International Journal of Pharmaceutics 407 (2011) 190–196

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Nanoparticles for paclitaxel delivery: A comparative study of different types of dendritic polyesters and their degradation behavior

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ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 7 January 2011
Accepted 13 January 2011
Available online 21 January 2011

Keywords:
Hyperbranched polyesters
Nanoparticles
Degradation
Toxicity
Paclitaxel

ABSTRACT

The aim of this study was to formulate nanoparticles from three different hyperbranched polymers, namely an unmodified dendritic polyester (Boltorn H40™), a lipophilic, fatty acid modified dendritic polymer (Boltorn U3000™) and an amphiphilic dendritic polymer (Boltorn W3000™) for drug delivery of paclitaxel and to investigate their properties. A solvent displacement method allowed preparation of nanoparticles from all three hyperbranched polymers. Nanoparticle sizes ranged from 70 to 170 nm. The lipophilic Boltorn U3000™ formed the biggest nanoparticles and the amphiphilic Boltorn W3000™ formed the smallest ones. Nanoparticles of amphiphilic Boltorn W3000™ displayed only a slightly negative zeta potential, while more negative zeta potentials were measured for nanoparticles based on the other two polymers. Degradation profiles were investigated by short time pH-stat titration. Boltorn H40™ showed a faster degradation rate then the two other fatty acid containing polymers. For Boltorn H40™, degradation rate was also investigated in longer term mass loss studies resulting in 30% degradation during 3 weeks. Cytotoxicity of the nanoparticles was studied by MTT assay displaying low cytotoxicity for all three polymers. All three types of nanoparticles were loaded with paclitaxel and their release profiles were studied. Sizes and zeta potentials remained stable after loading and did not change significantly. These three types of hyperbranched polymers show potential as nanoparticulate delivery systems and should be further studied. Due to their high loading efficiency, Boltorn U3000 and W3000 represent the most interesting candidates.

1. Introduction

Hyperbranched polymers (HBPs) and dendritic polymers have gained increasing interest for biomedical applications due to their unique architecture that provides them with a high number of functionalities and a supposedly globular shape (Aryal et al., 2009; Kontoyianni et al., 2008; Prabaharan et al., 2009b; Reul et al., 2009; Tziveleka et al., 2006; Zou et al., 2005). The hyperbranched polymers based on 2,2-bis-(methylol)-propionic acid show a very dense branching in their backbones due to small monomer units. The syntheses are less expensive and tedious than those of dendrimers and result in structures with a higher degree of imperfection (Paleos et al., 2007). However, both classes share many interesting features.

Boltorn™ H40 is a commercially available pseudo-fourth generation dendritic polyester based on 2,2-bis-(methylol)-propionic acid (bis-MPA) with 64 terminal OH groups (Zagar and Zigon, 2002). Boltorn™ U3000 is a more lipophilic dendritic polyester. It is esterified with 14 unsaturated fatty acids derived from sunflower oil consisting of C16 and/or C18 fatty acids (Domanska et al., 2009; Mezzenga et al., 2001). Boltorn™ W3000 is an amphiphilic dendritic polymer. It is non-ionic and self emulsifying and possesses a dendritic, globular structure from which chain ends are terminated by a combination of hydrophobic chains consisting of long unsaturated fatty acid and hydrophilic chains consisting of polyethylene glycol chains (Domaska and Zołek-Tryznowska, 2009).

A schematic representation of the three hyperbranched polyester structures is shown in Fig. 1.

Several nanosized systems based on Boltorn derivatives have been synthesized for biomedical applications. Based on Boltorn H 30, nanocomposite coatings using nanoclay filled hyperbranched polymers were created (Fogelström et al., 2006; Fogelstrom et al., 2010). Nanoparticles of Boltorn H20 modified by succinic anhydride and glycidyl methacrylate were used to encapsulate Daidzein (Zou et al., 2005). A hybrid nanocomposite was synthesized of modified Boltorn H20 and a tetraethoxysilane derivative (Zou et al., 2004)). After modification of Boltorn H40 with PEG and linear polyester chains like PCL or PLLA, sometimes followed by addition of folate targeting moieties, the resulting nanoparticles or micelles were applied for anti-cancer drug delivery (Aryal et al., 2009; Chen et al., 2008; Prabaharan et al., 2009a,b). Modification of Boltorn H40 with

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doi:10.1016/j.ijpharm.2011.01.028

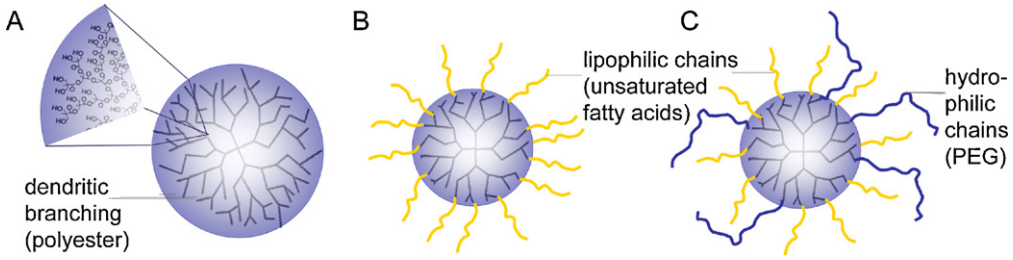

Fig. 1. Schematic representation of the hyperbranched polyesters: (A) Boltorn H40, (B) Boltorn U3000 and (C) Boltorn W3000.

tertiary amines created a non-toxic, biodegradable carrier for gene delivery (Reul et al., 2009). Carboxylic acid modified Boltorn H40 was utilized for controlled release of cisplatin (Haxton and Burt, 2008) and for the interaction with peptides (Guo et al., 2009). Monomolecular micelles from pegylated Boltorn H40 were utilized for encapsulating paclitaxel (Kontoyianni et al., 2008).

Paclitaxel is a poorly water soluble anti-cancer drug which exhibits a significant activity against a variety of solid tumors, including breast cancer, advanced ovarian carcinoma, lung cancer, head and neck carcinomas, and acute leukemia (Fonseca et al., 2002; Gaucher et al., 2010; Safavy, 2008). The diterpenoid pseudoalkaloid drug paclitaxel is with 0.6 mM almost insoluble in aqueous phases (Singla et al., 2002). To increase solubility and reach a pharmacological concentration, paclitaxel is dissolved in Cremophor EL® and ethanol in the standard formulation for clinical application, Taxol™, which has to be diluted prior to use. It has severe side effects caused by its Cremophor EL™ content (Hennenfent and Govindan, 2006). Therefore, alternative formulations are of great importance. Liposomal formulations can reduce the side effects, but also have some disadvantages such as relatively poor storage and physical stability, low entrapment efficiency and in case of non-pegylated liposomes also rapid blood clearance (Marcel Musteata and Pawliszyn, 2006; Torchilin, 2005; Yang et al., 2009).

In the last years, encapsulation of taxanes into polyester-based nanoparticles and micelles has gained increasing interest. The anticancer drugs have been loaded on several of these systems, mainly based on PLGA (Gaucher et al., 2010).

In this study, nanoparticles formed from three dendritic polymers, an unmodified, a lipophilic and an amphiphilic one, were compared. We were interested in how the different dendritic polymer compositions influence the properties and features of the resulting nanoparticles.

Our formulation approach for the three dendritic polymers involved a solvent displacement method. This is a facile, gentle method exploiting the Ouzo effect (Vitale and Katz, 2003) which is known to avoid shear forces and often results in small, well reproducible particles (Oster et al., 2006). Paclitaxel was chosen as a lipophilic drug which was encapsulated into the nanoparticles. Hyperbranched polymer nanoparticles were studied in terms of their physico-chemical properties and features, cytotoxicity and paclitaxel loading and release.

2. Methods and materials

2.1. Materials

The hyperbranched polyesters (Boltorn H40™, Boltorn U3000™ and Boltorn W3000™) were a gift from Perstorp (Perstorp, Sweden). Poloxamer 188 was supplied by BASF (Pluronic F 68™, BASF Germany). Paclitaxel (Genexol™) was kindly provided by San Yang Corp. (Seoul, Korea) and ^{14}C-paclitaxel [radiolabeled paclitaxel (paclitaxel-[2-benzoyl ring-UL-^{14}C])] were purchased from Hartmann (Hartmann Analytik GmbH, Germany). All other chemicals of analytical grade were purchased from Sigma (Sigma–Aldrich, Germany).

2.2. Nanoparticle formation

Nanoparticle preparation was performed by a solvent displacement method (Agueros et al., 2009; Jung et al., 2000). Therefore, 20 mg of polymer were dissolved in 1.5 ml acetone. Using a syringe, the resulting polymer solution was injected slowly and constantly into a magnetically stirred solution of 10 ml of 0.1% Poloxamer F 68 in distilled water. During injection, the opening of the needle (Sterican®, 0.9 mm × 40 mm, B. Braun, Melsungen, Germany) was submerged in the aqueous phase and pressed gently against the glass wall of the beaker. The resulting suspensions were stirred for 3 h under a fume hood to allow removal of residual acetone.

2.3. Photon correlation spectroscopy

Hydrodynamic diameter of nanoparticles was measured by photon correlation spectroscopy (PCS) using the Zetasizer, Nano ZS (Malvern Instruments, Germany). Nanoparticles prepared with 0.1% poloxamer solution as described above were measured at 25 °C with automatic settings. Average values and corresponding S.D. were calculated from three independent batches.

2.4. Zeta potential

The surface charges of nanoparticles were determined by measuring the zeta-potential in a standard capillary electrophoresis cell using a Zetasizer Nano ZS (Malvern Instruments, Germany). The average values and the corresponding S.D. were calculated from three independent batches.

2.5. pH-stat titration

Hydrolytic degradation was studied by a pH stat titration using a Mettler DL 50 titrator (Mettler, Germany). The titrator is equipped with a 10 ml burette and a titration stand for hydrolysis studies. The titration stand consisted of a jacketed beaker with an inlet and outlet for water circulation, a propeller stirrer attached to a stirring rod, the auto-burette tip and a pH electrode. The software LabX light V. 2.1 (Mettler-Toledo, Giessen, Germany) was used for data recording. The pH was adjusted by addition of NaOH once the pH changed allowing quantification of cleaved carboxylic acid that is released after hydrolytic degradation. Nanoparticles were prepared as described above and diluted to 20 ml using distilled water. PH stat titration was performed for 24 h at 37 °C to pH 7.4 titrating with 0.01 N NaOH. The amount of degraded polyester bonds was

calculated according to the following formula:

$$\text{Degraded esters (} = \text{free carboxylic acids) (\%)} = \left(\frac{\Delta V_{\text{NaOH}} \times 0.01\,\text{M}}{N_{\text{ester total}}}\right) \times 100$$

where ΔV_{NaOH} is the volume of NaOH solution (0.01 M) required to bring the pH value of the polymer solution back to 7.4 and $N_{\text{ester total}}$ is the total moles of ester groups in the polymer. Blank titrations with 0.9% NaCl in water were also performed. All experiments were performed in triplicates.

2.6. Mass loss of nano-carriers

Nanoparticles containing 20 mg of polymer in 8 ml of 0.1% poloxamer solution were prepared as described earlier. The suspensions were transferred to 15 ml screw-cap containers and diluted with 2 ml Hepes buffer, resulting in a particle concentration of 2 mg/ml and 20 mM buffer, pH (37 °C) 7.4. Incubation occurred at 37 °C while undergoing vertical rotation (Rotatherm, Gebr. Liebisch GmbH & Co., Bielefeld, Germany). At predetermined time intervals the incubation was stopped to determine the mass loss. Due to the fact that nano-carriers could not be isolated quantitatively by centrifugation, the suspensions were frozen at −20 °C for 24 h which led to aggregation of the particles. Centrifugation of the obtained aggregates at 6240 × *g* for 30 min led to distinct pellets. The supernatants were removed quantitatively and after washing three times with distilled water, the pellets were freeze-dried (Christ Beta I, Martin Christ GmbH, Osterode, Germany). The remaining mass of agglomerated nano-carriers was determined gravimetrically. Recovery was >90%. All measurements were carried out in triplicate.

2.7. Cytotoxicity (MTT assay)

Cell viability was evaluated using the MTT assay as described previously (Unger et al., 2007). Briefly, L929 cells were seeded into 96-well microtiter plates (NunclonTM, Nunc, Germany) at a density of 8000 cells/well. After 24 h the culture medium was replaced with serial dilutions of polymer stock solutions ($n = 8$) in antibiotic-free DMEM with 10% FCS. After an incubation period of 24 h, medium was replaced by DMEM without serum containing 0.5 mg/ml MTT (Sigma, Deisenhofen, Germany). After an incubation time of 4 h unreacted dye and DMEM were removed and the purple formazan product was dissolved in 200 μl/well dimethylsulfoxide (Merck, Darmstadt, Germany) and quantified by a plate reader (Titertek Plus MS 212, ICN, Germany) at wavelengths of 570 and 690 nm. Relative cell viability [%] related to control wells containing cell culture medium without polymer was calculated by ([*A*]test/[*A*]control) × 100. PEI 25K, a polycationic polymer widely used as gene transfer agent, was used as positive control. The IC_{50} was calculated as polymer concentration which inhibits growth of 50% of cells relative to non-treated control cells according to Unger et al. (2007). Data is presented as a mean of four measurements ±standard deviation. IC_{50} was calculated using the Boltzmann sigmoidal function from Origin® v 7.0 (OriginLab, Northampton, USA).

2.8. Drug loading with paclitaxel

Nanoparticles were loaded using 0.2% (w/w) paclitaxel. Nanoparticles were prepared by solvent displacement method as described in "Nanoparticle formation". Polymers and paclitaxel were both dissolved in acetone; polymer and paclitaxel solutions were then mixed and injected into an aqueous solution as described above. The resulting suspensions were stirred for 3 h under a fume hood to allow removal of residual acetone. Then, particles were centrifuged at 900 × *g* for 10 min and at 20 °C to remove unencapsulated paclitaxel crystals according to Yang et al. (2009). Nanoparticles were not affected by the centrifugation at 900 × *g* due to their much smaller size and stayed in solution.

2.9. Determination of encapsulation efficiency

Two ml of the paclitaxel loaded nanoparticle solutions were transferred into a 100 kDa Vivaspin device and centrifuged at 1000 × *g* for 20 min. One ml of the filtrate was mixed with scintillation cocktail (Packard BioScience, Germany). The activity of radio-labeled paclitaxel was quantified by liquid scintillation counting (LSC) (Tri-Carb 2100TR, Packard BioScience, Germany). The encapsulation efficiency was calculated by comparing the actual and theoretical loading in consideration of the ^{14}C-paclitaxel/paclitaxel ratio. Mass ratio of ^{14}C-labeled to unlabeled paclitaxel was 1/17. Experiments were determined in triplicates.

2.10. Release studies

Experiments investigating the release of paclitaxel (ratio: ^{14}C-paclitaxel:paclitaxel = 1:200) from the nanoparticles were performed using a dialysis-bag diffusion technique (Park et al., 2005). Therefore, 10 ml of nanoparticle suspension (40 μg paclitaxel) were placed in a dialysis membrane (regenerated cellulose; MWCO 10K) with standardized volume and membrane area. Dialysis was performed against 90 ml of 10 mM phosphate buffer pH 7.4 with 10% ethanol to create sink conditions for the poorly water soluble drug paclitaxel while stirring at 37 °C. At predetermined time intervals (0 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 36 h, 48 h), 1 ml aliquots of the aqueous solution were withdrawn from the release medium and replaced with the same volume of fresh medium to maintain non-saturation or sink conditions. The amount of paclitaxel released in each time interval was determined by liquid scintillation counting as described above.

2.11. Statistical analysis

Results are expressed as mean ± SEM (standard error of mean). Significance between the mean values was calculated using one-way ANOVA analysis with Bonferroni's post test. Predictive value (p) less than 0.05 was considered statistically significant, less than 0.01 as highly significant and less than 0.001 as extremely significant.

3. Results and discussion

Nanoparticles from the three different dendritic polymers were studied in comparison to evaluate which one is the most suitable for paclitaxel delivery. Due to their different properties and composition (see Table 1) with varying lipophilicity, they represent an interesting subject for such a comparison. Nanoparticles could be used for "passive tumor targeting" as they accumulate in certain solid tumors by the EPR effect (Danhier et al., 2010; Maeda et al., 2000). There is also a growing interest in more biocompatible formulations for paclitaxel and other taxanes (Gaucher et al., 2010).

3.1. Nanoparticle preparation

Nanoparticle preparation was performed by a solvent displacement method which is a very gentle (Nguyen et al., 2008a; Oster et al., 2006) and elegant method avoiding high shear forces. Particle formation occurs by the so-called Ouzo effect (Vitale and Katz, 2003). All three dendritic polymers formed stable, small nanopar-

Table 1
Properties of the nanoparticles formed from the different polymer types including size and zeta potential.

Polymer	Type	Functionalities	Mw[a] [g/mol]	Size [nm]	Zeta potential [mV]
Boltorn H40	Dendritic polyester	64 free OH-groups	5100	116 ± 4.8	−17.6 ± 9.9
Boltorn U3000	Lipophilic dendritic polymer	14 unsaturated fatty acids	6500	159.6 ± 6.1	−22.9 ± 0.3
Boltorn W3000	Amphiphilic dendritic polymer	Long unsaturated fatty acids. PEG chains	9000	72.5 ± 4.2	−4.8 ± 0.9

[a] As determined by GPC by the supplier (against PEG standards).

ticles. An overview of sizes and zeta potentials of nanoparticles formed of the different dendritic polymers can be found in Table 1.

3.2. Size and zeta potential

Sizes of nanoparticles were measured by dynamic light scattering. All dendritic polymers formed monomodally distributed nanoparticles in the desired range below 200 nm and therefore should not be detected by macrophages. Macrophage detection range starts between 200 and 250 nm (Dailey et al., 2006; Niven, 1995; Tsapis et al., 2002). Sizes showed extremely significant differences (***). Boltorn H40, the pure dendritic polyester, showed sizes of 116 nm, Boltorn U3000, the fatty acid modified dendritic polymer, resulted in larger nanoparticles (160 nm), Boltorn W3000, the amphiphilic dendritic polymer, displayed small sizes (73 nm). Zeta potentials for all three polymers were slightly negative. Zeta potentials of Boltorn U3000, the lipophilic one and Boltorn H40, the pure polyester, were roughly around −20 mV. A zeta potential in this range is often observed in polyester nanoparticles (e.g. prepared form PLGA) (Fonseca et al., 2002). The zeta potential of Boltorn W3000 was closer to zero, which could be due to PEG shielding. (Similar effects have been described for PEG-PEI where zeta potentials around zero were measured (Nguyen et al., 2008b).) Another explanation for this zeta potential could be due to the reduced hydrophobicity caused by the PEG chain. This zeta potential was significantly higher (*) than for Boltorn U3000 nanoparticles which only possess the fatty acid chains. In general, hydrophobic nanoparticles often display a slightly negative zeta potential (Tandon et al., 2008).

3.3. Hydrolytic degradation by pH stat titration

In order to investigate whether the dendritic polymers were hydrolytically degradable at a physiological pH and temperature, a degradation experiment was carried out in a pH stat titration at pH 7.4 and 37 °C (see Fig. 2). All formulations were prepared at a polymer concentration of 2 mg/ml. When the ester bonds of the hyperbranched polymer cleave, the resulting carboxylic acid can be titrated by NaOH. During pH stat titration, pH value is always adjusted to pH 7.4 by constantly neutralizing the free acids. The amount of required NaOH monitors the degradation (Ficara et al., 2003). The hyperbranched polyester Boltorn H40 showed hydrolytic degradation; 6.95% of ester bonds were cleaved during 24 h.

Degradation of the other two dendritic polymers, Boltorn U3000 and Boltorn W3000, was slower, showing only very few cleaved ester bonds after titration. This is probably caused by the presence of lipophilic fatty acid groups, which both polymers possess, as well as by the lower ester content. The ester degradation follows a first order kinetic and is therefore concentration dependent. Both dendritic polymers, Boltorn U3000 and Boltorn W3000, possess less ester in 2 mg/ml compared to Boltorn H40 which does not contain any hydrophilic or lipophilic chains. An overview of all degradation results can be seen in Table 2.

3.4. Mass loss

To obtain more information about degradation rate and kinetics, mass loss experiments were performed to screen a longer period of time on Boltorn H40 (see Fig. 3). This polymer was chosen as it

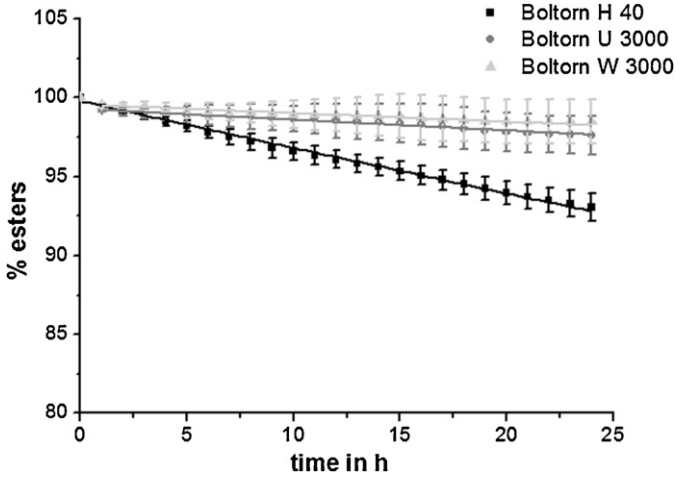

Fig. 2. pH stat titration profiles of all three dendritic polymers (expressed in % esters).

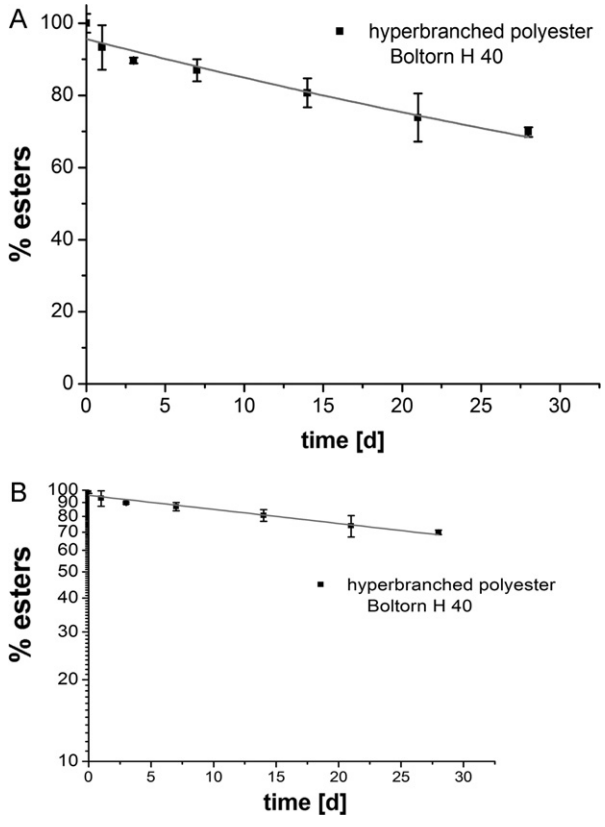

Fig. 3. Mass loss of the hyperbranched polyester at pH 7.4 and 37 °C during 4 weeks: (A) *y* axis in linear scale and (B) *y* axis in log scale.

Table 2
Degradation results of different dendritic polymers (Boltorn H40, Boltorn U3000, Boltorn W3000).

Polymer	Method	Conditions	Duration	% Degradation	k [s^{-1}]
Boltorn H40	pH-stat-titration	37 °C, pH 7.4	24 h	6.95 ± 0.86%	0.00302 (±0.00013)
Boltorn U3000	pH-stat-titration	37 °C, pH 7.4	24 h	2.39 ± 1.24%	0.00069 (±0.00020)
Boltorn W3000	pH-stat-titration	37 °C, pH 7.4	24 h	1.50 ± 1.42%	0.00055 (±0.00022)
Boltorn H40	Mass loss	37 °C, pH 7.4	28 days	30.1 ± 1.3%	0.0113 (±0.0012)

showed a faster degradation profile than the other dendritic polymers tested. (As pH stat titration revealed a very slow degradation process for Boltorn U3000 and Boltorn W3000 at 2 mg/ml due to their lipophilicity and lower ester content, these two were not included in the mass loss studies.) Mass loss was investigated by degrading the polymer at 37 °C and at pH 7.4 during 28 days, taking samples at different time points (0, 1, 3, 7, 14, 21, 28 days). The samples were gently mixed by vertical rotation. These experiments were conducted without any addition of enzymes, so that ester bonds degraded hydrolytically. The polymer itself is insoluble in an aqueous phase, while low molecular weight degradation products like the resulting bis-hydroxymethylpropylic acid (bis-MPA) are water-soluble. The insoluble polymer part was quantified after centrifugation. Ester degradation tends to show exponential decay of first order. This is described by the following equation: $y = A_1 \times e^{(-t_{k_1})}$ where y is the percentage of intact esters, A_1 is the initial concentration of the esters which were cleaved, k_1 is the decay constant from esters.

The decay constant k_1 of the hyperbranched polyester is 0.0113 (±0.00124816) and R^2 is 0.9556. During the period tested, half life has not been reached yet. After, 28 days, about 30% of the polymer was degraded (see Table 2). The reason why the degradation constant observed by the mass loss method is higher than by pH stat titration is most likely caused by the fact that oligomers might be already water soluble leading to a loss of mass.

3.5. Cytotoxicity by MTT assay

To evaluate the toxicity of the used dendritic polymers, MTT assays were performed (see Fig. 4). Tests were performed with L929 cells, a standard cell line for this type of toxicity evaluation (Varghese et al., 2011). The hyperbranched polyester displayed a relatively low cytotoxicity compared to commercially available 25 K bPEI. PEI 25K showed an IC_{50} value of 0.009 mg/ml. No IC_{50} value could be detected for the hyperbranched polyester Boltorn H40 for all concentrations tested. For concentrations of 0.01 mg/ml and smaller, cell viabilities were above 75%. In the concentration range from 0.1 to 1 mg/ml, cell viability decreased to levels between 56 and 65%. For Boltorn W3000, the IC_{50} value was 0.51 mg/ml and for Boltorn U3000, IC_{50} was 0.952 mg/ml. For concentrations up to 0.25 mg/ml, cell viabilities were above 85% for both, Boltorn U3000 and Boltorn W3000, showing that all tested hyperbranched polymers display a relatively low cytotoxicity.

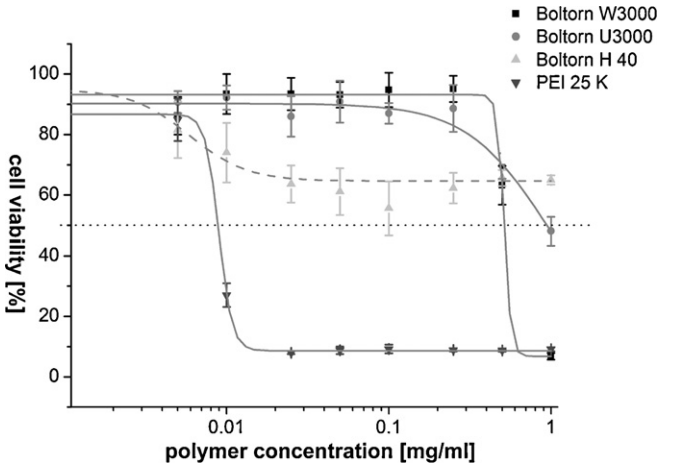

Fig. 4. Cytotoxicity assessed by MTT assay. PEI 25K was used as a positive control.

3.6. Paclitaxel loading

For all three types of nanoparticles, we aimed for 0.2% (w/w) of paclitaxel loading. Other paclitaxel loadings will be subject to future studies. The loading efficiencies determined were highest for Boltorn W3000, the amphiphilic dendritic polymer and Boltorn U3000, the lipophilic one (see Fig. 5). The unmodified dendritic polyester Boltorn H40 showed an extremely significantly (***) lower loading efficiency. Additional chains in Boltorn U3000 and W3000 seem to have a beneficial effect on the loading efficiency. The lipophilic chains increased loading probably by additional lipophilic interaction with paclitaxel. So paclitaxel interaction slightly improves due to the presence of fatty acid chains in the amphiphilic and lipophilic structure. Hydrophilic and lipophilic changes in the polymer composition did not seem to make such a large difference with regard to encapsulation as expected. We hypothesize that this is most likely due to the arrangement of the lipophilic and hydrophilic regions of the polymer when forming nanoparticles. Supposedly, the fatty acid and polyester regions of the polymer are in close contact to paclitaxel while PEG groups are probably directed to the nanoparticle surface to interact with water.

3.7. Sizes and zeta potential of paclitaxel loaded nanoparticles

When comparing loaded and unloaded nanoparticles with regard to their sizes and zeta potentials, very few differences were observed (see Fig. 6). After paclitaxel loading, zeta potentials did not show any significant changes. Sizes showed a slight increase after loading but were still in the desired range. This was the case for all three dendritic polymers. Each dendritic polymer together with paclitaxel loading yielded nanoparticles with similar sizes and zeta potentials like the ones observed for the corresponding pure dendritic polymer. So loading had no negative effect on these properties.

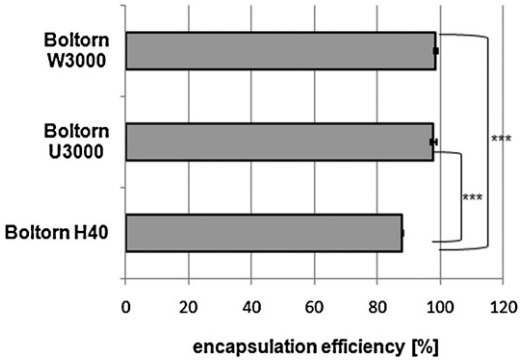

Fig. 5. Paclitaxel loading for different nanoparticles (Boltorn H40, Boltorn U3000 and Boltorn W3000).

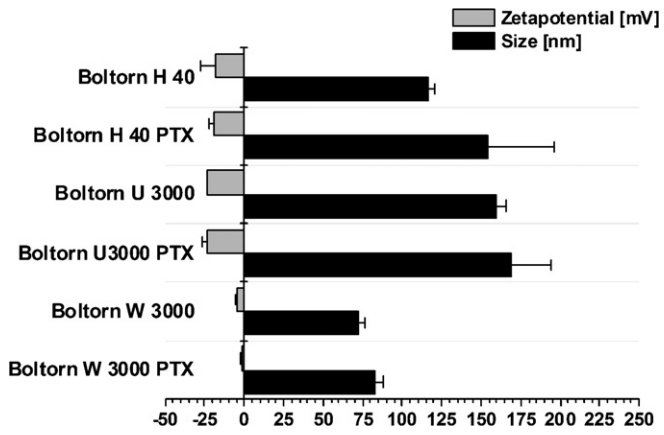

Fig. 6. Size and zeta potential of the nanoparticles from hyperbranched polymers paclitaxel (PTX) loaded and unloaded.

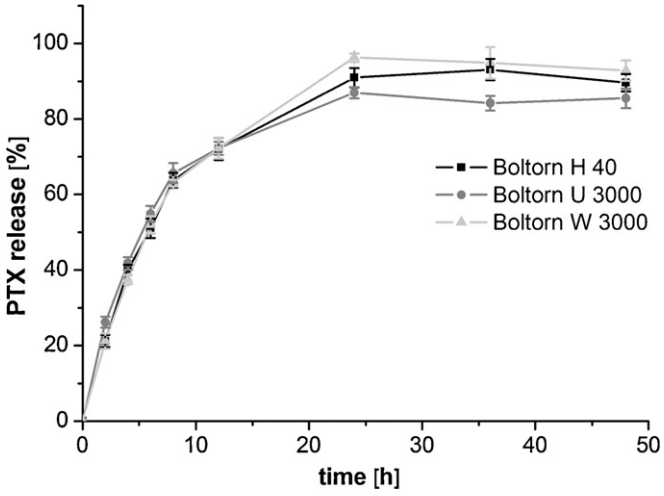

Fig. 7. Release profiles of nanoparticles of different hyperbranched polymers.

3.8. Release studies

Release profiles were generated using membrane dialysis which is a valuable system for drug release studies from nanocarriers (Park et al., 2005). The nanocarriers can be easily separated from the release buffer, without any shear forces affecting the particle integrity. For instance, centrifugation could lead to particle aggregation or rupture which could result in measurements of too high or too low release rates. To achieve sink conditions for the poorly water-soluble compound paclitaxel, 10% ethanol was added as a cosolvent (Fig. 7).

As tested in this simple dialysis model, the release of the nanoparticles was not influenced by the different compositions of the hyperbranched polymers. Release behavior of these polymers may be more related to particle size and therefore diffusion distance than to their lipophilicity or hydrophilicity. The release interval reaches more than 12 h which should be sufficient for delivery and targeting to specific regions.

4. Conclusion

All dendritic polymers were able to form stable nanoparticles by solvent displacement. Sizes and zeta potentials were in the desired range. Sizes were below 200 nm, zeta potentials were slightly negative. Sizes of Boltorn U3000, the most lipophilic one, were slightly larger than for the other nanoparticles. Nanoparticles from Boltorn W3000 displayed zeta potentials closer to zero which might be due to the neutral PEG chains. Degradation rates were fastest for the Boltorn H40 nanoparticles, the ones from the unmodified polymer. The other two dendritic polymers showed much slower degradation rates and constants, probably due to their content of lipophilic groups or their lower ester content. Therefore, only Boltorn H40 was tested for a 28 days period of time for mass loss study showing 30% mass loss. The degradation constant was slightly higher than for the pH stat titration, as the mass loss also includes soluble dimers or trimers of the bis-MPA. All tested nanoparticle types showed a low cytotoxicity. It was possible to load all dendritic polyesters tested with paclitaxel. Loading was lower for the hyperbranched polyester Boltorn H40, loadings for the amphiphilic compound Boltorn W3000 and Boltorn U3000, the lipophilic one, were very high. Their better loading capacity is probably due to favorable interactions of paclitaxel with their fatty acid chains. Sizes and zeta potentials were hardly influenced by the loading. All tested hyperbranched polymers showed a comparable release profile. This means, that all three types show potential as paclitaxel carriers and are worth further investigations *in vitro* and *in vivo*. But due to their higher loading capacities, Boltorn U3000 and W3000 represent the most promising candidates amongst them.

Acknowledgements

We would like to thank Eva Mohr for support in the cell culture laboratory. We thank Simon Harrisson for revising the manuscript.

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