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ScienceDirect
International Journal of Pharmaceutics 324 (2006) 56–66
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Nanoscopic core-shell drug carriers made of amphiphilic triblock and star-diblock copolymers

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Received 17 February 2006; received in revised form 9 July 2006; accepted 13 July 2006
Available online 16 July 2006

Abstract

The aim of this work was to design injectable nanocarriers for drug delivery based on PCL–PEO amphiphilic block copolymers with linear ABA triblock and 4-armed $(BA)_4$ star-diblock architectures (A = PEO, B = PCL). The copolymers were obtained by coupling of a monofunctional –COOH end-capped PEO (M_n = 2.0 kDa) with linear or 4-armed star-shaped PCL macromers bearing –OH terminal groups and were characterized by ^{1}H NMR spectroscopy and size exclusion chromatography. DSC and X-ray diffraction experiments showed that separate crystalline phases of PCL and PEO are present in bulk copolymers. Nanoparticles were produced by nanoprecipitation (NP) and by a new melting-sonication procedure (MS) without the use of toxic solvents, and characterized for size, polydispersity, zeta potential and core-shell structure. Nanoparticles were loaded with *all-trans*-retinoic acid (*at*RA) as a model drug and their release features assessed. Results demonstrate that both techniques allow the formation of PEO-coated nanoparticles with a hydrodynamic diameter that is larger for nanoparticles prepared by MS. *at*RA is released from nanoparticles at controlled rates depending on size, loading and, more important, preparation technique, being release rate faster for MS nanoparticles. Some biorelevant properties of the carrier such as complement activation were finally explored to predict their circulation time after intravenous injection. It is demonstrated that nanoparticles prepared by MS do not activate complement and are of great interest for future *in vivo* applications.

Keywords: Nanoparticles; Amphiphilic block copolymers; Poly(epsilon-caprolactone); Poly(ethylene oxide); *All-trans*-retinoic acid

1. Introduction

With the remarkable development of nanomedicine in recent years, new drug delivery approaches based on the state-of-the-art nanotechnology have been receiving significant attention (Emerich and Thanos, 2003; Moghimi et al., 2005; Couvreur and Vauthier, 2006). Such process involves the identification of precise targets (cells and receptors) related to specific clinical conditions and choice of the appropriate nanocarrier to achieve the required response while minimizing the side effects. Rational approaches in design and surface engineering of nanoscale systems are generally needed to impart suitable biological properties as well as optimized technological features. Nanocarriers for anticancer targeting, for example, should be long-circulating to escape the reticular endothelial system (RES) and able to extravasate in the target organs making advantage of enhanced permeability and retention (EPR) effect (Kim and Lim, 2002; Brannon-Peppas and Blanchette, 2004; Jain, 2005; Reddy, 2005; Vasir and Labhasetwar, 2005).

In their simplest design, long-circulating nanoparticles are formed by a solid core, made of biodegradable polymers, and a hydrophilic corona, made of flexible hydrophilic chains. Hydrophobic biodegradable core contains the drug, which is protected from *in vivo* inactivation, and controls drug release rate. Hydrophobic blocks generally originates from poly(lactic acid), poly(lactic-*co*-glycolic acid) and poly(alkylcyanoacrylate), although recently a renewed attention has been paid to poly(ε-caprolactone) (PCL). The presence of

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doi:10.1016/j.ijpharm.2006.07.020

a hydrophilic coating offers steric stabilization toward aggregation "in the bottle" while also dictating the pharmacokinetics and biodistribution of the carrier. A number of reviews are available where it is well illustrated how surface modification can be carried out to obtain long-circulating nanoparticles, making use of poly(ethylene oxide) (PEO) (Bhadra et al., 2002; Otsuka et al., 2003) or more recently polysaccharides (Lemarchand et al., 2003, 2004; Labarre et al., 2005). Core-shell nanocarriers can be obtained either by coating the hydrophobic core with hydrophilic polymers/surfactants or designing tailor-made block copolymers (Soppimath et al., 2001).

Amphiphilic block copolymers represent a large family of materials consisting of ordered sequences of two or more different monomers connected by chemical bonds and arranged with different architectures (Kumar et al., 2001; Qiu and Bae, 2006). In the simplest case a diblock copolymer AB consists of two different homopolymers linked end to end. Extension of this concept leads to ABA or BAB triblocks, $(AB)_n$ linear multiblocks and to radial arrangements of block copolymers, the simplest case being that of star-shaped structures, where *n* block copolymer chains are linked by one of their ends to a multifunctional moiety. Another structural possibility designated by heteroarm block copolymers is to link *n* homopolymer sequences to a given junction point. Resulting block copolymers can be used to form nanoparticles or micelles by different methods which are generally selected depending on copolymer solubility and drug features.

Among poly(ester)/PEO copolymers, diblock architecture is generally preferred to produce core-shell carriers. A number of papers have highlighted how PEO length and surface density can affect biomimetic properties of the nanocarrier, with special regard to opsonization process and *in vivo* fate (Gref et al., 2000; Kim et al., 2005). On the other hand very few is known about the applicability of novel polymer architectures in core-shell nanocarrier development, where they have the potential to offer a superior degree of nanoparticle coating.

Our aim was to develop PEO-coated nanocarriers for intravenous administration made of PCL–PEO amphiphilic block copolymers with linear ABA triblock and 4-armed $(BA)_4$ star-shaped architectures (A = PEO, B = PCL). The feasibility of producing nanoparticles by a novel melting-sonication procedure without the use of toxic solvents was explored too. To assess loading capacity of the nanoparticles, *all-trans*-retinoic acid (*at*RA) was employed as a model drug. Some biorelevant properties of the nanocarrier with special regard to complement activation were finally assessed to predict their ability to escape the RES.

2. Materials and methods

2.1. Reagents and materials

ε-Caprolactone, CL (Aldrich) was distilled from CaH_2 under vacuum. Pentaerythritol, PERT (Fluka) was purified by vacuum sublimation at 200 °C. Tin(2-ethylhexanoate)$_2$, Sn(oct)$_2$, (Aldrich), 4-(dimethylamino)pyridine, DMAP, (Fluka) and 1,3-dicyclohexylcarbodiimide, DCC (Fluka) were used as received. *N*,*N*′-dimethylformamide, DMF, and dichlorometane, DCM (Aldrich) were dried before use. Monomethoxy poly(ethylene glycol) with $M_n = 2.0$ kDa, *m*-PEO$_{2.0}$ (Aldrich) was dried by distillation from toluene of the water–toluene azeotrope. α-Methoxy-ω-carboxymethyl-PEO, *m*-PEO$_{2.0}$–COOH was obtained reacting *m*-PEO$_{2.0}$ with succinic anhydride as previously reported (Maglio et al., 2004). *All-trans*-retinoic acid (*at*RA), potassium phosphate dibasic and potassium phosphate monobasic were from Sigma–Aldrich. Analytical grade acetone, ethanol and tetrahydrofuran (THF) were from Carlo Erba Reagenti (Italy).

2.2. Synthesis of a linear PCL macromer (l-PCL$_{6.7}$)

CL (30.0 g, 263 mmol), 1,4-butanediol (0.394 g, 4.38 mmol) and 0.018 g (0.044 mmol) of Sn(oct)$_2$ (molar ratio: Sn/OH 1/200, CL/OH 60/1) dissolved in 0.22 ml of CL were charged in a flame dried vial under dry nitrogen. The vial was sealed under vacuum and heated at 140 °C for 24 h. After cooling to room temperature, the vial was opened and the polymer was dissolved in DCM (110 ml) and precipitated in 600 ml of methanol at 0 °C (26.9 g, 88% yield; $\eta_{inh} = 0.32$ dl/g; $CHCl_3$, 25 °C, $C = 0.5$ g/dl).

2.3. Synthesis of a 4-arm PCL star-shaped macromer (s-PCL$_{19.6}$)

PERT (0.259 g, 1.90 mmol) was dissolved at 105 °C under dry nitrogen atmosphere in 38.1 g (334 mmol) of CL and 4.75 ml of DMF. A 0.25 M solution of Sn(oct)$_2$ in DMF (0.15 ml, 0.038 mmol of Sn) was added to the reaction mixture and the polymerisation was carried out under stirring at 105 °C for 48 h (molar ratio: Sn/OH 1/200, CL/OH 43.9/1). The DMF was removed under vacuum and the residue was dissolved in chloroform and precipitated in methanol at 0 °C to give s-PCL$_{19.6}$ (37.6 g, 98% yield; $M_n = 19.6$ kDa as determined by ^{1}H NMR; $\eta_{inh} = 0.34$ dl/g; $CHCl_3$, 25 °C, $C = 0.5$ g/dl).

2.4. Synthesis of linear triblock and star-shaped diblock PCL–PEO copolymers

The coupling of PCL macromers with *m*-PEO$_{2.0}$–COOH was performed as previously reported (Maglio et al., 2004). The synthesis of s-PCL$_{19.6}$–PEO$_{2.0}$ is reported as an example. Briefly: a solution of DCC (1.26 mmol) in 5 ml of DCM and a solution of s-PCL$_{19.6}$ (0.25 mmol) in 10 ml of DCM were added under stirring to *m*-PEO$_{2.0}$–COOH (1.05 mmol) and DMAP (0.25 mmol) dissolved in 13 ml of DCM, under dry nitrogen atmosphere, and the reaction was carried for 48 h at room temperature. After purification, s-PCL$_{19.6}$–PEO$_{2.0}$ was obtained with 80% yield ($\eta_{inh} = 0.46$ dl/g, $CHCl_3$, 25 °C, $C = 0.5$ g/dl). The coupling of l-PCL with *m*-PEO$_{2.0}$–COOH to give l-PCL$_{6.7}$–PEO$_{2.0}$ was performed as above (70% yield; $\eta_{inh} = 0.38$ dl/g; $CHCl_3$, 25 °C, $C = 0.5$ g/dl).

2.5. Polymer characterization

The inherent viscosities were measured in chloroform at 25 °C using an Ubbelohde viscometer ($C = 0.5$ g/dl). Differential