PTMC AND MPEG-PTMC MICROPARTICLES FOR HYDROPHILIC DRUG DELIVERY

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Summary

In this study, the potential of PTMC and mPEG-PTMC microparticles for controlled delivery of hydrophilic drugs was investigated. PTMC and mPEG-PTMC microparticles, loaded with hydrophilic model compounds (BSA, lysozyme and Coomassie® Brilliant Blue G) were prepared by the double emulsion method. High loading efficiencies can be achieved, and first order release profiles during 60 days were observed.

Introduction

The controlled release of proteins, peptides and DNA fragments has attracted much attention because of developments in tissue- and protein engineering, peptide synthesis and recombinant DNA technology. Due to the short in vivo half-lives of these hydrophilic compounds, sustained release systems are of great importance.

Nano- and microparticles based on (co)polymers of lactide and glycolide have been developed as carriers in drug delivery. However, the degradation characteristics of these polymers result in the liberation of acidic compounds that can lead to denaturation and deactivation of the active components. Poly(trimethylene carbonate) (PTMC) degrades in vivo by surface erosion without the formation of acidic compounds [1]. In this study, we investigate the preparation of PTMC and mPEG-PTMC microparticles and the loading and delivery characteristics of hydrophilic model compounds.

Experimental methods

Polymer grade 1,3-trimethylene carbonate (TMC) was purchased from Boehringer Ingelheim (Germany). Monomethoxy poly(ethylene glycol) ($mPEG_3$, M_n=3000 g/mol) was obtained from Shearwater Polymers (USA). Stannous octoate ($SnOct_2$), bovine serum albumin (BSA) (minimum 98% pure) and lysozyme from chicken egg white (3× crystallised, dialysed and lyophilised) were purchased from Sigma (USA). Coomassie® Brilliant Blue G (blue dye) was purchased from Aldrich (USA). All materials were used as received. 1-Hexanol (Merck, Germany) was distilled over CaH_2 (Acros, Belgium) before use.

The $PTMC_{50}$ homopolymer and the $mPEG_3$-$PTMC_{50}$ diblock copolymer were prepared by ring opening polymerization of TMC using 1-hexanol or $mPEG_3$ as initiator and $SnOct_2$ as catalyst [2]. The polymer characteristics determined by ^1H NMR and by GPC are shown in Table 1.

Table 1
Characteristics of $PTMC_{50}$ homopolymer and $mPEG_3$-$PTMC_{50}$ diblock coplymer

Polymer	Targeted M_n (kg/mol)	Purified polymer			
		M_n (kg/mol)[a]	M_n (kg/mol)[b]	M_w/M_n[b]	$[\eta]$ (dL/g)[c]
$PTMC_{50}$	50	–	69.2	1.6	1.48
$mPEG_3$-$PTMC_{50}$	53	55.9	69.0	2.0	1.21

[a] By ^1H NMR, [b]by GPC, [c]Intrinsic viscosity, by GPC.

Microparticles were prepared by double emulsion using PVA (2 wt.%) as a stabilizer. In the first (o/w) emulsion, 25 mg protein or 20 mg dye was emulsified in 5 ml polymer solutions in dichloromethane. After the second (w/o/w) emulsion, particles were formed upon evaporation of dichloromethane; the particles were then rinsed with water and freeze-dried [3].

The protein loading efficiency was determined by elemental analysis. The loading efficiency and the release in water at 37 °C of the blue dye were determined by UV absorption at 616 nm. The release experiments were conducted in duplicate.

Results and discussion

The different microparticles could readily be loaded with the hydrophilic model compounds: BSA (66 kg/mol), lysozyme (14 kg/mol) and Coomassie® Brilliant Blue G (blue dye, 854 g/mol). Spherical microparticles of $PTMC_{50}$ and $mPEG_3$-$PTMC_{50}$, 1–50 μm in diameter, were

formed. Fig. 1 shows light microscopy images of the $mPEG_3$-$PTMC_{50}$ microparticles, the dye was evenly distributed in the microparticles. Also, the shape and size did not change after freeze-drying and redispersion.

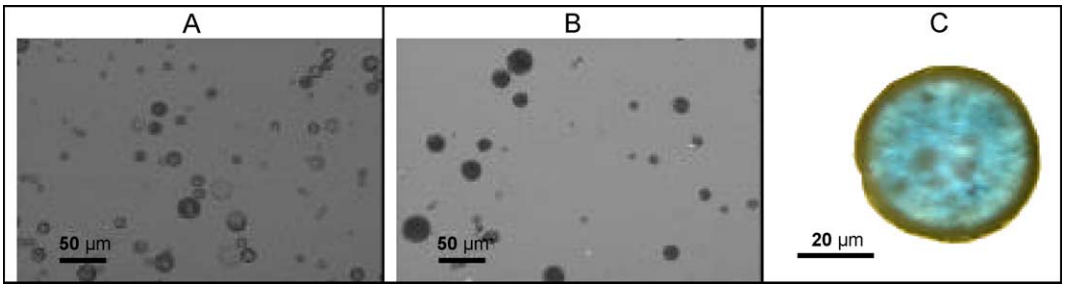

Fig. 1. Light microscopy images of $mPEG_3$-$PTMC_{50}$ microparticles. (A) microparticles loaded with lysozyme; (B) microparticles loaded with lysozyme, after freeze-drying and redispersion; (C) a microparticle loaded with Coomassie® Brilliant Blue G.

The loading efficiency of BSA and lysozyme in the different microparticles is shown in Table 2. The concentration of polymer in the organic phase mainly determines the loading efficiency. When 50 mg/ml polymer solutions were used, protein loading was negligible. At 100 mg/ml, high loading efficiency was achieved for $PTMC_{50}$ microparticles, while loading efficiency of $mPEG_3$-$PTMC_{50}$ microparticles was only 10–20%. Increasing the polymer concentration to 140 mg/ml resulted in high loading efficiency for both polymers.

When the polymer concentrations are higher than 100 mg/ml, $PTMC_{50}$ microparticles can be loaded with protein more efficiently than $mPEG_3$-$PTMC_{50}$ microparticles. This can be related to the difference in viscosity: at a same polymer concentration (100 mg/ml or 140 mg/ml), the $PTMC_{50}$ solution has a much higher viscosity than the $mPEG_3$-$PTMC_{50}$ solution. There is no significant difference in loading efficiency between BSA and lysozyme.

Table 2
Loading efficiency of $PTMC_{50}$ and $mPEG_3$-$PTMC_{50}$ microparticles

Polymer	Polymer concentration (mg/ml)	Loading efficiency(%)	
		BSA	Lysozyme
$PTMC_{50}$	50	0	0
	100	78.8	77.9
	140	95.3	90.2
$mPEG_3$-$PTMC_{50}$	50	0	0
	100	11.3	20.1
	140	71.2	77.4

Release profiles of the microparticles were studied using Coomassie® Brilliant Blue G, as it allows convenient visual observation and detection by UV.

Coomassie® Brilliant Blue G cannot be loaded into the microparticles when 60 mg/ml polymer solutions in dichloromethane are used in the preparation process. When 140 mg/ml $mPEG_3$-$PTMC_{50}$ solution or 100 mg/ml $PTMC_{50}$ solution is used, the loading efficiency is 80.0% for $mPEG_3$-$PTMC_{50}$ and 78.0% for $PTMC_{50}$ microparticles, respectively.

Fig. 2 shows the aqueous phases after the preparation of $mPEG_3$-$PTMC_{50}$ microparticles loaded with Coomassie® Brilliant Blue G. The polymer concentration in dichloromethane was 140 mg/ml. When fully released, the dye concentration is 200 μg/ml. A series of dye solutions in water (200–25 μg/ml) are also shown. The concentration of sample A, the supernatant directly after the second (w/o/w) emulsion step, is approximately 25 μg/ml. Sample B, the supernatant after solvent evaporation, has only a slightly higher concentration. This indicates that during solvent evaporation only little dye is released.

Fig. 3 shows release profiles of microparticles loaded with Coomassie® Brilliant Blue G. For microparticles prepared from both polymers, first-order release profiles were achieved. More than 90% of the dye was released in 60 days.

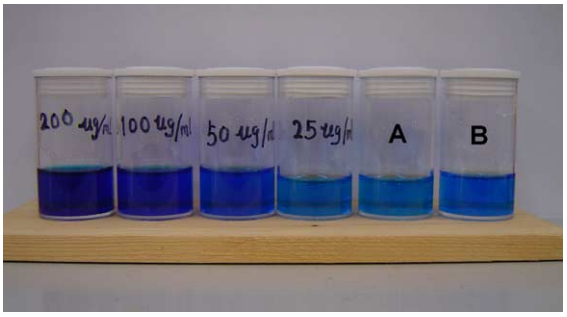

Fig. 2. Coomassie® Brilliant Blue G water solutions (200–25 μg/ml) and the aqueous phases after the preparation of $mPEG_3$-$PTMC_{50}$ microparticles (polymer concentration in dichloromethane is 140 mg/ml; dye concentration is 200 μg/ml when fully released). (A) supernatant after second (w/o/w) emulsion; (B) supernatant after solvent evaporation.

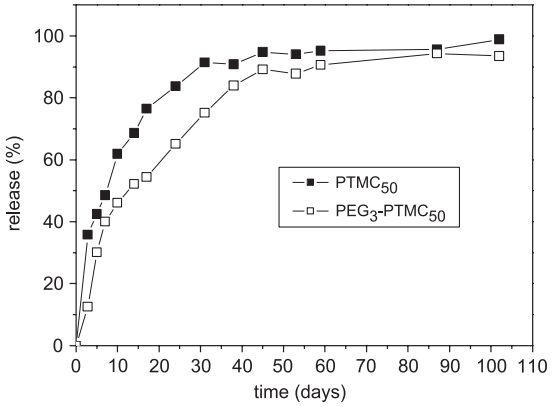

Fig. 3. Release profiles of microparticles loaded with Coomassie® Brilliant Blue G.

Conclusions

Microparticles of PTMC and mPEG-PTMC, 1–50 μm in diameter, were prepared by the double emulsion method. After freeze-drying and redispersion, the shape and size of the microparticles did not change. Hydrophilic model compounds (BSA, lysozyme and Coomassie® Brilliant Blue G) could be loaded efficiently. Microparticles loaded with Coomassie® Brilliant Blue G showed a first order release profile of the dye for 60 days, during which 90% of the dye was released.

References

[1] A.P. Pêgo, M.J.A. Van Luyn, L.A. Brouwer, P.B. Van Wachem, A.A. Poot, D.W. Grijpma, J. Feijen, J. Biomed. Mater. Res. 67A (2003) 1044.
[2] Z. Zhang, D.W. Grijpma, J. Feijen (2004), Macromol. Chem. Phys., in press.
[3] J. Bezemer, Protein Release Systems based on Biodegradable Amphiphilic Multiblock Copolymers, PhD thesis, University of Twente, 1999.