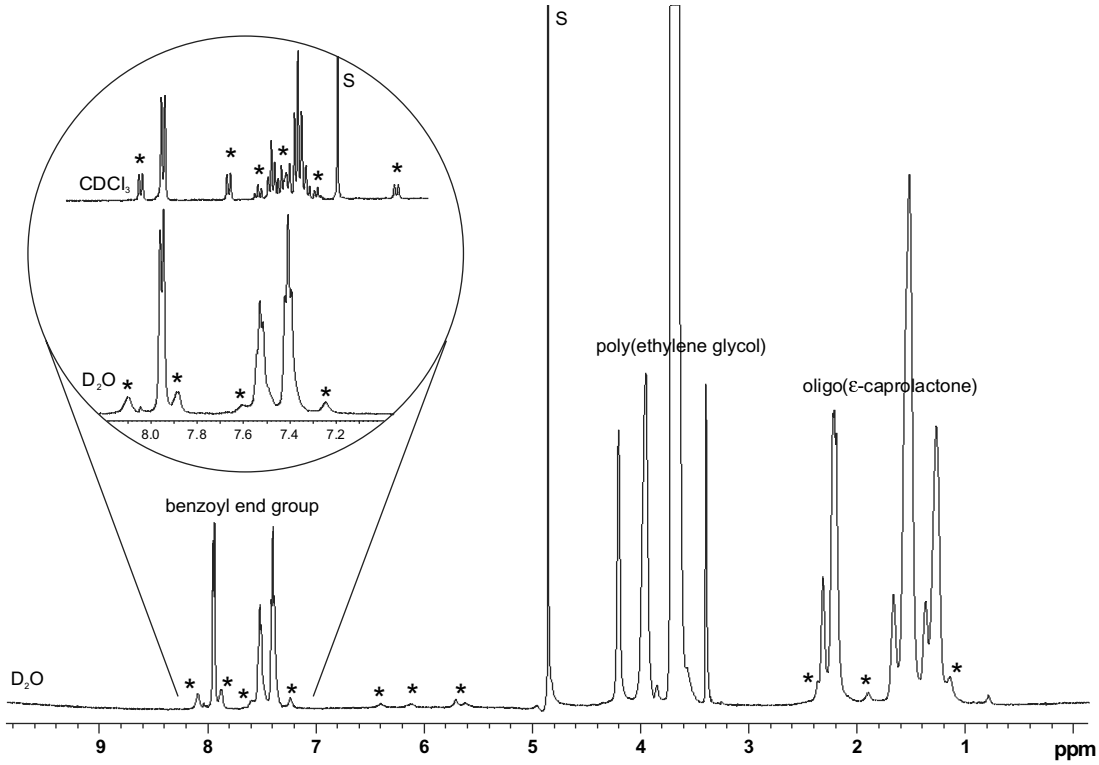

Fig. 4. ^{1}H NMR spectrum of 10% PTX encapsulated in mPEG750-*b*-OLC$_5$-Bz micelles dispersed in D_2O. The aromatic region of the samples dissolved in $CDCl_3$ (top) and dispersed in D_2O (bottom) is expanded. The (small) signals of the PTX-protons are indicated by *; S represents the solvent peak of HDO and $CHCl_3$, respectively.

6.25 and 7.90 ppm (D_2O), which is related to a different chemical environment. Furthermore, peak broadening is observed, which indicates that the mobility of the oligomer chains is restricted in the liquid core of the micelles, compared to chains dissolved in $CDCl_3$. Comparable results were obtained with Pluronics® (PEG-*b*-polypropyleneoxide (PPO)-*b*-PEG) [12] and PEG–PLA micelles above the glass transition temperature of PLA [45]. The presence of PTX signals in the D_2O spectrum suggests that these molecules are present in dissolved, rather than crystallised or aggregated, form. PTX also has a lower mobility than in $CDCl_3$, since it is dissolved in the viscous core, which has been shown for camptothecin and Reichardt's dye upon encapsulation in poly(glycerol succinic acid) dendrimers as well [48,49].

To investigate the molecular interactions in the micellar core, an NOE spectrum in D_2O was recorded and compared to an NOE spectrum in $CDCl_3$. Fig. 5 shows the expansion of the aromatic–aliphatic region in the spectrum in D_2O. The spectrum clearly demonstrates the presence of several cross-peaks between the aromatic protons (from the benzoyl-end group of the oligomer and PTX) and the aliphatic protons (from the OCL chain and the PTX molecule). Specific cross-peaks between the core forming block and the drug can be recognised between the methylene protons of the OCL-chain at 1.28 ppm and the aromatic protons of PTX at 8.11, 7.88 and 7.26 ppm. The recognition of other specific cross-peaks is hampered by

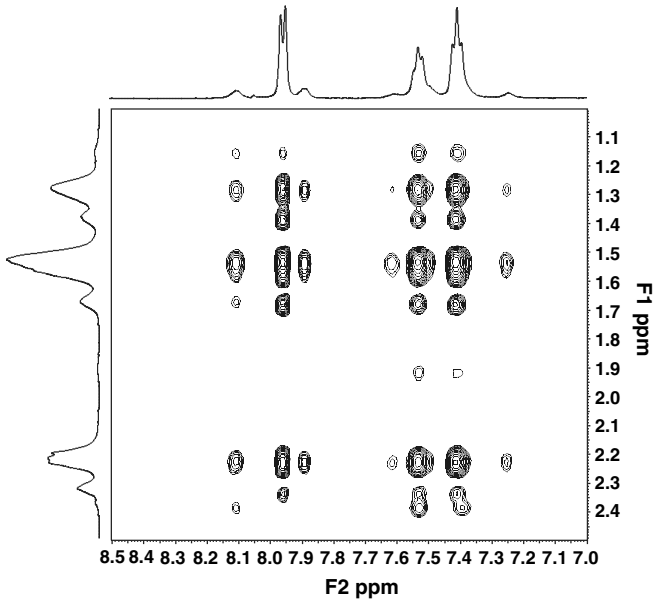

Fig. 5. The aromatic-aliphatic regionof the ^{1}H NMR NOESY of 10% PTX encapsulated in mPEG750-*b*-OLC$_5$-Bz micelles in D_2O ($\tau_m = 300$ ms).

the overlap of the OCL$_5$-Bz and PTX signals. All peaks observed in this region were absent in the $CDCl_3$ NOE spectrum. This indicates that in D_2O these protons are in close proximity [48,50], as a result of the formation of a micellar OCL$_5$-Bz core encapsulating PTX, corroborating

the results described above. NOE signals between the aromatic protons of PTX and the benzoyl end group of the block oligomer could not be detected due to peak overlap.

3.2. In vitro stability

To study their *in vitro* stability, 5% and 10% (w/w) PTX- and DCTX-loaded mPEG750-*b*-OCL_5-Bz micelles were incubated at 37 °C. The samples withdrawn were centrifuged to remove the precipitated taxane, and the concentration of block oligomer and taxane was determined by UPLC®-UV. Fig. 6 demonstrates that during the first hours of incubation the concentration of both the block oligomer and PTX remained constant. At 8 h, the concentration of PTX in the 10% (w/w) sample started to decrease, which was not observed in the 5% (w/w) sample, whereas the oligomer concentration remained constant for both formulations. Correspondingly, the Z_{ave} and PDI of mPEG750-*b*-OCL_5-Bz micelles loaded with 10% PTX started to increase after approximately 4 h of incubation at 37 °C, whereas at 5% (w/w) PTX loading, Z_{ave} and PDI remained constant during at least 15 h (results not shown). Similar results were obtained with DCTX loaded micelles (results not shown). In a subsequent experiment with 10-fold diluted 5% (w/w) loaded micelles, PTX release was also only observed after more than 24 h of incubation (*vide infra*, Fig. 7).

Previously, we demonstrated that empty mPEG750-*b*-OCL_5-Bz micelles are stable for two weeks at 37 °C in PBS [36], and the degradation half-life of these micelles in buffer (pH 7.4) was estimated at several years [51]. The constant oligomer concentration observed here indicates that the micelle stability is not affected by the presence of PTX, but PTX slowly leaks from the micelles at relatively high loading. This PTX leakage likely accounts for the observed increase in size, since PTX has a low solubility in water, and may crystallise into particles larger than the micelles. Eventually PTX precipitated, resulting in the decreased PTX concentration measured at 24 h.

Interaction with serum proteins is important for the *in vivo* fate of drug delivery systems such as micelles. Serum proteins may adsorb onto the micellar surface, thereby inducing opsonisation and subsequent clearance by the reticulo-endothelial system [29,52]. Furthermore, they may accelerate the release of drugs from the micelles, especially in case of drugs with a high protein affinity [27–29], such as PTX, which has a protein binding of 90% in serum [53,54]. Therefore, the effect of BSA on the micellar integrity was investigated. Because it was not possible to separate the intact micelles from soluble BSA, albumin immobilised on agarose beads (BSA-SepCL-4B) was used, which can be easily separated by centrifugation. Such beads have been used to remove a variety of albumin-binding compounds from plasma, such as bilirubin and digitoxin [39]. MPEG750-*b*-OCL_5-Bz micelles loaded with 5% (w/w) PTX were incubated with BSA-SepCL-4B at a molar ratio of PTX/BSA of 1/1.6, which represents an excess of BSA as it is known that albumin can bind more than one PTX molecule [55,56]. As presented in Fig. 7a, again, the oligomer concentration in the micellar dispersion remained constant over time, which suggests that at the experimental conditions, there is no adsorption of micelles or block oligomers to the BSA-coated beads. The absence of BSA adsorption at the micellar surface was also shown for ellipticine-loaded mPEG-*b*-P(5-benzyloxytrimethylene carbonate) (PBTMC) micelles [29], and Pluronics® [57], which may be related to sufficient protection by the PEG-shell of the micelles. Fig. 7b demonstrates that the presence of BSA-SepCL-4B did not affect the PTX release from 5% (w/w) loaded mPEG-*b*-OCL_5-Bz micelles, as both curves coincide. Although others did demonstrate binding of drugs loaded in polymeric micelles to BSA or serum proteins, their binding was always lower than the free drug [27,29]. This indicates that the encapsulation in micelles has a protective effect with regard to protein binding, which is corroborated by the data described here. Taken together, the presented data suggest that albumin neither destabilised the mPEG750-*b*-OCL_5-Bz micelles, nor extracted the PTX from the micellar core. This is an important result with regard to their stability *in vivo*, and encourages future biodistribution studies in an *in vivo* tumour model. Considering their small size, these micelles are expected to be

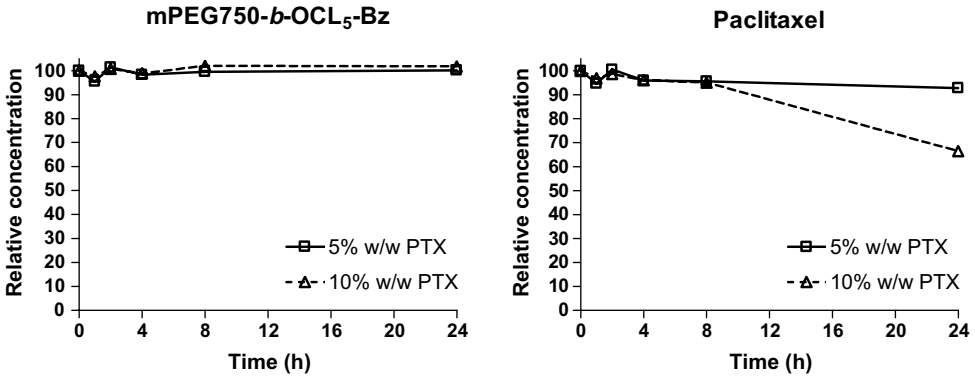

Fig. 6. Concentration of mPEG750-*b*-OCL-Bz (left) and PTX (right) upon incubation of 5% and 10% w/w loaded micelles at 37 °C. The results are presented as percentage of the concentration at time 0. The variation in the values is 10% ($n = 2$).

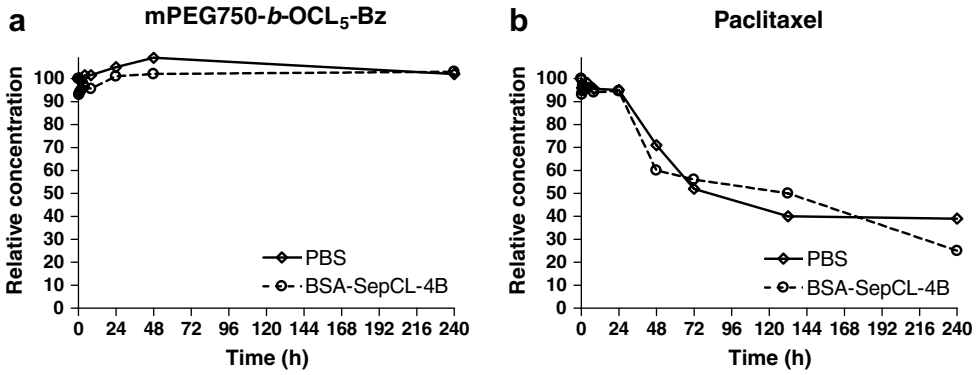

Fig. 7. mPEG750-*b*-OCL-Bz (left) and PTX (right) concentration upon incubation of 5% w/w loaded micelles with BSA immobilised on agarose beads (BSA-SepCL-4B) and in PBS at 37 °C. The results are presented as percentage of the concentration at time 0. The variation in the values is 10% ($n = 2$).

especially suitable for passive targeting of drugs to tumours with a low cut-off size of the vessel wall [58–60].

3.3. In vitro cytotoxicity

The cytotoxic effect of empty and 10% w/w taxane-loaded mPEG750-*b*-OCL_5-Bz micelles on C26 murine colon carcinoma cells was tested, and compared with the commercially available formulations Taxol® and Taxotere®. Table 4 shows that the cytotoxicity of the PTX- and DCTX-loaded micelles is comparable to that of Taxol® and Taxotere®, respectively. Importantly, the empty micelles are far less toxic than the CrEl®/EtOH 1/1 (v/v) used in the Taxol® formulation, as reflected by the higher cell viability upon incubation with the empty vehicles (Table 4). Moreover, neither the empty mPEG750-*b*-OCL_5-Bz micelles nor Tw80 (used in the Taxotere® formulation) showed any cytotoxicity at the concentrations tested. Comparison of the cell viability after incubation with the loaded vehicles and the empty ones (Table 4), reveals that the cytotoxicity of Taxol® at 10 μg/mL PTX may be (partly) due to CrEL®/EtOH, whereas the effect of the loaded micelles and Taxotere® is solely caused by the drug. The results are in line with those obtained elsewhere with other PTX-loaded micelles, which were tested on C26 and other cancer cell lines [22–24]. The cytotoxicity of other DCTX-loaded micelles, which was similar to that of Taxotere®, has been demonstrated in literature as well [25]. The absence of vehicle-related cytotoxicity of Taxotere® may be explained by the better toxicity profile of Tw80, when compared to empty Taxol®, combined with the presence of a lower amount of excipient in the formulation (only 25/1 (w/w) Tw80/DCTX vs 88/1 (w/w) CrEl®/PTX in Taxol®). However, since some of the clinically observed side effects of Taxotere® have been ascribed to Tw80, rather than DCTX [2,4,5], a Tw80-free formulation may still be preferred, which can be obtained by using the biodegradable and biocompatible block oligomers described here. It may be anticipated that the low toxicity of the micelles allows higher dosing than Taxol® (and possibly Taxotere®), thereby improving the anti-tumour effect as observed with other PTX-loaded micelles [15,19,22].

At the reported IC_{50} values of 0.03 and 0.01 μg taxane/mL for 10% (w/w) PTX- and DCTX-loaded oligomeric micelles, the block oligomer concentrations are 0.3 and 0.1 μg oligomer/mL, respectively. It is important to note that these values are below the critical aggregation concentration (CAC) of the block oligomers (10 μg/mL) [36], and therefore the taxanes are likely present in their free form. Calculations based on the administered dose of Taxol® and Taxotere® in patients and their plasma volume revealed a taxane concentration around 100 and 50 μg/mL in plasma, respectively. In this study, at a taxane concentration of 10 μg/mL, where the mPEG750-*b*-OCL_5-Bz concentration (100 μg/mL) is above the CAC, the taxane-loaded micelles have a cytotoxicity of 70%, which is comparable to the toxicity of the commercial formulations at the same

Table 4
In vitro cytotoxic effect of taxane-loaded oligomeric micelles and Taxol®/Taxotere® on C26 colon carcinoma cells after 3 days of incubation

	IC_{50} (μg taxane/mL)[a]	Cell viability at 10 μg/mL taxane (% of PBS control)	Cell viability empty vehicle[b] (% of PBS control)
Taxol® (PTX)	0.02 ± 0.02	38 ± 5%	42 ± 4%
Taxotere® (DCTX)	0.01 ± 0.02	30 ± 2%	96 ± 10%
PTX-loaded mPEG750-*b*-OCL_5-Bz micelles	0.03 ± 0.01	31 ± 2%	87 ± 17%
DCTX-loaded mPEG750-*b*-OCL_5-Bz micelles	0.01 ± 0.01	29 ± 1%	87 ± 17%

Data are presented as mean ± SD ($n = 3$).
[a] IC_{50}: concentration (μg/mL) of taxane-loaded formulation that causes 50% cell death or growth inhibition.
[b] The concentration of the excipients (CrEL/EtOH, Tw80, block oligomer) is the same as in the loaded formulations at 10 μg/mL of taxane.

drug concentrations (around 60% and 70% for Taxol® and Taxotere®, respectively; Table 4). At this concentration, the taxanes may exert their intracellular effect after uptake of the loaded micelles or after extracellular release. The mechanism responsible for the cytotoxic effect will be subject of future studies.

4. Conclusions

In this study, both paclitaxel and docetaxel were successfully loaded in small (<20 nm) oligomeric micelles based on mPEG750-*b*-oligo(ε-caprolactone)$_5$. It was shown that the presence of an aromatic end group on the core-forming block is necessary to improve the loading and enhance the stability of the formulation of taxanes, indicating the importance of a good compatibility between the loaded drug and the micellar core. The micellar core has a liquid nature, in which paclitaxel is present in a dissolved state. *In vitro*, the release of paclitaxel occurs through leakage from the micelles, which was not affected by the presence of albumin. This, in combination with the observed low cytotoxic effect of the empty micelles on C26 cells demonstrates the feasibility of a novel taxane formulation based on mPEG-*b*-oligo(ε-caprolactone) micelles.

Acknowledgements

The authors gratefully acknowledge M.C. Lok for his assistance in the cell culture experiments. This research was financially supported by OctoPlus N.V., Leiden, The Netherlands.

References

[1] K. Gelmon, The taxoids: paclitaxel and docetaxel, Lancet 344 (1994) 1267–1272.
[2] K.L. Hennenfent, R. Govindan, Novel formulations of taxanes: a review. Old wine in a new bottle? Ann. Oncol. 17 (2006) 735–749.
[3] L. He, G.A. Orr, S.B. Horwitz, Novel molecules that interact with microtubules and have functional activity similar to Taxol, Drug Discov. Today 6 (2001) 1153–1164.
[4] L. van Zuylen, J. Verweij, A. Sparreboom, Role of formulation vehicles in taxane pharmacology, Invest. New Drug 19 (2001) 125–141.
[5] F.K. Engels, R.A. Mathot, J. Verweij, Alternative drug formulations of docetaxel: a review, Anticancer Drug 18 (2007) 95–103.
[6] Z. Xie, H. Guan, X. Chen, C. Lu, L. Chen, X. Hu, Q. Shi, X. Jing, A novel polymer-paclitaxel conjugate based on amphiphilic triblock copolymer, J. Control. Release 117 (2007) 210–216.
[7] S.S. Ng, A. Sparreboom, Y. Shaked, C. Lee, S. Man, N. Desai, P. Soon-Shiong, W.D. Figg, R.S. Kerbel, Influence of formulation vehicle on metronomic taxane chemotherapy: albumin-bound versus cremophor EL-based paclitaxel, Clin. Cancer Res. 12 (2006) 4331–4338.
[8] V.P. Torchilin, Structure and design of polymeric surfactant-based drug delivery systems, J. Control. Release 73 (2001) 137–172.
[9] G. Gaucher, M.H. Dufresne, V.P. Sant, N. Kang, D. Maysinger, J.C. Leroux, Block copolymer micelles: preparation, characterization and application in drug delivery, J. Control. Release 109 (2005) 169–188.
[10] G.S. Kwon, T. Okano, Polymeric micelles as new drug carriers, Adv. Drug Deliv. Rev. 21 (1996) 107–116.
[11] M.-C. Jones, J.-C. Leroux, Polymeric micelles – a new generation of colloidal drug carriers, Eur. J. Pharm. Biopharm. 48 (1999) 101–111.
[12] G.S. Kwon, Polymeric micelles for delivery of poorly water-soluble compounds, Crit. Rev. Ther. Drug 20 (2003) 357–403.
[13] V.P. Torchilin, Micellar nanocarriers: pharmaceutical perspectives, Pharm. Res. 24 (2007) 1–16.
[14] C.F. van Nostrum, Polymeric micelles to deliver photosensitizers for photodynamic therapy, Adv. Drug Deliv. Rev. 56 (2004) 9–16.
[15] R.T. Liggins, H.M. Burt, Polyether–polyester diblock copolymers for the preparation of paclitaxel loaded polymeric micelle formulations, Adv. Drug Deliv. Rev. 54 (2002) 191–202.
[16] T.Y. Kim, D.W. Kim, J.Y. Chung, S.G. Shin, S.C. Kim, D.S. Heo, N.K. Kim, Y.J. Bang, Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clin. Cancer Res. 10 (2004) 3708–3716.
[17] X. Zhang, H.M. Burt, D.V. Hoff, D. Dexter, G. Mangold, D. Degen, A.M. Oktaba, W.L. Hunter, An investigation of the antitumour activity and biodistribution of polymeric micellar paclitaxel, Cancer Chemother. Pharm. 40 (1997) 81–86.
[18] X. Zhang, J.K. Jackson, H.M. Burt, Development of amphiphilic diblock copolymers as micellar carriers of taxol, Int. J. Pharm. 132 (1996) 195–206.
[19] S.C. Kim, D.W. Kim, Y.H. Shim, J.S. Bang, H.S. Oh, S. Wan Kim, M.H. Seo, *In vivo* evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy, J. Control. Release 72 (2001) 191–202.
[20] X. Zhang, H.M. Burt, G. Mangold, D. Dexter, D.V. Hoff, L. Mayer, W.L. Hunter, Anti-tumor efficacy and biodistribution of intravenous polymeric micellar paclitaxel, Anti-Cancer Drug 8 (1997) 696–701.
[21] T. Hamaguchi, Y. Matsumura, M. Suzuki, K. Shimizu, R. Goda, I. Nakamura, I. Nakatomi, M. Yokoyama, K. Kataoka, T. Kakizoe, NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend *in vivo* antitumour activity and reduce the neurotoxicity of paclitaxel, Brit. J. Cancer 92 (2005) 1240–1246.
[22] D. Le Garrec, S. Gori, L. Luo, D. Lessard, D.C. Smith, M.A. Yessine, M. Ranger, J.C. Leroux, Poly(*N*-vinylpyrrolidone)-block-poly(D,L-lactide) as a new polymeric solubilizer for hydrophobic anticancer drugs: *in vitro* and *in vivo* evaluation, J. Control. Release 99 (2004) 83–101.
[23] O. Soga, C.F. van Nostrum, M. Fens, C.J.F. Rijcken, R.M. Schiffelers, G. Storm, W.E. Hennink, Thermosensitive and biodegradable polymeric micelles for paclitaxel delivery, J. Control. Release 103 (2005) 341–353.
[24] S.C. Lee, C. Kim, I.C. Kwon, H. Chung, S.Y. Jeong, Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(e-caprolactone) copolymer as a carrier for paclitaxel, J. Control. Release 89 (2003) 437–446.
[25] M. Elsabahy, M.E. Perron, N. Bertrand, G. Yu, J.C. Leroux, Solubilization of docetaxel in poly(ethylene oxide)-block-poly(butylene/styrene oxide) micelles, Biomacromolecules (2007) 2250–2257.
[26] X. Shuai, T. Merdan, A.K. Schaper, F. Xi, T. Kissel, Core-cross-linked polymeric micelles as paclitaxel carriers, Bioconjugate Chem. 15 (2004) 441–448.
[27] G.S. Kwon, M. Naito, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Physical entrapment of adriamycin in AB block copolymer micelles, Pharm. Res. 12 (1995) 192–195.
[28] P. Opanasopit, M. Yokoyama, M. Watanabe, K. Kawano, Y. Maitani, T. Okano, Influence of serum and albumins from different species on stability of camptothecin-loaded micelles, J. Control. Release 104 (2005) 313–321.
[29] J. Liu, F. Zeng, C. Allen, Influence of serum protein on polycarbonate-based copolymer micelles as a delivery system for a hydrophobic anti-cancer agent, J. Control. Release 103 (2005) 481–497.
[30] H.M. Burt, X. Zhang, P. Toleikis, L. Embree, W.L. Hunter, Development of copolymers of poly(D,L-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel, Coll. Surf. B 16 (1999) 161–171.

[31] J. Liu, Y. Xiao, C. Allen, Polymer-drug compatibility: a guide to the development of delivery systems for the anticancer agent, ellipticine, J. Pharm. Sci. 93 (2004) 132–143.
[32] M. Yokoyama, P. Opanasopit, T. Okano, K. Kawano, Y. Maitani, Polymer design and incorporation methods for polymeric micelle carrier system containing water-insoluble anti-cancer agent camptothecin, J. Drug Target. 12 (2004) 373–384.
[33] M. Watanabe, K. Kawano, M. Yokoyama, P. Opanasopit, T. Okano, Y. Maitani, Preparation of camptothecin-loaded polymeric micelles and evaluation of their incorporation and circulation stability, Int. J. Pharm. 308 (2006) 183–189.
[34] M.L. Forrest, A. Zhao, C.Y. Won, A.W. Malick, G.S. Kwon, Lipophilic prodrugs of Hsp90 inhibitor geldanamycin for nanoencapsulation in poly(ethylene glycol)-*b*-poly(epsilon-caprolactone) micelles, J. Control. Release 116 (2006) 139–149.
[35] A. Lavasanifar, J. Samuel, G.S. Kwon, The effect of fatty acid substitution on the *in vitro* release of amphotericin B from micelles composed of poly(ethylene oxide)-block-poly(*N*-hexyl stearate–aspartamide), J. Control. Release 79 (2002) 165–172.
[36] M.G. Carstens, J.J.L. Bevernage, C.F. van Nostrum, M.J. van Steenbergen, F.M. Flesch, R. Verrijk, L.G.J. de Leede, D.J.A. Crommelin, W.E. Hennink, Small oligomeric micelles based on end group modified mPEG-oligocaprolactone with monodisperse hydrophobic blocks, Macromolecules 40 (2007) 116–122.
[37] B.M. Rao, A. Chakraborty, M.K. Srinivasu, M.L. Devi, P.R. Kumar, K.B. Chandrasekhar, A.K. Srinivasan, A.S. Prasad, J. Ramanatham, A stability-indicating HPLC assay method for docetaxel, J. Pharm. Biomed. 41 (2006) 676–681.
[38] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A. Bax, NMRPipe: a multidimensional spectral processing system based on UNIX pipes, J. Biomol. NMR 6 (1995) 277–293.
[39] M. Wilchek, T. Miron, Immobilization of enzymes and affinity ligands onto agarose via stable and uncharged carbamate linkage, Biochem. Int. 4 (1982) 629–635.
[40] Sanofi-Aventis U.S. LLC. Taxotere (docetaxel) Injection Concentrate. Preparation and Administration. July 2005 [cited May 4. 2007]; Available from: <http://www.taxotere.com/professional/about/preparation.do>.
[41] D.A. Scudiero, R.H. Shoemaker, K.D. Paull, A. Monks, S. Tierney, T.H. Nofziger, M.J. Currens, D. Seniff, M.R. Boyd, Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines, Cancer Res. 48 (1988) 4827–4833.
[42] Daylight Chemical Information Systems Inc. CLogP. [cited June 4. 2007]; Available from: <http://www.daylight.com/daycgi/clogp>.
[43] W.J. Lin, L.W. Juang, C.C. Lin, Stability and release performance of a series of pegylated copolymeric micelles, Pharm. Res. 20 (2003) 668–673.
[44] J.S. Hrkach, M.T. Peracchia, A. Bomb, N. Lotan, R. Langer, Nanotechnology for biomaterials engineering: structural characterization of amphiphilic polymeric nanoparticles by 1H NMR spectroscopy, Biomaterials 18 (1997) 27–30.
[45] C.R. Heald, S. Stolnik, K.S. Kujawinski, C. De Matteis, M.C. Garnett, L. Illum, S.S. Davis, S.C. Purkiss, R.J. Barlow, P.R. Gellert, Poly(lactic acid)–poly(ethylene oxide) (PLA–PEG) nanoparticles: NMR studies of the central solid like PLA core and the liquid PEG corona, Langmuir 18 (2002) 3669–3675.
[46] Y. Yamamoto, K. Yasugi, A. Harada, Y. Nagasaki, K. Kataoka, Temperature-related change in the properties relevant to drug delivery of poly(ethylene glycol)–poly(D,L-lactide) block copolymer micelles in aqueous milieu, J. Control. Release 82 (2002) 359–371.
[47] O. Soga, C.F. van Nostrum, A. Ramzi, T. Visser, F. Soulimani, P.M. Frederik, P.H.H. Bomans, W.E. Hennink, Physicochemical characterization of degradable thermosensitive polymeric micelles, Langmuir 20 (2004) 9388–9395.
[48] M.T. Morgan, M.A. Carnahan, C.E. Immoos, A.A. Ribeiro, S. Finkelstein, S.J. Lee, M.W. Grinstaff, Dendritic molecular capsules for hydrophobic compounds, J. Am. Chem. Soc. 125 (2003) 15485–15489.
[49] M.T. Morgan, Y. Nakanishi, D.J. Kroll, A.P. Griset, M.A. Carnahan, M. Wathier, N.H. Oberlies, G. Manikumar, M.C. Wani, M.W. Grinstaff, Dendrimer-encapsulated camptothecins: Increased solubility, cellular uptake, and cellular retention affords enhanced anticancer activity *in vitro*, Cancer Res. 66 (2006) 11913–11921.
[50] R. Vakil, G.S. Kwon, Poly(ethylene glycol)-*b*-poly(e-caprolactone) and PEG-phospholipid form stable mixed micelles in aqueous media, Langmuir 22 (2006) 9723–9729.
[51] M.G. Carstens, C.F. van Nostrum, R. Verrijk, L.G.J. de Leede, D.J.A. Crommelin, W.E. Hennink, A mechanistic study of the chemical and enzymatic degradation of PEG-oligo(e-caprolactone) micelles, J. Pharm. Sci., in press, doi:10.1002/jps.21092.
[52] H.M. Patel, S.M. Moghimi, Serum-mediated recognition of liposomes by phagocytic cells of the reticuloendothelial system – the concept of tissue specificity, Adv. Drug Deliv. Rev. 32 (1998) 45–60.
[53] D.S. Sonnichsen, M.V. Relling, Clinical pharmacokinetics of paclitaxel, Clin. Pharm. 27 (1994) 256–269.
[54] G.N. Kumar, U.K. Walle, K.N. Bhalla, T. Walle, Binding of taxol to human plasma, albumin and alpha 1-acid glycoprotein, Res. Commun. Chem. Pathol. 80 (1993) 337–344.
[55] M. Purcell, J.F. Neault, H.A. Tajmir-Riahi, Interaction of taxol with human serum albumin, Biochim. Biophys. Acta 1478 (2000) 61–68.
[56] K. Paal, J. Muller, L. Hegedus, High affinity binding of paclitaxel to human serum albumin, Eur. J. Biochem. 268 (2001) 2187–2191.
[57] A.V. Kabanov, I.R. Nazarova, I.V. Astafieva, E.V. Batrakova, V.Y. Alakhov, A.A. Yaroslavov, V.A. Kabanov, Micelle formation and solubilization of fluorescent probes in poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene) solutions, Macromolecules 28 (1995) 2303–2314.
[58] S.K. Hobbs, W.L. Monsky, F. Yuan, W.G. Roberts, L. Griffith, V.P. Torchilin, R.K. Jain, Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment, Proc. Natl. Acad. Sci. USA 95 (1998) 4607–4612.
[59] V. Weissig, K.R. Whiteman, V.P. Torchilin, Accumulation of protein-loaded long-circulating micelles and liposomes in subcutaneous lewis lung carcinoma in mice, Pharm. Res. V15 (1998) 1552–1556.
[60] N. Nishiyama, K. Kataoka, Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery, Pharmacol. Therapeut. 112 (2006) 630–648.