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Research paper

# Polymeric Micelles for parenteral delivery of Sagopilone: Physicochemical characterization, novel formulation approaches and their toxicity assessment *in vitro* as well as *in vivo*

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ABSTRACT

*Purpose:* The block copolymers $PEG_{2000}$-*b*-$PLA_{2200}$, $PEG_{2000}$-*b*-$PCL_{2600}$ and $PEG_{5000}$-*b*-$PCL_{5000}$ have been currently identified as optimal solubilizing agents for Sagopilone, a poorly water-soluble anticancer drug. In the present study, the stability, formulation feasibility and *in vitro* as well as *in vivo* toxicity were evaluated.

*Methods:* Dispersion media, storage conditions, and dilutions were varied for stability assessment. The critical micelle concentration (CMC) was determined using a fluorescent probe technique. Lyophilizates and polymeric films were investigated as formulation options. Furthermore, the toxicity was studied *in vitro* and *in vivo* using HeLa/MaTu cells and a nude mouse model, respectively.

*Results:* A drug–polymer ratio as low as 1:20 (w/w) was sufficient to solubilize Sagopilone effectively and to obtain stable dispersions (24 h: drug content $\geqslant$95%). Although the micelles exhibited a similar thermodynamic stability (CMC: $10^{-7}$–$10^{-6}$ M), PEG-*b*-PCL micelles were kinetically more stable than $PEG_{2000}$-*b*-$PLA_{2200}$ (24 h at 37 °C: drug content $\geqslant$90% compared to 30%, respectively). Lyophilization of PEG-*b*-PCL micelles and storage stability of solid drug-loaded $PEG_{2000}$-*b*-$PLA_{2200}$ films (3 m, 6 °C: drug content of (95.6 ± 1.4)%) were demonstrated for the first time. The high antiproliferative activity has been maintained *in vitro* ($IC_{50}$<1 nM). Carrier-associated side effects have not been observed *in vivo* and the maximum tolerated dose of micellar Sagopilone was determined to be 6 mg/kg.

*Conclusion:* The results of this study indicate that polymeric micelles, especially PEG-*b*-PCL micelles, offer excellent potential for further preclinical and clinical cancer studies using Sagopilone.



## 1. Introduction

Solubilization represents one of the major challenges in formulation development nowadays since approximately 40% of the new compounds in drug discovery are poorly water-soluble [1]. This is of particular concern in the parenteral delivery field because the number of approved excipients is limited. Furthermore, currently used solubilizers such as Cremophor®EL have been implicated in clinically important adverse effects and unfavourable alterations of the pharmacokinetics of drugs as shown for paclitaxel [2].

Sagopilone (Fig. 1) is a novel, poorly water-soluble anticancer drug belonging to the group of epothilones that is administered parenterally [3,4]. The epothilones present a novel class of microtubule-stabilizing anticancer drugs originally occurring in *Sorangium cellulosum*. Their mechanism of action is similar to paclitaxel, but they exhibit superior features relative to the latter. Besides their activity against various tumour types, they show low susceptibility to key tumour resistance mechanisms *in vitro*, and most importantly, *in vivo* [5]. Thus, they are effective in tumours resistant to paclitaxel making them very likely to become successors to taxane therapy. Sagopilone (Fig. 1) is a synthetic epothilone derivative, which is currently under clinical trial evaluation [6]. Dosing of Sagopilone is limited due to the occurrence of peripheral neuropathy. This is a typical side effect of epothilones, which recently gave reason to the refusal of the marketing authorisation for the epothilone derivative Ixabepilone by the European Medicines Agency (EMEA) [7]. The agency concluded that the benefits in the treatment of breast cancer with Ixabepilone did not outweigh its risks due to neuropathy.

Thus, an optimal delivery system for this class of anticancer drugs requires (a) solubilization of the drug, (b) accumulation of

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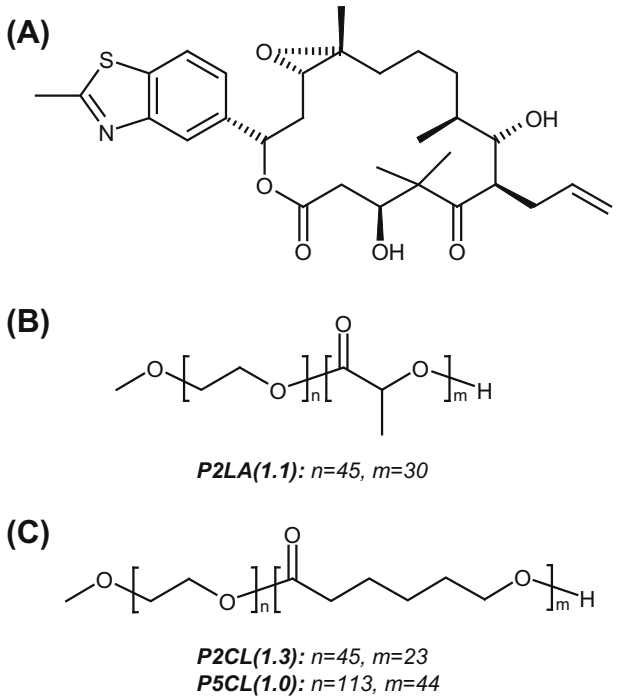


**Fig. 1.** Structural formula of (A) Sagopilone, (B) PEG-*b*-PLA and (C) PEG-*b*-PCL.

the drug at the tumour site due to enhanced permeation and retention (EPR-effect) [8,9], and (c) reduction of drug-related adverse effects at non-tumour sites. Among several approaches, polymeric micelles offer great potential to meet these demands [10–13] with regard to solubilization [1,14–16], vehicle safety after administration [17,18] and passive tumour targeting [19,20].

Until now, numerous publications have described various polymeric micellar systems with respect to solubilization and *in vivo* performance using different drugs and various animal models. For this reason, the results are difficult to compare. In our previous study, amphiphilic block copolymers composed of poly(ethylene glycol) (PEG) and a biodegradable polyester block of poly(lactide) (PEG-*b*-PLA) or poly(ε-caprolactone) (PEG-*b*-PCL) were investigated with regard to the solubilization of Sagopilone for parenteral delivery [21]. As a result, three polymers along with the appropriate method of preparation were selected as optimal solubilizing agents. The polymers used were: $PEG_{2000}$-*b*-$PLA_{2200}$, $PEG_{2000}$-*b*-$PCL_{2600}$ and $PEG_{5000}$-*b*-$PCL_{5000}$ (Fig. 1) abbreviated as P2LA(1.1), P2CL(1.3) and P5CL(1.0), respectively, in which the number in parentheses details the hydrophobic/hydrophilic ratio (w/w) of the block copolymer.

A critical point for formulation development is the stability of polymeric micelles [22]. They have to be stable both prior to clinical application and after intravenous (i.v.) administration, since intact micelles are considered an important prerequisite for passive tumour targeting. The stability of polymeric micelles is often considered sufficient in general due to their low critical micelle concentration (CMC) values. However, this view disregards the kinetic stability, which may exhibit serious differences depending on the nature and state of the micellar core [1], especially important in the field of drug delivery. Thus, the selection of a core-forming block providing a high degree of kinetic stability in conjunction with a slow rate of disassembly is described as a strategy for the preparation of micelles that stay intact until reaching the tumour site [23] besides other approaches such as core-crosslinking [24] or the chemical modification of the core-forming block [25,26]. Examining a set of PEG-*b*-PCL polymers, Liu et al. showed superior *in vitro* as well as *in vivo* stability of P5CL(1.0) [23]. A significant portion of the copolymer remained assembled as intact micelles even 24 h after administration of thermodynamically unstable micelles (2 mg/kg body weight) that would likely fall to concentrations below the CMC following distribution [23]. In the present work, a comparative study of the physicochemical stability of PCL- and PLA-containing micelles was performed, assuming that PCL-containing cores exhibit a higher stability due to their nature (higher hydrophobicity) and state (semi-crystalline) compared to amorphous poly(D,L-lactide).

In addition, the applicability of polymeric micelles to clinical development requires stable formulations with sufficient shelf-life. Since the polymers used are sensitive to hydrolytic degradation, aqueous dispersions of the micelles are not suitable for ready-to-use formulations. This issue has been rarely addressed, especially for PEG-*b*-PCL micelles. With regard to the semi-crystalline nature of PCL, potential aggregation has to be taken into account during freeze-drying. With this in mind, the feasibility of lyophilization was studied using different conditions to prevent crystallization of PCL and provide a storable formulation of PEG-*b*-PCL micelles. As an alternative to lyophilization, solid drug-loaded polymeric films of PEG-*b*-PLA were investigated as a novel approach for stabilizing parenteral formulations.

Following the physicochemical and formulation studies, the *in vitro* as well as the preclinical *in vivo* toxicity has been studied to determine the safety profile of the carriers and the maximum tolerated dose (MTD) of the drug-loaded micelles for future *in vivo* tumour efficacy studies.

## 2. Materials and methods

### 2.1. Materials

Sagopilone was obtained from Bayer Schering Pharma AG (Berlin, Germany). The block copolymers poly(ethylene glycol)-*b*-poly(ε-caprolactone), namely $PEG_{2000}$-*b*-$PCL_{2600}$ and $PEG_{5000}$-*b*-$PCL_{5000}$ (abbr.: P2CL(1.3) and P5CL(1.0), respectively), and the poly(ethylene glycol)-*b*-poly(D,L-lactide) $PEG_{2000}$-*b*-$PLA_{2200}$ (abbr.: P2LA(1.1)) were purchased from Polymer Source Inc. (Dorval, Canada). Pyrene, sucrose, trehalose and mannitol were obtained from Merck KGaA (Darmstadt, Germany). Hydroxypropyl-β-cyclodextrin (abbr.: HPβCD) was purchased from Roquette (Lestrem, Fance). Polyvinylpyrrolidone (abbr.: PVP, Kollidon® 17PF, $M_r$ = 7000–11,000 g/mol) was purchased from BASF (Ludwigshafen, Germany). All other ingredients were obtained in analytical quality.

### 2.2. Micelle preparation and drug loading

Loading of Sagopilone within block copolymer micelles was done by the appropriate method of preparation as described previously [21]. In brief, sonication was used to prepare PEG-*b*-PCL micelles by simply weighing the polymer and Sagopilone, adding phosphate buffer (0.05 M, pH 7.4) and sonication for 10 min. Micelles composed of the PEG-*b*-PLA polymer P2LA(1.1) were prepared by a film formation method. The polymer and the drug were dissolved in acetonitrile, and the organic solvent was evaporated under reduced pressure at room temperature with subsequent drying at 0.1 mbar for 1 h. Micelle formation took place upon redispersion of the resulting film with phosphate buffer (0.05 M, pH 7.4) while shaking without additional heating or sonication. Unloaded micelles and blanks were prepared according to the same procedures in the absence of Sagopilone or the polymer, respectively. The resulting dispersions were sterilized by filtration through 0.22-μm syringe filters (Millex®-GV 0.22 μm, Millipore, USA).

### 2.3. Determination of drug content and micelle size

The final Sagopilone concentration present in the micelles was determined by reversed-phase high performance liquid chromatography (RP-HPLC) using two Chromolith® Performance RP-18e columns (100 × 4.6 mm, Merck, Germany) and an Agilent 1100 Series chromatography system (quaternary pump, auto-injector, column heater at 25 °C and UV-detector) from Agilent Technologies (Santa Clara, USA). The method used has been described in detail previously [21].

The solubilization efficiency (SE) and the loading (% and mol/mol) of Sagopilone were calculated according to Eq. (1)–(3), respectively.

$$\text{SE}\ (\%) = \frac{\text{mass of Sagopilone-loaded in mg}}{\text{mass of Sagopilone fed in mg}} \times 100\% \quad (1)$$

$$\text{Loading}\ (\%) \frac{m_{\text{Sagopilone}} \text{in mg}}{m_{\text{polymer}} \text{in mg}} \times 100\% \quad (2)$$

$$\text{Loading}\ (\text{mol/mol}) \frac{m_{\text{Sagopilone}} \text{in mol}}{m_{\text{polymer}} \text{in mol}} \quad (3)$$

The micelle sizes and size distributions were measured by Dynamic Light Scattering (DLS) at a scattering angle of 173° using a Zetasizer Nano (Malvern Instruments Ltd., Worcestershire, UK) with a temperature controller set at 25 °C. Autocorrelation functions were calculated and analyzed using the DTS v5.1 software provided by Malvern. Measurements were done in triplicate with 15–20 runs each, and the calculated mean values of the hydrodynamic diameter ($d_H$) and the size distribution (PDI: polydispersity index) were used.

### 2.4. Determination of critical micelle concentration

The critical micelle concentration (CMC) of the amphiphilic block copolymers was determined by a fluorescent dye assay as reported previously [15]. In brief, excitation spectra of pyrene were obtained at a constant pyrene concentration of $6 \times 10^{-7}$ M in the presence of amphiphilic block copolymers at concentrations ranging from $1 \times 10^{-5}$ to 10 g/L using a Spex Fluorolog-2 spectrofluorometer (Horiba Jobin Yvon, Edison, NJ). The ratio of the intensity at 338–333 nm was plotted against the concentration of the block copolymer on a logarithmic scale to determine the CMC.

### 2.5. Lyophilization

Different lyoprotectants were added to the micellar dispersions ($c$(polymer) = 20 g/L) at varying polymer–lyoprotectant weight ratios ranging from 1:0 to 1:20. Two millilitres of the dispersions were filled in 6R-glass vials fitted with 13-mm lyophilization stoppers. The samples were frozen by either immersion in liquid nitrogen (−196 °C) or at −45 °C over 4.5 h. They were lyophilized for 56.5 h at 0.09–0.01 mbar in a Genesis Super XL (VirTis, USA) with a condenser temperature of −60 °C. The resulting lyophilizates were redispersed by adding 2 mL water and subsequent shaking. When redispersion was complete, the drug content and the micellar characteristics were determined.

### 2.6. X-ray powder diffraction (XRPD)

Data collection was carried out in transmission mode on the automated STOE Powder Diffractometer STADI P using germanium-monochromatized Cu $K\alpha_1$-radiation (=1.5406 Å). The X-ray tube with copper anode was operated at 40 kV and 30 mA. The $2\Theta$ scans were performed using the small linear position sensitive detector with an angular resolution of 0.08° between $12° \leqslant 2\Theta \leqslant 23°$ (step width 0.1°). The samples were enclosed between two polyacetate films. Data acquisition and evaluation were performed using Version 2.07 of the STOE WinX$^{pow}$ software package.

### 2.7. In vitro cytotoxicity

For the *in vitro* cytotoxicity study, the human cervix carcinoma cell line HeLa/MaTu (Epo GmbH Berlin) was used. The cytotoxic activity was evaluated at five dilutions ranging from $10^{-6}$ to 200 μM Sagopilone using the crystal violet assay according to the standard method [27]. In brief, cells were harvested from exponential phase cultures growing in DMEM/HAMS F12 (Biochrom AG) medium supplemented with 2 mM L-Glutamine and 10% fetal calf serum, counted and seeded onto 96-well plates with a density of 3000 cells per well. After a 24-h recovery at 37 °C in a humidified atmosphere with 5% $CO_2$, the cells were incubated with 200 μL of medium containing free Sagopilone or Sagopilone-loaded micelles. Each sample and concentration step was plated in octuplicate. Untreated (medium) and positive controls (paclitaxel) were included as well. Following 4 days of exposure, the cells were treated with glutaraldehyde solution (10%) for 15 min and washed three times. Afterwards, the viable cells were stained with crystal violet for 20 min, which was detected at 595 nm using a Tecan Sunrise Microplate reader after the addition of 10% acetic acid. As a result, the inhibitory concentration $IC_{50}$, which is the concentration of Sagopilone producing 50% inhibition of cell proliferation, was determined as a mean from three independent experiments.

### 2.8. In vivo toxicity

*In vivo* tolerability studies were performed in healthy female, adult NMRI: nu/nu mice (6–8 weeks of age, lack of mature T-lymphocytes, Taconic, 4623 Lille Skensved, Denmark). To determine the acute tolerability of the carriers, unloaded micellar dispersions were administered intravenously at a dose of 200 mg/kg to two animals per carrier type. Mice were monitored daily for acute reactions and variation in body weight over 1 week. In the absence of toxic effects of the carriers, the maximum tolerated doses (MTD) of the drug-loaded dispersions were determined according to OECD guideline No. 425 for one drug. Therefore, groups of adult NMRI: nu/nu mice (female, 33.5 ± 2 g) with three animals per group received slow i.v. bolus injections of Sagopilone-loaded micellar dispersions (application volume: 0.2 mL per 20 g mouse body weight) at a dose of 6, 8 and 10 mg/kg. Mice were inspected daily for treatment-related toxicity. The body weight was determined daily, and changes in the body weight served as a parameter of toxicity. The MTD was defined as the dose where the median body weight loss does not exceed 15% nor leads to remarkable changes in general behaviour or to death due to toxic side effects within 2 weeks after administration. Animals showing weight loss exceeding 20% were sacrificed.

All animal experiments were conducted in accordance with Recommendations from the Declaration of Helsinki, the UKCCCR regulations for the welfare of animals and the German animal protection law, in addition to approval by local authorities.

### 2.9. Statistics

Data were recorded as mean ± standard deviation. All experiments were done at least in triplicate as specified in the results section. Means were analyzed for statistical significance using unpaired student's $t$-test. Differences were considered significant at $p$-values <0.05.

**Table 1**
Solubilization of Sagopilone ($n = 3$).

| Sample | Polymer $c$ (g/L) | Preparation Method | Sagopilone | | Loading of Sagopilone | |
|---|---|---|---|---|---|---|
| | | | $c$ (mg/L) | SE (%) | (wt.%) | (mol/mol) |
| Blank | – | FF | 56.7 ± 8.5 | 5.7 | – | – |
| P2LA(1.1) | 10 | FF | 739.8 ± 8.1 | 74.0 | 7.40 ± 0.08 | 0.571 ± 0.006 |
| P2LA(1.1) | 20 | FF | 997.2 ± 11.4 | 99.7 | 4.98 ± 0.24 | 0.385 ± 0.004 |
| Blank | – | SO | 8.3 ± 0.2 | 0.8 | – | – |
| P2CL(1.3) | 10 | SO | 761.3 ± 15.9 | 76.1 | 7.61 ± 0.16 | 0.644 ± 0.013 |
| P2CL(1.3) | 20 | SO | 996.8 ± 48.5 | 99.7 | 5.06 ± 0.16 | 0.422 ± 0.021 |
| P5CL(1.0) | 10 | SO | 703.1 ± 41.9 | 70.3 | 7.03 ± 0.42 | 1.293 ± 0.078 |
| P5CL(1.0) | 20 | SO | 1011.0 ± 31.8 | 101.1 | 4.99 ± 0.06 | 0.930 ± 0.029 |

## 3. Results and discussion

### 3.1. Solubilization capacity and stability of micellar dispersions

Sagopilone was solubilized by polymeric micelles with the appropriate method of preparation for PLA- and PCL-containing block copolymers, using the film formation and sonication method, respectively. The aim was to reach the clinically relevant Sagopilone concentration of 1 g/L necessitating an 83-fold solubility enhancement compared to the solubility in water (12 μg/mL). Using a polymer concentration of 10 g/L resulted in comparable molar drug-loading capacities of (0.57 ± 0) and (0.64 ± 0.01) mol Sagopilone per mol polymer for P2LA(1.1) and P2CL(1.3), respectively (Table 1). This is distinctly lower compared to the loading capacity of the higher molecular weight polymer P5CL(1.0) at (1.29 ± 0.08) mol Sagopilone per mol polymer (Table 1). Assuming that the drug was solubilized by the hydrophobic blocks within the micellar core, the corresponding loading capacities were almost equal at 14% and 13–14% (w/w hydrophobic block) for PLA and PCL, respectively. However, the block copolymer concentration was too low to reach the target concentration of Sagopilone. Therefore, the amount of the polymers was increased to 20 g/L, resulting in micellar dispersions that contained Sagopilone at a satisfactory concentration of 1 g/L (Table 1), equivalent to a Sagopilone loading of 5% (w/w). The corresponding solubilization efficiency was 100%, indicating the absence of any drug loss during the preparation for all polymers used.

The hydrodynamic diameters of the drug-loaded micelles were (20.2 ± 0.1), (38.6 ± 0.9) and (68.4 ± 3.3) nm for P2LA(1.1), P2CL(1.3) and P5CL(1.0), respectively (Fig. 2A). PEG-*b*-PCL micelles exhibited a higher polydispersity (PDI: 0.13–0.21) compared to PEG-*b*-PLA micelles (PDI: 0.01–0.05), independent of the drug loading and polymer concentration (Fig. 2A). As previously shown in a cryoTEM study [21], the PEG-*b*-PCL micelles exhibited a uniform size distribution despite their higher PDI values, and aggregation was not observed. In contrast to the clear dispersions comprised of P2LA(1.1), unloaded as well as drug-loaded PCL-containing formulations showed a slight or intense white to pale blue opalescence (Fig. 2B), indicative of crystalline light scattering structures in the submicron size range. The particle sizes as well as the PDI did not differ between the unloaded and drug-loaded micelles except for P5CL(1.0) at a concentration of 10 g/L (Fig. 2A). The exception revealed a significant increase in the micellar size ($p = 0.01$) while size distribution (PDI) did not change significantly ($p = 0.25$). This phenomenon may be due to the formation of a small amount of drug nanocrystals with diameters less than 0.22 μm. However, they may only account for a marginal proportion of the total number of particles, since they have not been detected as a single size population at DLS, and the corresponding blank samples exhibited negligible Sagopilone concentrations (Table 1).

The dispersions were stored at room temperature, and their remaining drug content was determined after 24 h to assess their stability. As shown in Fig. 3A, all dispersions were stable at a polymer concentration of 20 g/L while precipitation of the drug occurred at a P2LA(1.1)-concentration of 10 g/L. Thus, a further requirement for clinical development or processing, namely the stability of the dispersions for a specific time period, was met. The previously described 'supersaturation' effect of P2LA(1.1)-dispersions prepared by a film formation method was not observed at the higher polymer concentration. The lower Sagopilone loading (5 wt.%, Table 1) did not exceed the loading capacity of the P2LA(1.1)-micelles, circumventing a subsequent precipitation of excessive Sagopilone, and (97.2 ± 1.3)% of the drug still remained solubilized after 24 h (Fig. 3A). In addition, the time-dependent behaviour of the P2LA(1.1)-micelles was monitored by DLS (Fig. 3B). The "supersaturated" dispersions revealed a slightly ascending PDI during the first 9 h (PDI: 0.05–0.11) with a subsequent sharp increase from 0.11 to 0.20, whereas the polydispersity

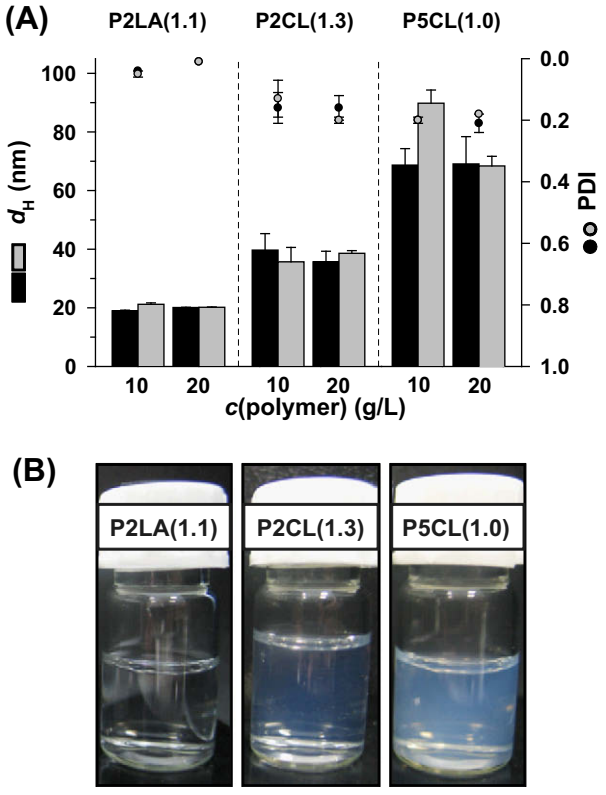


**Fig. 2.** Size characteristics of micellar dispersions. (A) Particle Characteristics of unloaded (▬/●) and Sagopilone-loaded (▭/○) polymeric micelles as a function of the polymer concentration. (B) Appearance of Sagopilone-loaded micellar dispersions at a polymer concentration of 20 g/L. $n = 3$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

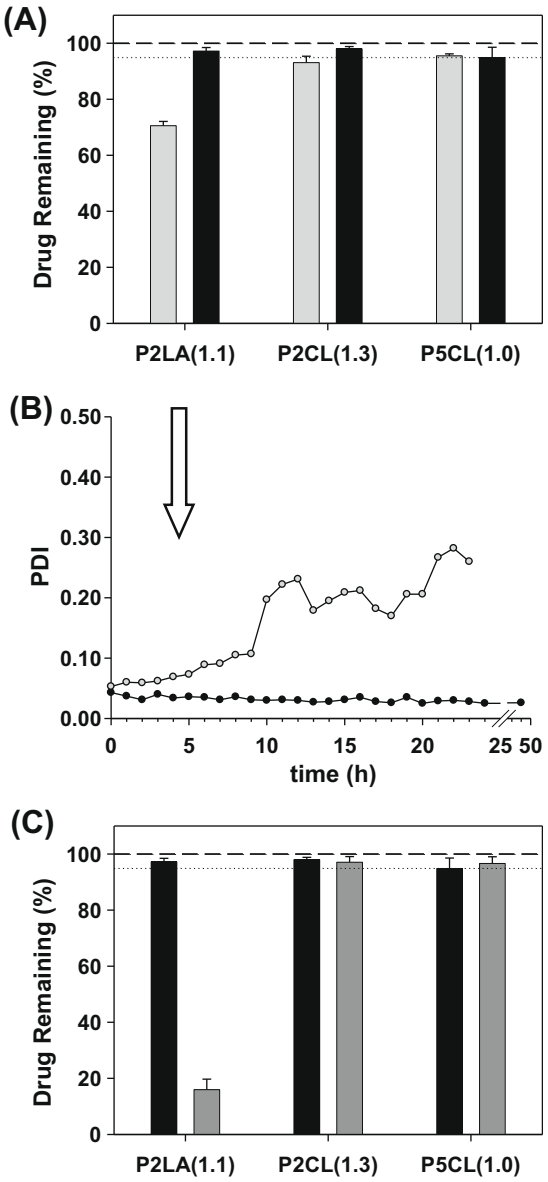


**Fig. 3.** Stability of micellar dispersions. 24-h Stability of Sagopilone-containing micellar dispersions (target conc. 1 g/L) at room temperature. (A) Remaining drug content after 24 h at a polymer concentration of 10 g/L (▭) and 20 g/L (▬) in phosphate buffer (0.05 M, pH 7.4). (B) Absence of the 'supersaturation' effect of P2LA(1.1) at *c*(polymer) of 20 g/L (•) compared to 10 g/L (∘) shown by a 24-h DLS-measurement. (C) Remaining drug content after 24 h in dispersions comprising phosphate buffer (0.05 M, pH 7.4) (▬) and phosphate buffer saline (0.05 M, pH 7.4) (▭) at a polymer concentration of 20 g/L. $n = 3$.

of the micelles comprising a higher polymer concentration of 20 g/L did not change for at least 48 h (Fig. 3B). This points to the importance of determining the drug content at multiple time points in addition to a single measurement after preparation. The latter often leads to a misinterpretation of the micellar loading capacity especially if the film formation method is used due to the 'supersaturation' effect.

The previously described dispersions were prepared in phosphate buffer (0.05 M, pH 7.4) as a dispersion medium. The higher concentration of sodium chloride present in the phosphate buffer saline (0.05 M, pH 7.4) remarkably decreased the stability of P2LA(1.1)-micelles as drug content dropped to (16.0 ± 3.8)% after 24 h (Fig. 3C). Conversely, the stability of the PEG-*b*-PCL micelles was not affected after 24 h. Thus, phosphate buffer (0.05 M, pH 7.4) was used for further investigations.

### 3.2. Critical micelle concentration and stability upon dilution

Using a fluorescent probe technique, the critical micelle concentration (CMC) was determined as a thermodynamic parameter characterizing the micelles' stability during dissolution. The three polymers tested exhibited a very low CMC on an order of magnitude of $10^{-7}$–$10^{-6}$ M as shown in Table 2. Furthermore, the free energy ($\Delta G^\circ$) of the micelle formation process was calculated according to Eq. (4), where the CMC is expressed in units of mole fraction, R is the gas constant and $T$ is the absolute temperature of the system [28].

$$\Delta G^\circ = RT \ln(\mathrm{CMC}) \quad (4)$$

The obtained $\Delta G^\circ$ values were negative, independent from the polymers used, indicating a self-assembly process. Thus, the spherical nanoparticles detected at DLS (Fig. 2A) and visualized by cryoTEM [21] were proven to be thermodynamically stable, self-assembled micelles. Based on their CMC values, the micellar dispersions (*c*(polymer) = 20 g/L) of P2LA(1.1), P2CL(1.3) and P5CL(1.0) may be diluted by a factor of 2740, 2990 and 3780, respectively, to fall below the CMC. As previously stated, the micelles are not necessarily destroyed after dilution below the CMC, depending on their kinetic stability [1,20]. By definition, unimers exist in equilibrium with polymeric micelles at concentrations above the CMC. The rate of the exchange of polymer unimers between the micelles as well as the dissociation defines the kinetic stability. It may occur rapidly, gradually or not at all, depending on the state of the core (liquid-like, glassy or crystalline), whereas the latter are known as 'frozen' micelles [1].

To define the kinetic stability at concentrations above the CMC, dilution experiments were performed by mixing the micellar dispersions (1 g/L Sagopilone, 20 g/L polymer) with normal saline (0.9%) in a ratio of 1:10. Subsequently, the dilutions were stored at 4 and 37 °C for 24 h, and the remaining drug content was determined thereafter (Fig. 4). In contrast to P2LA(1.1), the PEG-*b*-PCL micelles exhibited a high stability upon dilution. (97.2 ± 2.2)% and (97.3 ± 0)% of the drug content remained solubilized after 24 h at 4 °C using P5CL(1.0) and P2CL(1.3), respectively, in contrast

**Table 2**
CMC-values (determined at 25 °C, $n = 3$).

| Polymer | CMC (μg/mL) | CMC ($10^{-6}$ M) | $\Delta G^\circ$ (kJ/mol) |
|---|---|---|---|
| P2LA(1.1) | 7.3 ± 1.9 | 1.7 ± 0.5 | −32.9 ± 0.7 |
| P2CL(1.3) | 6.7 ± 2.7 | 1.5 ± 0.6 | −33.5 ± 1.1 |
| P5CL(1.0) | 5.3 ± 3.2 | 0.5 ± 0.3 | −36.1 ± 1.4 |

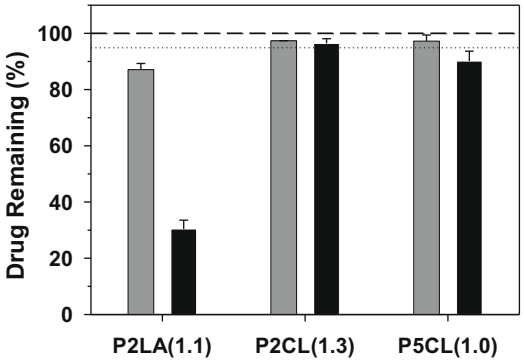


**Fig. 4.** Stability upon dilution. Stability demonstrated by the remaining drug content of the dilutions after 24 h stored at 4 °C (▭) and 37 °C (▬). Dilution of micellar dispersions (1 g/L Sagopilone; 20 g/L polymer) in phosphate buffer (0.05 M, pH 7.4) with normal saline (0.9%) in a ratio of 1:10 (v/v). $n = 3$.

to (87.1 ± 2.2)% for P2LA(1.1). The superior stability of the PCL-containing micelles was even more obvious at 37 °C. At this temperature, more than 70% of the initially solubilized drug substance precipitated in micellar dispersions of P2LA(1.1), whereas (96.4 ± 1.7) and (90.1 ± 3.6)% of the drug remained solubilized in P2CL(1.3) and P5CL(1.0), respectively.

By summarizing the dilution (Fig. 4) and stability experiments in the presence of sodium chloride (Fig. 2C), a remarkable difference in the stability of PLA- and PCL-containing micelles was observed. P2LA(1.1) and P2CL(1.3) exhibited similar values in their CMC, but P2LA(1.1)-micelles were less stable, both in phosphate buffer saline and after dilution with normal saline despite the fact that the polymer concentration was beyond the CMC for all polymers tested. Hence, PEG-*b*-PCL micelles exhibited a superior kinetic stability despite the similar thermodynamic stability parameters for the PLA- and PCL-containing polymers. This is most likely due to the semi-crystalline core of the PEG-*b*-PCL micelles. As the amorphous portion of PCL solubilizes Sagopilone [21], the partial crystallinity leads to "frozen" micelles with very slow exchange rates between unimers and micelles and consequently an increased kinetic stability. In contrast, P2LA(1.1)-micelles comprise glassy cores at ambient temperature, which are kinetically less stable due to the absence of crystalline structures and subsequent unhampered exchange between unimers and polymeric micelles. Consequently, these systems are more susceptible to having their equilibrium affected by a higher amount of sodium chloride towards a destabilized state. The kinetic stability was additionally decreased at 37 °C since the glass transition temperature of PLA (approximately 38 °C) was reached, resulting in (a) an increased fluidity and exchange rate of the liquid-like core and (b) a shift towards free unimers since the polymer is more soluble at higher temperatures. The observed superior stability of P5CL(1.0) at concentrations above the CMC coincides with the described superior kinetic stability and a slow rate of disassembly of P5CL(1.0) at concentrations below the CMC [23]. Thus, CMC values can be used to provide evidence of a self-assembly process, but all facets of stability have to be considered.

### 3.3. Formulation development

Using different types and amounts of lyoprotective agents, both blank and Sagopilone-containing dispersions (1 g/L Sagopilone, 20 g/L polymer) were freeze-dried as described in Table 3. Lyophilized P2LA(1.1)-micelles were completely redispersed even without the addition of lyoprotective agents. As shown in Fig. 5a, the micelles did not change in size after lyophilization (prior: 20 nm; after: 19–22 nm), and this behaviour was not altered in the presence of Sagopilone (prior: 21 nm; after: 21–24 nm).

For PEG-*b*-PCL micelles, a lyoprotective agent such as polyvinylpyrrolidone (PVP) or Hydroxypropyl-*β*-cyclodextrin (HPβCD) was necessary to obtain complete redispersion of the unloaded as well as drug-loaded samples. In addition, the dispersions were frozen by immersion in liquid nitrogen prior to lyophilization to avoid potential sedimentation and aggregation of the micelles. Interestingly, redispersion of lyophilizates containing P5CL(1.0) and a sufficient amount of sucrose were completely redispersible, but their drug-loaded counterparts were not.

The addition of PVP or HPβCD to the micelle dispersions prior to lyophilization led to an alteration in the sizes measured by DLS as seen in Fig. 5b and c. This is very likely to be due to a measurement artefact by the altered composition and viscosity of the dispersion medium affecting the micelle mobility, which in turn presents the basis for size calculations at DLS. A change in the micelle morphology is very unlikely, since the polydispersity and size distributions did not change and precipitation phenomena were not detected. The altered sizes were used as comparative values in the assessment of the redispersion behaviour of the corresponding lyophilizates. Micelles comprised of P5CL(1.0) revealed similar sizes prior to and after lyophilization (Fig. 5c), independent of the lyoprotective agent and drug loading. On the other hand, lyophilizates comprised of P2CL(1.3) and HPβCD exhibited a remarkable increase in their micellar size despite complete redispersion. This was also observed for the drug-loaded micelles of P2CL(1.3) using PVP as a lyoprotectant. Irrespective of the polymer used, the drug content did not change after redispersion (data not shown).

Overall, lyophilization was feasible using the specified conditions and can be considered a viable option for parenteral formulations. The results of P2LA(1.1) are in good agreement with the described freeze-drying of Paclitaxel-loaded PEG-*b*-PLA micelles [29]. To date, the preparation of freeze-dried PEG-*b*-PCL micelles for storage and redispersion later on has not been addressed extensively. There are only few reports addressing refreezing that present storable forms for PEG-*b*-PCL micelles [30]. Application of the same conditions used for PEG-*b*-PLA was not feasible for the lyophilization of PEG-*b*-PCL micelles. Again, the different nature of the hydrophobic blocks had a great impact. The amorphous structure of PLA itself was superior to semi-crystalline PCL with regard to the redispersion behaviour and the need for lyoprotection. Embedding of the latter within a dense matrix of PVP or HPβCD preserved the micellar structures resulting in complete redispersion. Despite the large molecular weight of PEG and PCL, P5CL(1.0) was superior to P2CL(1.3) with regard to aggregation behaviour. This may be due to the higher PEG content of P2CL(1.3) (0.22 mmol PEG per g polymer) compared to P5CL(1.0) (0.1 mmol PEG per g polymer). Previous freeze-drying studies of PEG-*b*-PLA nanoparticles [31] showed a clear relationship between

**Table 3**
Redispersion behaviour after lyophilization ($n = 3$).

| Lyo-protectant | Ratio[a] | Freezing[b] | P2LA(1.1)[c] | | P2CL(1.3)[c] | | P5CL(1.0)[c] | |
|---|---|---|---|---|---|---|---|---|
| | | | No drug | Sago. 1 g/L | No drug | Sago. 1 g/L | No drug | Sago. 1 g/L |
| No lyoprot. | – | | ✓ | ✓ | >1 μm | >1 μm | >1 μm | >1 μm |
| Mannitol | 1:1 | | ✓ | ✓ | >1 μm | >1 μm | >1 μm | >1 μm |
| Sucrose | 1:1 | | ✓ | ✓ | >1 μm | >1 μm | >1 μm | >1 μm |
| Sucrose | 1:20 | × | – | – | >1 μm | >1 μm | ✓ | >1 μm |
| Trehalose | 1:5 | × | – | – | >1 μm | >1 μm | >1 μm | >1 μm |
| Trehalose | 1:20 | × | – | – | >1 μm | >1 μm | >1 μm | >1 μm |
| PVP | 1:5 | × | – | – | >1 μm | >1 μm | >1 μm | >1 μm |
| PVP | 1:20 | × | – | – | ✓ | ✓ | ✓ | ✓ |
| HPβCD | 1:20 | × | – | – | ✓ | ✓ | ✓ | ✓ |

✓ Complete redispersion possible.
[a] Polymer–lyoprotectant ratio (w/w).
[b] Freezing by immersion with liquid nitrogen (−196 °C) prior to lyophilization.
[c] $c$(polymer) = 20 g/L.

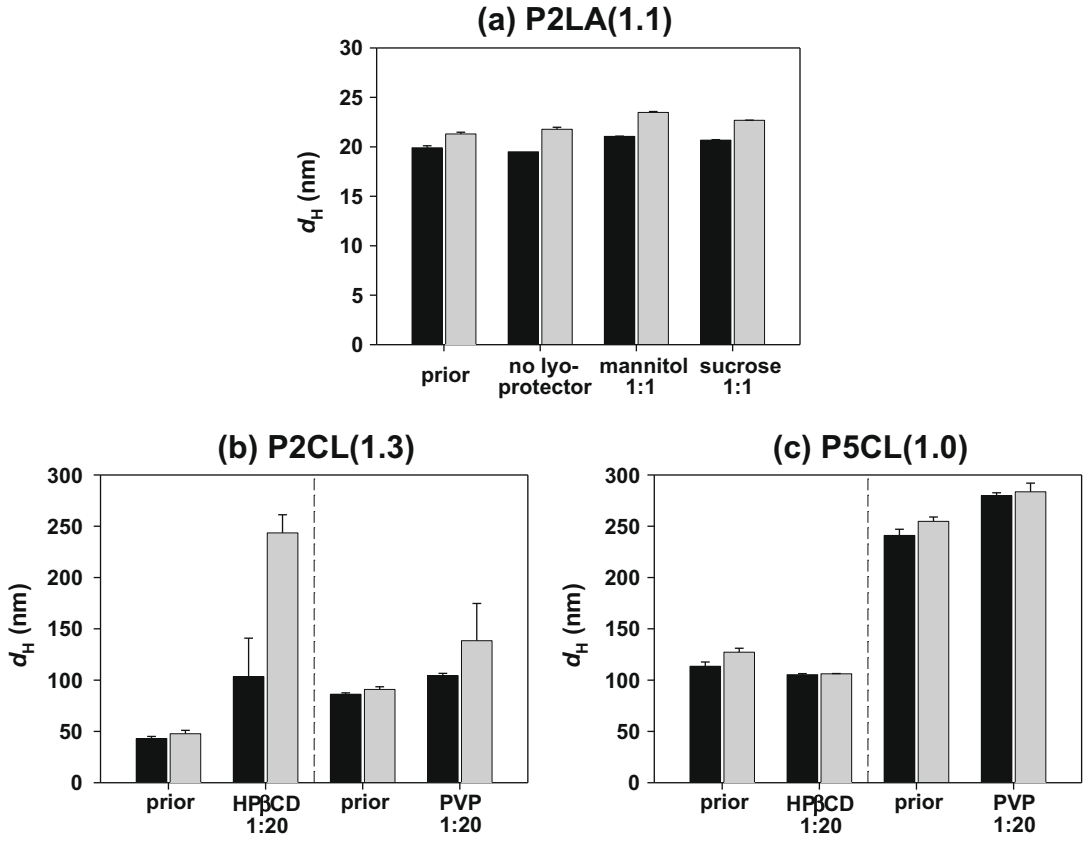


**Fig. 5.** Lyophilization. Hydrodynamic diameter ($d_H$) of unloaded (▬) and Sagopilone-loaded (▬) micelles, prior to lyophilization and after redispersion of the lyophilizates, as a function of the lyoprotective agent added and its amount (polymer–lyoprotectant ratio (w/w)). The particle sizes of (b) and (c) prior to freeze-drying were measured in the presence of HPβCD or PVP. $n = 3$.

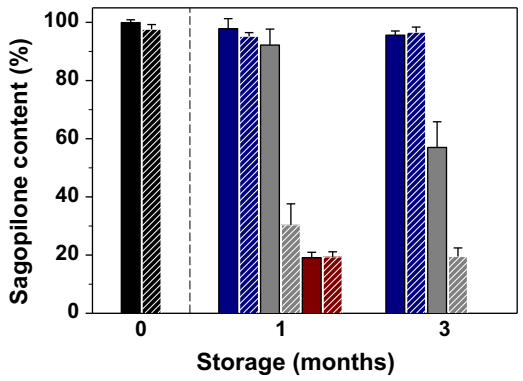


**Fig. 6.** Film stability. Stability of Sagopilone-loaded P2LA(1.1)-films as a function of the storage time and temperature (6 °C: ▬, 25 °C: ▬, 40 °C: ▬) represented by the mean Sagopilone content of the micellar dispersions after redispersion (solid bars) and subsequent storage at 2–8 °C for 12 h (hatched bars). $n = 6$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the amount of grafted PEG and the degree of aggregation because of the formation of stable PEG crystallized bridges between neighbouring particles. This may also be due to an efficient shielding of the PCL core by longer PEG chains and subsequent prevention of the formation of PCL-aggregates. Optimization of the procedure as well as an elaborative elucidation of the change in the particle sizes will be the focus of future studies.

As an alternative to freeze-drying, solid polymeric films for redispersion prior to clinical application were investigated. The films were composed of P2LA(1.1) and Sagopilone at a drug loading of 5% (w/w) to avoid 'supersaturation' phenomena after redispersion.

At a storage temperature of 6 °C, no crystallization of Sagopilone was observed as shown in the XRPD pattern (Fig. 7D, blue diffractogram). The observed peak at 19 $2\Theta$ corresponded to the crystalline PEG phase of the films, which was present in the blank films of P2LA(1.1) as well (Fig. 7C). Redispersion by the use of simple shaking by hand was easy and complete resulting in micellar dispersions with a mean drug content of (96 ± 1)% (Fig. 6, blue bars). Precipitation did not occur afterwards, and the dispersions still exhibited (97 ± 2)% in drug content after 12-h storage at 2–8 °C (Fig. 6).

Thus, polymeric films of P2LA(1.1) comprising a Sagopilone content of 5% have been demonstrated as a novel solid formulation stable for at least 3 months of storage at 6 °C. Adjuvant excipients were not required to maintain the capability to form micellar dispersions in an aqueous medium. This is in contrast to previous publications [32] describing the necessity of the addition of PEG to obtain storable liquid formulations because redispersion had failed without it. The storage temperature has been identified as a key factor in stability. Although complete film redispersion was still possible after 1 month of storage at 25 °C, the resulting dispersions were not stable, and more than 60% of the initially solubilized drug precipitated within the following 12 h (Fig. 6, grey bars). This precipitation phenomenon was very likely to be due to an enhanced degradation of PLA resulting in shorter PLA blocks with a subsequent decreased drug-loading capacity of the corresponding micelles. After storage for another 2 months, Sagopilone crystallization occurred (Fig. 7D), impeding complete redispersion. At 40 °C, crystalline Sagopilone patterns were detected even after 1 month (Fig. 7B, red diffractogram) along with incomplete

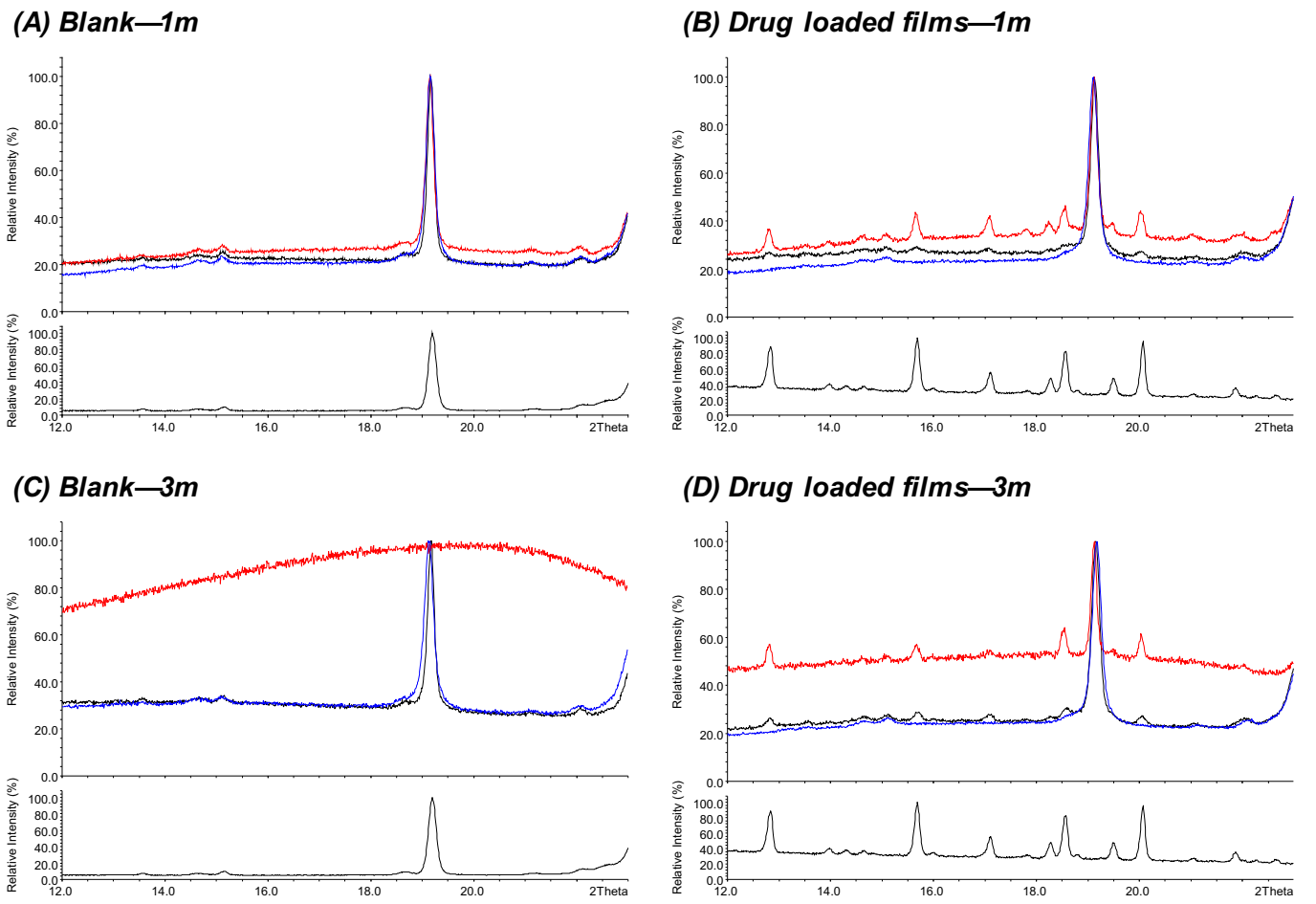


**Fig. 7.** XRPD pattern of polymeric films. XRPD pattern of blank and Sagopilone-loaded P2LA(1.1)-films stored at 6 °C (——), 25 °C (——) and 40 °C (——) after 1 and 3 months (m). As a comparison, the XRPD pattern of PEG (1500 Da) and Sagopilone is displayed below the blank and drug-loaded diffractograms, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

redispersion (Fig. 6, red bars). Crystallization was promoted since the glass transition temperature of PLA (approximately 38 °C) was reached increasing the fluidity and coalescence of drug-loaded phases. Additionally, the blank matrix changed to a liquid state after 3 months with complete disappearance of the PEG peak (Fig. 7C). This is a clear indication of a high degree of degradation of PLA resulting in lactic acid, which is a colourless to slightly yellow syrupy liquid [33] dissolving the residual crystalline PEG. Thus, the storage temperature has to be maintained at 6 °C to prevent matrix degradation and drug crystallization.

### 3.4. In vitro cytotoxicity

The *in vitro* cytotoxic activity of micellar and free Sagopilone was investigated in a proliferation assay using a human cervix carcinoma cell line (HeLa/MaTu). The activity is given as a concentration that inhibits cell proliferation by 50% ($IC_{50}$). For comparison, Sagopilone solutions containing HPβCD (drug–excipient ratio of 1:200) or ethanol (0.1%) were tested. Paclitaxel (ethanol solution) was used as an internal standard to verify the reliability of the results obtained with the given *in vitro* test system. Sagopilone's high antiproliferative activity ($IC_{50}$ < 1 nM) compared to Paclitaxel ($IC_{50}$ > 1 nM) [3] was maintained. The $IC_{50}$-values of the Sagopilone samples tested were in a range of 0.14–0.26 nM (Table 4), showing no significant differences between the respective formulations and the ethanol solution ($p$ > 0.05). Corresponding blanks did not show any cytotoxicity within the effective concentration range of Sagopilone. Thus, micellar Sagopilone was proven to be still highly active on a cellular level. Encapsulation in polymeric micelles did not prevent drug internalization into the cells, a mandatory premise for Sagopilone's activity. Although *in vitro* experiments do not allow a prediction of the *in vivo* behaviour of nanocarriers, they are considered as a necessary step towards *in vivo* testing.

**Table 4**
*In vitro* cytotoxicity of different Sagopilone-loaded polymeric micelles compared to free Sagopilone and Paclitaxel in human HeLa/MaTu cells ($n = 3 \times 8$).

| Vehicle | Sagopilone | Paclitaxel | $IC_{50}$ (nM) |
|---|---|---|---|
| P2LA(1.1) | × | | 0.21 ± 0.03 |
| P2CL(1.3) | × | | 0.17 ± 0.04 |
| P5CL(1.0) | × | | 0.14 ± 0.05 |
| HPβCD | × | | 0.19 ± 0.08 |
| Ethanol/medium | × | | 0.26 ± 0.07 |
| | | × | 1.17 ± 0.21 |

### 3.5. In vivo toxicity

Following the *in vitro* investigations, the toxicity of the polymeric micelles as well as the maximum tolerated dose (MTD) of the drug-loaded micelles were determined *in vivo*.

The polymeric micelles ($c$(polymer) = 20 g/L) revealed no acute toxicity or signs of hypersensitivity reactions after i.v. application to non-tumour-bearing nude mice at a polymer dose of 200 mg/kg

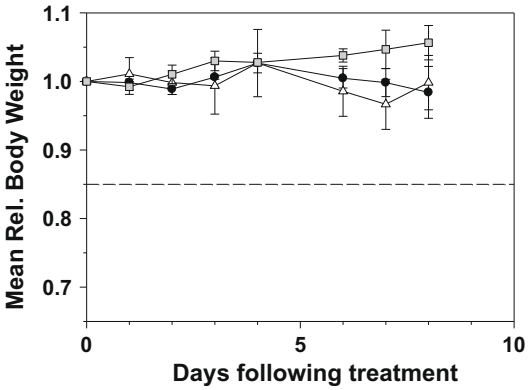


**Fig. 8.** Safety of vehicles. Mean relative body weight after i.v. injection of unloaded polymeric micelles ($c$ = 20 g/L) comprising P2LA(1.1) (•), P2CL(1.3) (Δ) and P5CL(1.0) (□) at a dose of 200 mg/kg (Mean ± Min/Max). $n$ = 2.

(Fig. 8). The body weight of the mice did not change during 1 week. Thus, the PEG-*b*-PLA as well as the PEG-*b*-PCL vehicles were proven to be safe, which is an important requirement for the subsequent dose-finding study. For this study, drug-loaded micelles (drug–polymer ratio 1:20) were administered at increasing doses as shown in Fig. 9.

The MTD was determined to be 6 mg/kg, independent of the polymer used. At the higher dose of 8 mg/kg, the animals died or had to be sacrificed due to a weight loss exceeding 20% with the exception of the group receiving P2CL(1.3) micelles, in which only 1 of 3 mice died (Fig. 9*).

The MTD of micellar Sagopilone was decreased compared to a cyclodextrin-based formulation of Sagopilone (MTD = 10 mg/kg, data not shown). This may be due to an enhanced effective dose and biodistribution of Sagopilone accompanied by an enhanced toxicity. Furthermore, the degradation of Sagopilone by serum esterases may be hampered due to its encapsulation within polymeric micelles resulting in higher plasma levels of the effective drug after i.v. administration. Comparatively, the MTD of micellar Paclitaxel (Genexol®-PM, 60 mg/kg) was threefold higher than that of conventional Paclitaxel (Taxol®, 20 mg/kg) using a polymer similar to P2LA(1.1) in a nude mouse model [17]. Taxol® uses Cremophor®EL, which is known to cause severe side effects limiting the dose of Paclitaxel. Since the polymeric micelles (Genexol®-PM) did not exhibit any hypersensitivity reactions, dosing of Paclitaxel could be increased, resulting in a higher MTD. As shown in the present study, the comparison with a cyclodextrin-based formulation revealed a decreased MTD, suggesting an improved stability after i.v. administration and an enhanced lysosomal internalization of micellar Sagopilone into cells. The recommended dose was identified to be 6 mg/kg. The described micelles are believed to show an increased *in vivo* antitumour efficacy due to a decreased degradation of the drug and an enhanced permeation and retention of micellar Sagopilone in solid tumours.

## 4. Conclusion

Polymeric micellar dispersions of P2LA(1.1), P2CL(1.3) and P5CL(1.0) were successfully used to solubilize Sagopilone at a clinically relevant concentration of 1 g/L, requiring a drug–polymer ratio as low as 1:20 (w/w). The resulting micellar dispersions exhibited sufficient stability, independent of the polymer type and composition. Precipitation phenomena due to a 'supersaturation' following particular preparation methods such as the film formation must not be mistaken with instability of the micelles and could be circumvented by simply adjusting the drug loading to values not exceeding the loading capacity as seen for P2LA(1.1).

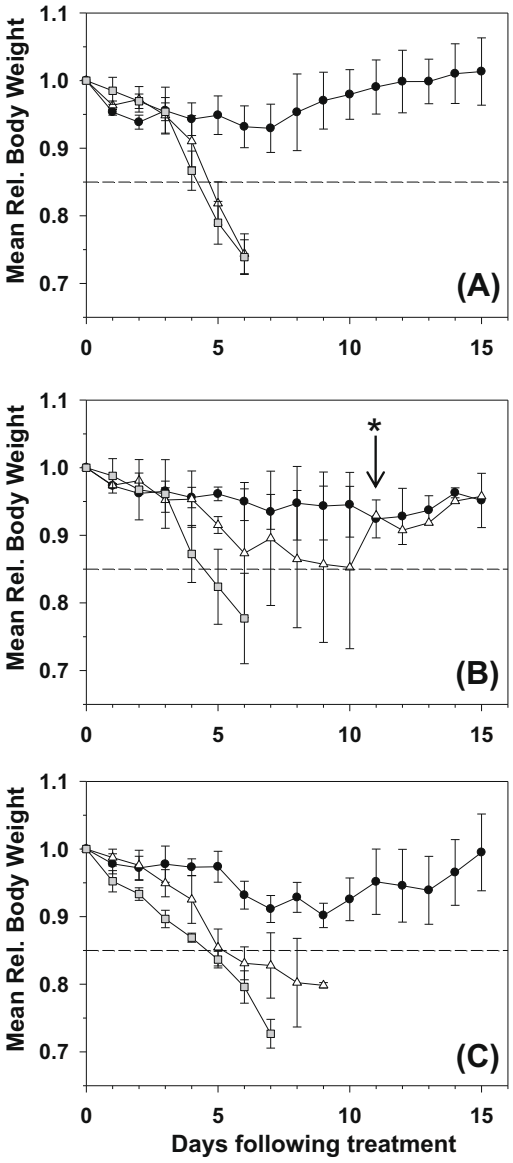


**Fig. 9.** Determination of MTD. Mean relative body weight after i.v. application of Sagopilone-loaded polymeric micelles of (A) P2LA(1.1), (B) P2CL(1.3) and (C) P5CL(1.0) at Sagopilone doses of 6 (•), 8 (Δ), and 10 (□) mg/kg and polymer doses of 120, 160 and 200 mg/kg, respectively. $n$ = 3, except *8 mg/kg: one animal has died.

The demonstrated lyophilization of these dispersions, shown for the first time with PEG-*b*-PCL, promotes the further development of this kind of block copolymers as solubilizing agents. A novel solid formulation concept, namely drug-loaded polymeric films for redispersion prior to parenteral administration, was demonstrated to be feasible for PEG-*b*-PLA even without additional excipients, in contrast to previous studies. This approach could be of considerable commercial interest due to the prevention of complex and costly lyophilization and the absence of water during the production process. Altogether, amphiphilic block copolymers could lend themselves to become standard solubilizing excipients. The PEG-*b*-PCL micelles revealed a distinctly higher kinetic stability both in the presence of isotonic additives and upon dilution. For this reason, they may demonstrate superior stability after i.v. application and passive tumour targeting. The *in vivo* evaluations revealed no carrier-related side effects and decreased MTDs of Sagopilone-loaded polymeric micelles, independent of the polymer used and despite their different kinetic stability. The polymeric micelles

are believed to be superior in terms of delivering higher amounts of drugs to tumour tissue despite lower dosing due to an increased stability of the encapsulated drug against blood esterases as well as an enhanced permeation and retention of the delivery system in solid tumours. To provide evidence of this EPR-effect, tumour efficacy studies are needed, preferably using tumour models that represent the *in vivo* situation of leaky vessels.

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## References

[1] G.S. Kwon, Polymeric micelles for delivery of poorly water-soluble compounds, Crit. Rev. Ther. Drug Carr. Syst. 20 (2003) 357–403.
[2] A.J. ten Tije, J. Verweij, W.J. Loos, A. Sparreboom, Pharmacological effects of formulation vehicles: implications for cancer chemotherapy, Clin. Pharmacokinet. 42 (2003) 665–685.
[3] U. Klar, B. Buchmann, W. Schwede, W. Skuballa, J. Hoffmann, R.B. Lichtner, Total synthesis and antitumor activity of ZK-EPO: the first fully synthetic epothilone in clinical development, Angew. Chem. Int. Edit. 45 (2006) 7942–7948.
[4] J. Hoffmann, I. Vitale, B. Buchmann, L. Galluzzi, W. Schwede, L. Senovilla, W. Skuballa, S. Vivet, R.B. Lichtner, J.M. Vicencio, T. Panaretakis, G. Siemeister, H. Lage, L. Nanty, S. Hammer, K. Mittelstaedt, S. Winsel, J. Eschenbrenner, M. Castedo, C. Demarche, U. Klar, G. Kroemer, Improved cellular pharmacokinetics and pharmacodynamics underlie the wide anticancer activity of Sagopilone, Cancer Res. 68 (2008) 5301–5308.
[5] H. Reichenbach, G. Höfle, Discovery and development of the epothilones: a novel class of antineoplastic drugs, Drugs R. D. 9 (2008) 1–10.
[6] US National Institutes of Health, Clinical Trials of Sagopilone. <http://www.clinicaltrial.gov/ct2/results?term=Sagopilone> (accessed 02.01.10).
[7] European Medicines Agency, Doc.Ref. EMEA/602569/2008 – questions and answers on recommendation for the refusal of the marketing authorisation for Ixempra. <http://www.emea.europa.eu/pdfs/human/opinion/IxempraQ&A_60256908en.pdf> (accessed 09.10.09).
[8] Y. Matsumura, H. Maeda, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs, Cancer Res. 46 (1986) 6387–6392.
[9] H. Maeda, G.Y. Bharate, J. Daruwalla, Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect, Eur. J. Pharm. Biopharm. 71 (2009) 409–419.
[10] Y. Bae, K. Kataoka, Intelligent polymeric micelles from functional poly(ethylene glycol)-poly(amino acid) block copolymers, Adv. Drug Deliv. Rev. 61 (2009) 768–784.
[11] V.P. Torchilin, Micellar nanocarriers: pharmaceutical perspectives, Pharm. Res. 24 (2007) 1–16.
[12] N. Nishiyama, K. Kataoka, Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery, Pharmacol. Ther. 112 (2006) 630–648.
[13] H.M. Aliabadi, A. Lavasanifar, Polymeric micelles for drug delivery, Expert Opin. Drug Deliv. 3 (2006) 139–162.
[14] 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.
[15] H.M. Aliabadi, A. Mahmud, A.D. Sharifabadi, A. Lavasanifar, Micelles of methoxy poly(ethylene oxide)-*b*-poly(ε-caprolactone) as vehicles for the solubilization and controlled delivery of cyclosporine A, J. Control. Release 104 (2005) 301–311.
[16] O. Molavi, Z. Ma, A. Mahmud, A. Alshamsan, J. Samuel, R. Lai, G.S. Kwon, A. Lavasanifar, Polymeric micelles for the solubilization and delivery of STAT3 inhibitor cucurbitacins in solid tumors, Int. J. Pharm. 347 (2008) 118–127.
[17] S.C. Kim, D.W. Kim, Y.H. Shim, J.S. Bang, H.S. Oh, S.W. Kim, M.H. Seo, In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy, J. Control. Release 72 (2001) 191–202.
[18] T. Kawaguchi, T. Honda, M. Nishihara, T. Yamamoto, M. Yokoyama, Histological study on side effects and tumor targeting of a block copolymer micelle on rats, J. Control. Release 136 (2009) 240–246.
[19] H.M. Aliabadi, M. Shahin, D.R. Brocks, A. Lavasanifar, Disposition of drugs in block copolymer micelle delivery systems: from discovery to recovery, Clin. Pharmacokinet. 47 (2008) 619–634.
[20] A.S. Mikhail, C. Allen, Block copolymer micelles for delivery of cancer therapy: transport at the whole body, tissue and cellular levels, J. Control. Release 138 (2009) 214–223.
[21] A. Richter, C. Olbrich, M. Krause, T. Kissel, Solubilization of Sagopilone, a poorly water-soluble anticancer drug, using polymeric micelles for parenteral delivery, Int. J. Pharm. 389 (2010) 244–253.
[22] Y.H. Bae, H. Yin, Stability issues of polymeric micelles, J. Control. Release 131 (2008) 2–4.
[23] J. Liu, F. Zeng, C. Allen, In vivo fate of unimers and micelles of a poly(ethylene glycol)-block-poly(caprolactone) copolymer in mice following intravenous administration, Eur. J. Pharm. Biopharm. 65 (2007) 309–319.
[24] 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.
[25] M.G. Carstens, P.H.J.L.F. de Jong, C.F. van Nostrum, J. Kemmink, R. Verrijk, L.G.J. de Leede, D.J.A. Crommelin, W.E. Hennink, The effect of core composition in biodegradable oligomeric micelles as taxane formulations, Eur. J. Pharm. Biopharm. 68 (2008) 596–606.
[26] M.H. Seo, Y.W. Yi, J.W. Yu, Stable polymeric micelle-type drug composition and method for the preparation thereof, European Patent Specification EP1282447B1, 2009.
[27] W. Kueng, E. Silber, U. Eppenberger, Quantification of cells cultured on 96-well plates, Anal. Biochem. 182 (1989) 16–19.
[28] R. Liu, N. Sadrzadeh, P.P. Constantinides, Micellization and drug solubility enhancement, in: R. Liu (Ed.), Water-insoluble Drug Formulation, Interpharm Press, Englewood, 2000, pp. 213–354.
[29] M.H. Seo, Y.W. Yi, J.W. Yu, Stable polymeric micelle-type drug composition and method for the preparation thereof, European Patent Specification 1, 2009.
[30] S. Cai, K. Vijayan, D. Cheng, E.M. Lima, D.E. Discher, Micelles of different morphologies – advantages of worm-like filomicelles of PEO-PCL in paclitaxel delivery, Pharm. Res. 24 (2007) 2099–2109.
[31] F. De Jaeghere, E. Allémann, J. Feijen, T. Kissel, E. Doelker, R. Gurny, Freeze-drying and lyopreservation of diblock and triblock poly(lactic acid)–poly(ethylene oxide) (PLA–PEO) copolymer nanoparticles, Pharm. Dev. Technol. 5 (2000) 473–483.
[32] M.H. Seo, I.J. Choi, Polymeric composition for solubilizing poorly water-soluble drugs and process for the preparation thereof, US Patent US6616941B1, 2003.
[33] Sigma–Aldrich, Safety Data Sheet: D,L-Lactic Acid, <http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do> (accessed 09.04.09).