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Poly(trimethylene carbonate) and monomethoxy poly(ethylene glycol)-*block*-poly(trimethylene carbonate) nanoparticles for the controlled release of dexamethasone

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Abstract

In this study, single emulsion and salting out methods were employed to prepare poly(trimethylene carbonate) (PTMC) and monomethoxy poly (ethylene glycol)-*block*-poly(trimethylene carbonate) (mPEG–PTMC) nanoparticles. Well-defined nanoparticles of a PTMC homopolymer were prepared using poly(vinyl alcohol) (PVA) as a stabilizer. The average size of the nanoparticles can be adjusted by varying the stirring speed and polymer concentration. These particles can be readily freeze-dried and redispersed, with little influence on the average particle size and size distribution. Nanoparticles based on amphiphilic mPEG–PTMC can be prepared without an additional stabilizer. In this case, the size of the obtained nanoparticles did not vary much and ranged between 95 and 120 nm. These nanoparticles could be freeze-dried and redispersed as well. Using the salting out method, dexamethasone was loaded into PTMC and mPEG–PTMC nanoparticles at a highest efficiency of respectively 54% and 88%. With the single emulsion method, the loading efficiencies were, respectively, 91% and 72%. These drug-loaded particles were stable in time for at least 20 weeks. It was found that the release of dexamethasone from these nanoparticles was diffusion-controlled and could be sustained for 14 to 60 days. Depending on the nature of the polymer employed and the preparation method, dexamethasone diffusion coefficients varied between 4.8×10^{-18} and 22.6×10^{-18} cm^2/s.

Keywords: Controlled release; Nanoparticles; Poly(trimethylene carbonate); Poly(ethylene glycol)-*block*-poly(trimethylene carbonate); Dexamethasone

1. Introduction

The first polymeric nanoparticles for the controlled drug delivery were developed in 1970s [1–4]. Since then, much research has been carried out to prepare such systems from biodegradable polyesters [5–7]. However, the degradation products resulting from polyester hydrolysis can lead to an increase in acidity, which could be deleterious to the loaded active drug components [8]. Also the bulk degradation behavior of polyesters [9] may result in an accelerated release of the drug at the late stages of the degradation process. Poly(trimethylene carbonate) (PTMC) degrades in vivo by surface erosion without the formation of acidic compounds [10,11]. Recently, we found that PTMC homopolymer and its block copolymer with monomethoxy poly(ethylene glycol) (mPEG–PTMC) is stable in water, but can be degraded in lipase solutions by an enzymatic surface erosion process [11,12].

In spite of their advantageous properties with regard to degradation, TMC-based (co)polymers have not been widely used in the preparation of drug delivery systems. A few examples are the release of hydrophobic drugs from films based on poly(1,4-dioxan-2-one-*co*-TMC) random copolymers [13], PTMC–PEG–PTMC triblock copolymers [14], and PTMC and poly(adipic anhydride) blends [15]. Also self-assembling mPEG–poly(ε-caprolactone-*co*-TMC) micelles for the oral delivery of poorly water-soluble drugs has been reported [16]. We have reported on the characteristics of microparticles based on PTMC and mPEG–PTMC block copolymers for the loading and release of hydrophilic model compounds (Coomassie® Brilliant Blue G, lysozyme and BSA) as well [17].

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Very recently, the properties of PTMC–PEG–PTMC nanoparticles for the controlled release of methotrexate (a hydrophobic, anticancer drug) were reported [18]. These nanoparticles were prepared by a dialysis technique, which resulted in relatively low loading efficiencies of 7% to 30%. Hydrophobic drugs can be loaded into polymeric nanoparticles at higher loading efficiencies using salting out [19] and single emulsion [20] methods. The salting out method consists of the emulsification of a polymer solution in a water-miscible solvent in an aqueous salt solution. Due to the high salt concentration, the phases are immiscible. Upon addition of water, nanoparticles are formed as the organic solvent diffuses into the water phase. In the single emulsion method, a water-immiscible polymer solution is emulsified in an aqueous phase. Evaporation of the organic solvent then leads to the formation of nanoparticles.

In this study, nanoparticles based on PTMC and mPEG–PTMC block copolymer were prepared by the salting out and the single emulsion methods. The effects of polymer structure, preparation method, stirring speed, polymer concentration, and freeze-drying and redispersion on the resulting nanoparticle properties were investigated. These nanoparticles were loaded at high efficiencies with dexamethasone, a poorly water-soluble, anti-inflammatory and immuno-suppressive drug [21,22]. The stability of the drug-loaded nanoparticles and the release profiles of dexamethasone were evaluated.

2. Materials and methods

2.1. Materials

Polymer grade 1,3-trimethylene carbonate (TMC) was purchased from Boehringer Ingelheim (Germany). Monomethoxy poly(ethylene glycol) ($M_n = 3\times10^3$ g/mol, $mPEG_3$) was obtained from Shearwater Polymers (USA) and was used without further purification. Stannous octoate ($SnOct_2$) and dexamethasone were purchased from Sigma (USA). Poly(vinyl alcohol) (PVA, 80% hydrolysed poly(vinyl acetate) with M_n of approximately 9.5×10^3 g/mol), sodium dodecyl sulphate (SDS), and deuterated dimethyl sulfoxide (DMSO) were purchased from Aldrich (USA). Deuterated chloroform, magnesium chloride hexahydrate ($MgCl_2\cdot6H_2O$) and 1-hexanol were purchased from Merck (Germany). 1-Hexanol was distilled over CaH_2 (Acros, Belgium). Phosphate buffered saline (PBS, pH = 7.4) was purchased from NPBI (Emmer Compascuum, The Netherlands). Cellulose ester membranes with a molecular weight cut off value (MWCO) of 3500 (Spectrum Laboratories, USA) were used in dialysis experiments. Water was deionised using a Milli-Q water purification system (Millipore, France).

2.2. Synthesis of polymers and their characterization

Poly(TMC) (PTMC) and a block copolymer of $mPEG_3$ and PTMC ($mPEG_3$–PTMC) were synthesized in dried, freshly silanized glass ampoules. The ampoules were purged with dry argon, charged with TMC monomer, initiator and catalyst (2×10^{-4} mol $SnOct_2$ per mol TMC) and heat-sealed under vacuum. In the polymerization of TMC, 1-hexanol (2.0×10^{-3} mol per mol TMC) was used as an initiator. In the preparation of $mPEG_3$–PTMC block copolymer, 1.0×10^{-2} mol $mPEG_3$ per mol TMC was employed. Assuming each hydroxyl group initiates a polymer chain, it can be expected that PTMC with a molecular weight of 50×10^3 and $mPEG_3$–PTMC with a total molecular weight of 13×10^3 g/mol are prepared. The polymerizations were conducted at 130 ± 1 °C for 3 days. Under these conditions monomer conversion was in excess of 99%. The polymers were purified by precipitating polymer solutions in chloroform into an excess of hexane and drying in vacuo.

Matrix-assisted laser desorption ionisation time-of-flight (MALDI–TOF) mass spectrometry (Voyager-DE-RP, Applied Biosystems/PerSeptive Biosystems, USA) was performed to determine the M_n and M_w/M_n of the employed $mPEG_3$. As a matrix, 2,5-dihydroxybenzoic acid (DHBA) was used. The M_n and M_w/M_n values were found to be 3.2×10^3 g/mol and 1.02, respectively. Nuclear magnetic resonance (^{1}H NMR) spectroscopy at 300 MHz (Varian Inova, USA) was used to verify the number average molecular weight (M_n) of $mPEG_3$, and to determine the composition of the prepared diblock copolymer. Deuterated chloroform was used as solvent unless mentioned otherwise. Gel permeation chromatography (GPC) using chloroform as eluent was employed to determine molecular weights and molecular weight distributions [23]. Table 1shows characteristics of the employed polymers. From the result of ^{1}H NMR, the diblock copolymer is abbreviated as $mPEG_3$–$PTMC_{11}$.

2.3. Preparation of nanoparticles and their characterization

2.3.1. Nanoparticle preparation by the salting out method [24]

For the preparation of PTMC nanoparticles, 5.5 ml THF solutions containing 1 to 4 wt.% polymer were emulsified in 10 ml aqueous solutions under mechanical stirring at 6500 to 24 000 rpm for 40 s using a homogenizer (T25 Ultraturrax equipped with a S25 dispersing tool, Ika Labortechnik, Germany). The aqueous phase contained 60 wt.% $MgCl_2\cdot6H_2O$ and 2 wt.% PVA. Then another 10 ml of a 0.5 wt.%

Table 1
Characteristics of $mPEG_3$ and the synthesized PTMC homopolymer and $mPEG_3$–$PTMC_{11}$ block copolymer

Polymer[a]	Initiator	M_n[b] (10^3 g/mol)	M_n[c] (10^3 g/mol)	M_w/M_n[c]
$mPEG_3$	–	3.1	4.0[d]	1.04[d]
PTMC	1-hexanol	not determined	69.2	1.8
$mPEG_3$–$PTMC_{11}$	$mPEG_3$	$mPEG_3$ block: 3.1 PTMC block: 10.8	21.4	1.7

[a] The subscript refers to the number average molecular weight (M_n) of the polymer (blocks) in 10^3 g/mol.
[b] Determined by ^{1}H NMR.
[c] Determined by GPC.
[d] MALDI–TOF determinations gave values of 3.2×10^3 g/mol for M_n and 1.02 for M_w/M_n.

PVA solution in water was quickly added and the stirring was continued for another 30 s. As more water is added, the THF and water phases become miscible and stable PTMC nanoparticles form.

The nanoparticles were purified by diluting with an equal volume of a 0.5 wt.% PVA solution in water, ultra-centrifugation at 25 000 rpm for 30 min (Centrikon T-2180, Kontron Instruments, UK) and removal of the supernatant. Then the nanoparticles were redispersed in 0.5 wt.% PVA solution in water. The ultra-centrifugation and redispersion steps were repeated twice.

Nanoparticles from $mPEG_3$–$PTMC_{11}$ could be prepared without the use of an additional stabilizer. The same preparation and purification methods as described above were employed, except that the aqueous phase did not contain PVA.

2.3.2. Nanoparticle preparation by the single emulsion method

PTMC nanoparticles were prepared by emulsifying 7.5 ml of a 1 to 4 wt.% solution of polymer in CH_2Cl_2 in 20 ml of water containing 2 wt.% PVA under mechanical stirring at 6500 to 24 000 rpm for 40 s. This emulsion was then added to 30 ml of a 0.5 wt.% PVA solution in water under mechanical stirring at 600 rpm. The stirring was continued at room temperature for 1 h to evaporate CH_2Cl_2. The formed nanoparticles were purified by ultra-centrifugation and redispersion as described above.

For the preparation and purification of $mPEG_3$–$PTMC_{11}$ nanoparticles the same procedure was followed except that the aqueous phase did not contain PVA as an additional stabilizer.

2.3.3. Freeze-drying of nanoparticles

After purification, the nanoparticle dispersions were frozen in liquid nitrogen for 10 min and freeze-dried in vacuum for 2 days. Then the freeze-dried nanoparticles were redispersed as described above.

2.3.4. Size determination of nanoparticles by dynamic light scattering

The average sizes and the size distributions of the prepared nanoparticles were determined by dynamic light scattering (DLS, Zetasizer 4000, Malvern Instruments, UK) at 25 °C from the intensity of light (633 nm) scattered by the particles at an angle of 90°, taking the average of four measurements. The particle size and polydispersity index (P.I.) were determined by the CONTIN-method.

2.4. Loading and release of dexamethasone from PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles

As dexamethasone is soluble in organic solvents, dexamethasone-loaded nanoparticles can be prepared from common solutions of drug and polymer. Dexamethasone-loaded PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles were prepared by the previously described methods at a stirring speed of 24 000 rpm. In the preparation of dexamethasone-loaded nanoparticles by the salting out method, the polymer was dissolved in a dexamethasone solution in THF. The polymer concentration was either 2.0 wt.% (100 mg polymer in 5.5 ml THF) or 5.3 wt.% (150 mg polymer in 3 ml THF), while the amount of dexamethasone was varied between 16 and 21 wt.% relative to the total weight of the polymer and the drug. In the preparation by the single emulsion method, solutions in chloroform were prepared. The polymer concentration was 2 wt.% and the amount of dexamethasone was approximately 17 wt.% relative to the total weight of the polymer and the drug. The characterization of the drug-loaded nanoparticles was done as previously described after purification by centrifugation and redispersion.

The loading and the loading efficiency of dexamethasone in the nanoparticles were determined by ^{1}H NMR in deuterated DMSO after the water was removed by freeze-drying after ultra-centrifugation. The integral values of characteristic resonances of dexamethasone (1*H*, δ=7.28–7.31 ppm, doublet; 1*H*, δ=6.20–6.24 ppm, doublet; 1*H*, δ=6.01 ppm, singlet) were compared to those of methylene protons in TMC (4*H*, δ=4.11–4.17 ppm, triplet), which allows the calculation of the loading and loading efficiency. To calculate loading and loading efficiency in the prepared PTMC nanoparticles, PVA was not added in the redispersion steps. It should be noted that this leads to aggregation of the particles.

To investigate the release profiles of dexamethasone, the PTMC nanoparticles loaded with dexamethasone were redispersed in water containing 0.5 wt.% PVA, while $mPEG_3$–$PTMC_{11}$ nanoparticles were redispersed in water. The final nanoparticle concentrations were 7.5 mg/ml.

Of the nanoparticle dispersions, 1 ml was added in a dialysis bag and dialyzed at 37 °C against 75 ml water containing 0.03 wt.% SDS as the release medium. Under these conditions, the maximum concentration of dexamethasone that can be reached (15 μg/ml) was less than 10% of the maximum solubility of dexamethasone in the release medium, as it was found that dexamethasone could be dissolved in the SDS-containing water at a concentration of 150 μg/ml. Sink conditions are therefore ensured [25].

At regular time intervals, 3 ml samples were taken from the release medium, which was replenished with 3 ml water containing 0.03 wt.% SDS. The amount of released dexamethasone was determined by UV at a wavelength of 242 nm (Cary 300 Bio UV–Visible spectrophotometer, Varian, The Netherlands). The release experiments were performed in duplicate.

2.5. Nanoparticle stability

The stability of dexamethasone-loaded PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticle dispersions (7.5 mg/ml) at 37 °C was investigated by determining the particle size at regular time intervals by DLS. In the case of PTMC nanoparticles, the water contained 0.5 wt.% PVA.

To evaluate potential changes in composition of the nanoparticles due to polymer degradation, a non-loaded $mPEG_3$–$PTMC_{11}$ nanoparticle dispersion in water (5 mg/ml) was prepared by the single emulsion method and kept at 37 °C. At regular time intervals, the size and molecular composition of the nanoparticles were determined by DLS and ^{1}H NMR, respectively. The experiments were performed in duplicate.

3. Results and discussion

3.1. Preparation and characterization of PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles

In controlled release applications using systemically injected particles, the size of the particles is important as it was found that particles with sizes ranging from 70 nm to 200 nm have the most prolonged circulation time due to the least accumulation in the liver and in the spleen [26]. In vitro, phagocytosis of polystyrene and phenylated polyacrolein microspheres by macrophages was most pronounced for particles with a diameter of 1 to 2 μm, and particle uptake gradually decreased as their size decreased [27]. We employed two commonly used methods (salting out and single emulsion methods) to prepare nanoparticles of PTMC and $mPEG_3$–$PTMC_{11}$ polymers. The properties of the polymers are shown in Table 1. The effects of stirring speed and polymer concentration on the size of the nanoparticles were investigated.

The PTMC homopolymer is hydrophobic; therefore, the surface that is created during an emulsification step needs to be stabilized. For this reason, in the preparation of PTMC nanoparticles by both methods, the aqueous phase contained water-soluble PVA (2 wt.%) as a stabilizer. The relationships of the stirring speed and the polymer concentration with the size of the prepared PTMC particles are shown in Fig. 1.

Using the single emulsion method, well-defined PTMC nanoparticles with sizes between 285 and 426 nm and a polydispersity index (P.I.) smaller than 0.2 could be obtained at stirring speeds above 13 500 rpm. The particle size strongly depended on the stirring speed and polymer concentration. The smallest particles were formed at the highest stirring speed and at the lowest concentration of polymer in the organic phase. This result can be expected as the size of the formed droplets in the emulsification process is related to the total energy input and viscosity of the polymer solutions.

When applying the salting out method, PTMC nanoparticles with sizes between 183 and 251 nm were formed. The effects of stirring speed and polymer concentration on nanoparticle size were less pronounced than in the single emulsion method and always much smaller particles were obtained under comparable conditions. These differences are probably due to the fact that the organic solvent used in the salting out method (THF) is water miscible, while CH_2Cl_2, which is used in the single emulsion method, is not. The required energy in forming droplet surfaces is therefore expected to be less in the salting out method.

Often, in biomedical applications, the use of a stabilizer such as PVA is not desired. Therefore, we investigated the preparation of nanoparticles from self-stabilizing, amphiphilic $mPEG_3$–$PTMC_{11}$ block copolymers as well. Such particles have the additional advantage that circulation times are much increased as their clearance in vivo is decreased or delayed [20].

From $mPEG_3$–$PTMC_{11}$ block copolymers, well-defined nanoparticles with sizes of approximately 100 nm could readily be prepared by both the salting out and the single emulsion methods without the use of an additional stabilizer. Interestingly, the size of the nanoparticles did not vary significantly in spite

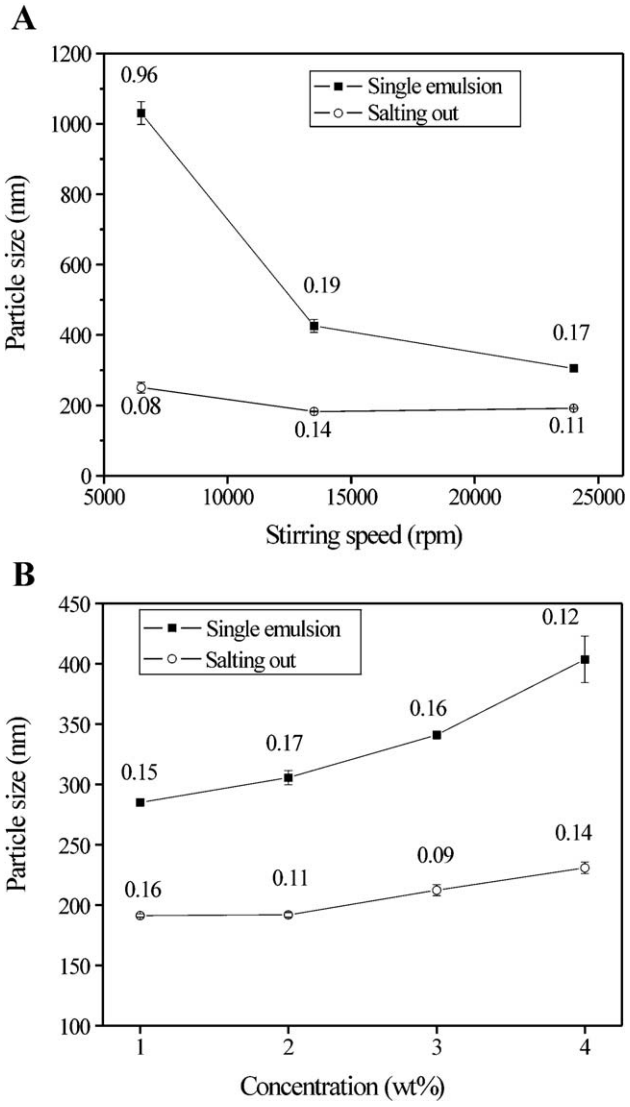

Fig. 1. (A) Average size of PTMC particles prepared at different stirring speeds when the polymer concentration in the organic phase is 2 wt.%. (B) Average size of the prepared PTMC particles at different polymer concentrations when the stirring speed is 24 000 rpm. The numbers correspond to the polydispersity index values of the particle size distributions. All experiments were performed in duplicate.

of the large variations in stirring speeds (between 600 and 24 000 rpm) and polymer concentrations (between 1 and 10 wt.%), and was always between 95 and 120 nm. The polydispersity index values were always smaller than 0.2. These results illustrate the low energy input required to prepare the nanoparticles and the self-stabilizing properties of the mPEG segment in the $mPEG_3$–$PTMC_{11}$ polymer.

In pharmaceutical applications, it is important to be able to store the (drug-loaded) nanoparticles. Often, the particles are freeze-dried and then redispersed at a later stage. Table 2 shows the average sizes and the P.I. of the redispersed PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles before and after freeze-drying, and after keeping the dispersed nanoparticles for 2 weeks at room temperature.

PTMC nanoparticles were obtained by freeze-drying a dispersion in water containing 0.5 wt.% PVA. The freeze-

Table 2
Average sizes and P.I. values of redispersed nanoparticles before and after freeze-drying, and after keeping the redispersed nanoparticles for 2 weeks at room temperature

Polymer[a]	Method[b]	Before freeze-drying		After freeze-drying and redispersion		2 weeks after freeze-drying and redispersion	
		Size (nm)	P.I.	Size (nm)	P.I.	Size (nm)	P.I.
PTMC	s.o.	181±1	0.17	184±3	0.21	183±2	0.18
PTMC	s.e.	316±3	0.13	334±4	0.17	321±5	0.16
$mPEG_3$–$PTMC_{11}$	s.o.	113±1	0.19	270±5	0.50	188±7	0.18
$mPEG_3$–$PTMC_{11}$	s.e.	110±5	0.13	214±4	0.52	179±7	0.13

Experiments were performed in duplicate.
[a] PTMC nanoparticles were obtained by freeze-drying a dispersion in water containing 0.5 wt.% PVA. $mPEG_3$–$PTMC_{11}$ nanoparticles were obtained by freeze-drying a dispersion in water.
[b] s.o.=salting-out method; s.e.=single emulsion method.

dried nanoparticles could be redispersed in water at room temperature in a few hrs, without significant changes in average size and P.I. of the nanoparticles. $mPEG_3$–$PTMC_{11}$ nanoparticles, obtained after freeze-drying aqueous dispersions only, i.e. without PVA stabilizer in the aqueous phase, could also be redispersed. The required redispersion time had extended to 1 day. Although the average size and P.I. of the nanoparticles increased significantly after freeze-drying and redispersion, keeping the dispersions at room temperature for 2 weeks decreased these values. More effective redispersion methods to prepared $mPEG_3$–$PTMC_{11}$ nanoparticles, such as sonication, might be required in preparing the final dispersions before use.

PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles could also be redispersed in PBS solutions (pH=7.4), giving dispersions that were stable for at least 7 days.

3.2. Loading and release of dexamethasone from PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles

Dexamethasone is a well-known compound that inhibits many of the pathways involved in restenosis due to its anti-inflammatory and immuno-suppresive properties. It is a hydrophobic drug, which is poorly soluble in water, but has good solubility in organic solvents such as THF and chloroform. Therefore, dexamethasone is a relevant model compound to perform loading and release studies with. It can be loaded into the PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles by dissolving the drug in the organic phase during preparation of the nanoparticles.

Table 3 shows characteristics of dexamethasone-loaded nanoparticles prepared by the salting out method and the single emulsion method as previously described. In drug delivery applications, a high loading efficiency is desired. In our studies, we aimed at loading the nanoparticles with an amount of dexamethasone that ranged between 16.0 wt.% and 20.7 wt.%. The addition of dexamethasone to the organic phase did not influence average sizes and polydispersity index values of the formed nanoparticles. In all cases, the average sizes of dexamethasone-loaded nanoparticles agreed well with those of non-loaded nanoparticles. Also the values of the polydispersity index remained low.

In the salting out method, dexamethasone is dissolved in the organic phase (THF). When the polymer concentration in this phase was 2.0 wt.%, PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles were prepared in which the dexamethasone loading was only 2.0 wt.% and 3.6 wt.%, respectively. These values correspond to loading efficiencies below 20%. During the solidification process of the particles, THF is rapidly extracted with water. As dexamethasone is readily soluble in THF, a significant loss of the drug could occur. To increase the efficiency of the drug loading process, the amount of THF needs to be decreased. Therefore, the polymer concentration in the organic phase was increased to 5.3 wt.%. Now, much higher loading efficiencies, 54% and 88%, were achieved for PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles, respectively, prepared with the salting out method.

When the polymer concentration was only 2 wt.%, the single emulsion method allowed the preparation of PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles with very high dexamethasone loading efficiencies of 91% and 72%, respectively. These high loading efficiencies are probably because chloroform, the organic solvent employed in the single emulsion method, is immiscible with water and slowly evaporates during the

Table 3
Characteristics of dexamethasone-loaded PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles

Entry	Polymer	Stabilizer	[Polymer] (wt.%)	Size (nm)	P.I.	Loading (wt.%)		Loading efficiency (%)
						Aim	Achieved	
Salting out method								
1	PTMC	PVA	2.0[a]	181	0.17	20.7	2.0	9.7
2	$mPEG_3$–$PTMC_{11}$	None	2.0[a]	116	0.21	20.7	3.6	17.4
3	PTMC	PVA	5.3[b]	186	0.05	16.0	8.7	54.4
4	$mPEG_3$–$PTMC_{11}$	None	5.3[b]	95	0.20	16.0	14.1	88.1
Single emulsion method								
5	PTMC	PVA	2.0[c]	261	0.10	17.1	15.6	91.2
6	$mPEG_3$–$PTMC_{11}$	None	2.0[c]	103	0.17	16.4	11.8	72.0

[a] 100 mg polymer per 5.5 ml THF was emulsified in 20 ml aqueous phase.
[b] 150 mg polymer per 3 ml THF was emulsified in 20 ml aqueous phase.
[c] 150 mg polymer per 5 ml chloroform was emulsified in 20 ml aqueous phase.

solidification process. Consequently, loss of dexamethasone into the aqueous phase is limited.

To investigate the effect of the polymer used and the preparation method employed on the dexamethasone release properties of the nanoparticles, release experiments were conducted using dispersions of nanoparticles with the highest drug loading efficiencies (entries 3 to 6 in Table 3). The amount of drug-releasing particles was chosen to ensure that sink conditions were met throughout the experiment.

At regular time intervals, the amount of dexamethasone released in the medium was determined using UV spectroscopy. The cumulative dexamethasone release profiles are shown in Fig. 2.

Fig. 2 shows that the release of dexamethasone from both PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles prepared by the salting out method is relatively fast; most of the drug was released within 2 weeks. The release of dexamethasone from nanoparticles prepared by the single emulsion method was slower, especially in the case of PTMC nanoparticles. From these particles, dexamethasone was released throughout a period of 60 days. NMR analyses of the nanoparticles at the end of the experiments confirmed the complete release of dexamethasone.

3.3. Stability of dexamethasone-loaded PTMC and $mPEG_3$–$PTMC_{11}$ nanoparticles

As a change in the average particle size of a dispersion of the nanoparticles would imply a reduced stability, we carried out DLS particle size determinations of dexamethasone-loaded nanoparticles for prolonged periods of time at 37 °C. The same nanoparticles as used in the release experiments (Fig. 2), which were prepared from different polymers and by different preparation methods (entries 3 to 6 in Table 3), were used. Fig. 3 clearly shows that all drug-loaded nanoparticles were stable in time, without significant changes in their average sizes. At all times the polydispersity index values also remained below 0.2.

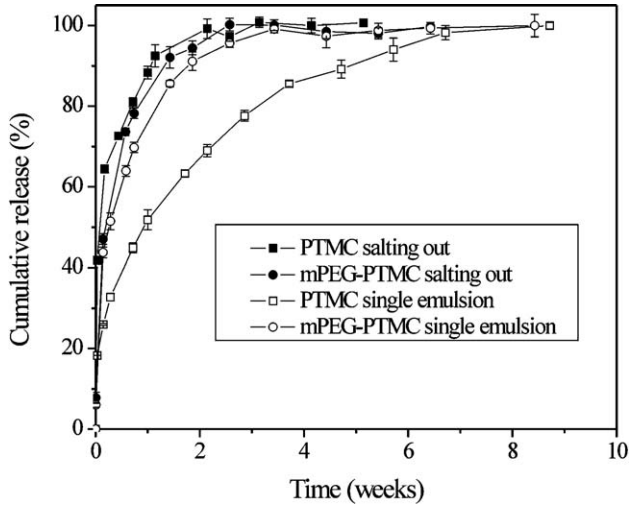

Fig. 2. Cumulative dexamethasone release profiles from drug-loaded nanoparticles (entries 3 to 6 in Table 3) in water containing 0.03 wt.% SDS at 37 °C. The experiments were performed in duplicate.

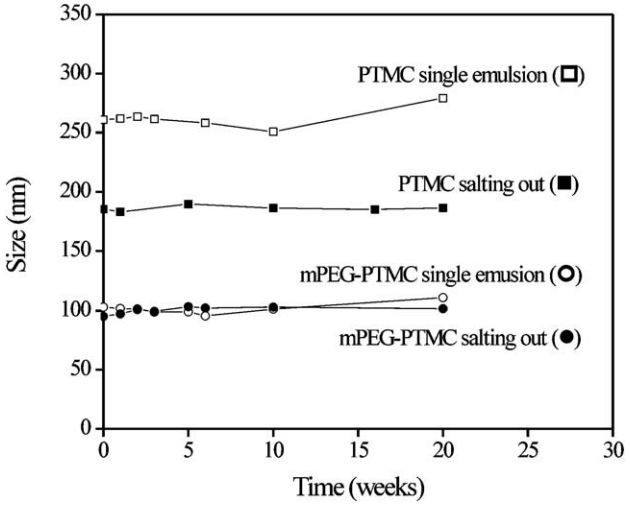

Fig. 3. Average size of dispersed dexamethasone-loaded nanoparticles at 37 °C as a function of time.

To evaluate possible changes in chemical composition of $mPEG_3$–$PTMC_{11}$ nanoparticles, a separate batch of $mPEG_3$–$PTMC_{11}$ nanoparticles (not loaded with dexamethasone) was prepared by the single emulsion method. NMR analysis was employed to determine the mPEG content. The mPEG content in the polymer used for the nanoparticle preparation was 22.3 wt.%. Immediately after nanoparticle preparation, the mPEG content in the nanoparticles had decreased to 15.1 wt.%. After evaporation of water from the supernatant, the mPEG content in this fraction was found to be 53.9 wt.%. Apparently part of the mPEG–PTMC with a relatively high mPEG content had dissolved in the aqueous phase during the nanoparticle preparation step. During incubation of the nanoparticle dispersion at 37 °C, the mPEG content in the nanoparticles remained constant for a time period of 12 weeks. In this time period, the size of the nanoparticles also remained constant. The polydispersity index values remained below 0.2 at all times.

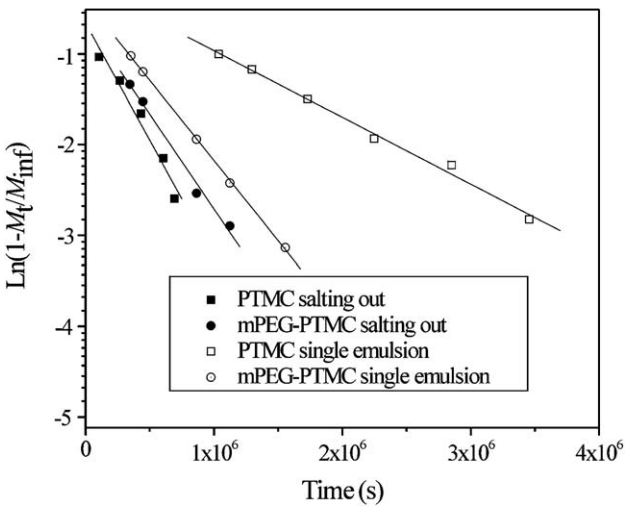

Fig. 4. Semi-logarithmic plots of $1-M_t/M_\infty$ (with $M_t/M_\infty > 0.6$) as a function of time for the release of dexamethasone from the different nanoparticles.

3.4. Diffusion-controlled release of dexamethasone from PTMC and mPEG$_3$–PTMC$_{11}$ nanoparticles

In diffusion-controlled release of drugs that are dissolved in spherical particles, the relationship between the size of the particles and the released fraction of the drug at a time t can be described by well-known equations [28]:

$$\frac{M_t}{M_\infty} = 6\sqrt{\frac{Dt}{\pi r^2}} - \frac{3Dt}{r^t} \qquad \text{for } M_t/M_\infty < 0.4, \tag{1}$$

and

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\exp\left(-\frac{\pi^2 Dt}{r^2}\right) \qquad \text{for } M_t/M_\infty > 0.6. \tag{2}$$

In these equations, M_t is the amount of drug released at a time t, M_∞ is the total amount of drug loaded into the particles of an average radius r, and D is the diffusion coefficient of the drug in the polymer matrix.

For PTMC and mPEG$_3$–PTMC$_{11}$ nanoparticles loaded with dexamethasone, we have shown that the size of the nanoparticles was stable for a time period of up to 20 weeks (see Fig. 3). Previously we also showed that the molecular weights of a PTMC homopolymer ($M_n = 69.2 \times 10^3$ g/mol) and of an mPEG–PTMC diblock copolymer (M_n of the mPEG and PTMC blocks are respectively 3.1×10^3 and 53.1×10^3 g/mol) did not decrease during conditioning at 37 °C in water for a time period of up to 8 weeks [11,12]. Therefore, it can be assumed that the diffusion coefficients of dexamethasone in the drug-loaded nanoparticles remained constant during the release experiments described in Fig. 2, allowing us to calculate their values. As the release was rapid and essentially complete at the end of the experiment, we used Eq. (2) to perform the calculations.

Fig. 4 shows semi-logarithmic plots of $1 - M_t/M_\infty$ as a function of time for the release of dexamethasone from the different nanoparticles. The released fraction M_t/M_∞ was larger than 0.6. In all cases, linear plots are obtained ($R^2 > 0.97$), which demonstrates that the release of dexamethasone from these nanoparticles is indeed controlled by diffusion. From the slopes and the average sizes of the nanoparticles, the values of the diffusion coefficients of dexamethasone in different nanoparticles can be determined. It can be seen from the figure that the nature of the polymer and the method of nanoparticle preparation have significant effects on the diffusion properties of the drug. The values of the diffusion coefficients are listed in Table 4.

In the case of PTMC nanoparticles, the value of the diffusion coefficient of dexamethasone in nanoparticles prepared by the salting out method (22.6×10^{-18} cm^2/s) is higher than that in nanoparticles prepared by the single emulsion method (12.7×10^{-18} cm^2/s). This can be expected, as in the salting out method the fast extraction of THF during particle formation might result in a relatively porous structure of the nanoparticles. Interestingly, the value of the diffusion coefficient of dexamethasone in mPEG$_3$–PTMC$_{11}$ nanoparticles did not depend on the preparation method. This result is in agreement with the fact that the size of the mPEG$_3$–PTMC$_{11}$ nanoparticles is independent of the preparation method and variations of experimental conditions as we described above. For mPEG$_3$–PTMC$_{11}$ nanoparticles prepared by both the salting out and the single emulsion methods, a relatively low value of D (4.8×10^{-18} cm^2/s) is obtained. As dexamethasone is a hydrophobic compound, the highest rates of diffusion could be expected in PTMC particles, which is made of the more hydrophobic polymer.

Table 4
Values of the diffusion coefficients (D) of dexamethasone in PTMC and mPEG$_3$–PTMC$_{11}$ nanoparticles prepared by salting out and single emulsion methods

Polymer	Preparation method	D(10^{-18} cm^2/s)
PTMC	Salting out	22.6
PTMC	Single emulsion	12.7
mPEG$_3$–PTMC$_{11}$	Salting out	4.8
mPEG$_3$–PTMC$_{11}$	Single emulsion	4.8

4. Conclusions

Well-defined nanoparticles based on PTMC can be prepared by single emulsion and salting out methods using PVA as a stabilizer. The size of the nanoparticles can be controlled by adjusting the stirring speeds and the polymer concentrations employed. These particles can readily be freeze-dried and redispersed without changes in average size and size distribution. With an amphiphilic mPEG$_3$–PTMC$_{11}$ diblock copolymer, nanoparticles can be prepared without using an additional stabilizer. The size of the obtained mPEG$_3$–PTMC$_{11}$ nanoparticles always ranged between 95 and 120 nm. These nanoparticles can be freeze-dried and redispersed as well.

Dexamethasone can be efficiently loaded in PTMC and mPEG$_3$–PTMC$_{11}$ nanoparticles during preparation by both the salting out and the single emulsion methods. The release of dexamethasone was sustained and diffusion controlled. The diffusion coefficients of dexamethasone ranged between 4.8×10^{-18} and 22.6×10^{-18} cm^2/s and depended on the nature of the polymer matrix and on the nanoparticles preparation method used. Complete release of the drug could be achieved in times ranging from 2 weeks to 60 days.

Our results show that PTMC and mPEG$_3$–PTMC$_{11}$ nanoparticles are attractive for the controlled delivery of hydrophobic drugs.

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