

BIODEGRADABLE NANOPARTICLES FOR LOCAL DRUG DELIVERY

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Introduction

To reduce restenosis after balloon angioplasty, local drug administration has the intuitive appeal of a higher site-specificity with lower drug doses needed than in the case of systemic administration. Whereas free drug administered locally diffuses out within a couple of minutes after restoration of blood circulation [1,2], the use of nanoparticles as drug carriers increases the drug uptake and the residence time considerably. In the preparation of these nanoparticles, a stabilizer is usually employed. However, 90–95% of the stabilizer is washed away during purification, which may be a drawback when further surface modification is anticipated. To obtain biodegradable nanoparticles that allow further surface modification, particles were prepared from poly(ethylene oxide)-poly(DL-lactic-co-glycolic acid) (PEO-PLGA) block copolymers. In aqueous environment the hydrophilic PEO-block may serve as a stabilizer, whereas the PEO-endgroup offers possibilities for nanoparticle surface modification. Moreover, drug-loaded particles were prepared and the drug loading efficiency was determined. Probucol was chosen as a drug, as it has an anti-restenosis activity [3]. Since drug release from the nanoparticles takes place by diffusion from and degradation of the matrix, the *in vitro* degradation of the PEO-PLGA nanoparticles was studied. The *in vitro* characteristics of these particles were compared with conventionally stabilized poly(DL-lactic acid) (PDLLA) and PLGA nanoparticles.

Methods

The (co)polymers were prepared by ring-opening polymerization of DL-lactide (Purac, Gorinchem, The Netherlands) and glycolide (Purac) with either hexanol (Merck, Darmstadt, Germany) (in case of PDLLA and PLGA) or methoxy-poly(ethylene glycol) ($M_n = 3,000$ g/mol) (Shearwater, Huntsville, USA) (in case of PEO-PLGA) as initiator and stannous(II)octoate (Sigma, St. Louis, USA) as catalyst at 130 °C and a polymerization time of 24 hours.

(Drug-loaded) Nanoparticles were prepared by using the salting-out method. In the case of PDLLA/PLGA, a polymer solution in acetone (containing various amounts of probucol (Sigma)) was emulsified under mechanical stirring in an aqueous solution of $MgCl_2 \cdot 6H_2O$ (Merck) as a salting-out agent and poly(vinyl alcohol) ($M = 9,000$ – $10,000$ g/mol, 80% hydrolysed from poly(vinyl acetate)) (Aldrich, Milwaukee, USA) as a stabilizer. In the case of PEO-PLGA, the polymer solution in acetone was emulsified in an aqueous solution of $MgCl_2 \cdot 6H_2O$ only. After emulsification, pure water was added, causing acetone to diffuse into the water phase, inducing the formation of nanoparticles. The nanoparticles were purified by rinsing with water using centrifugation (3 times, 30 min*65,000 g) and were subsequently lyophilized.

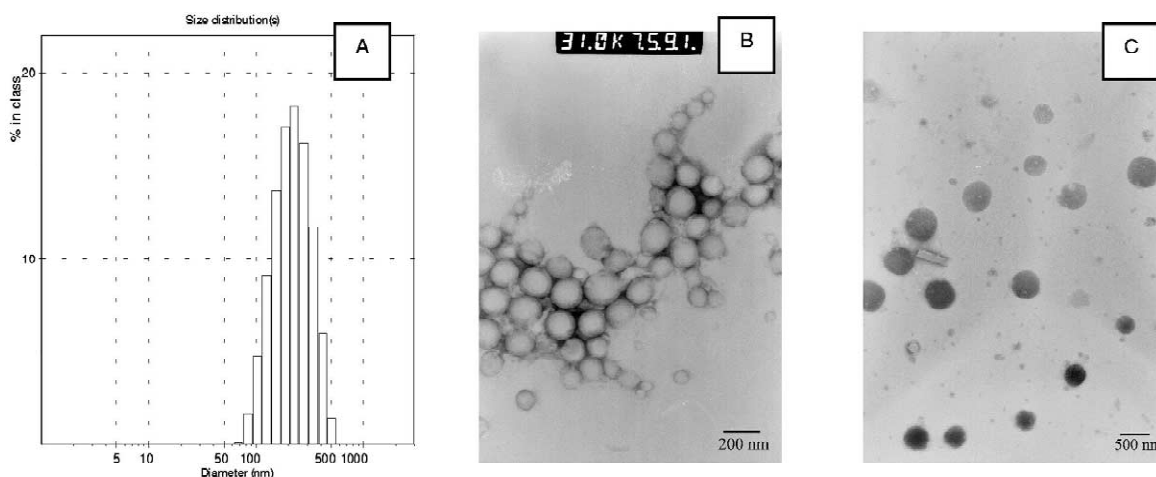


Fig. 1. PEO-PLGA nanoparticles as characterized by DLS (A) and TEM (B) and drug-loaded PEO-PLGA nanoparticles as characterized by TEM (C).

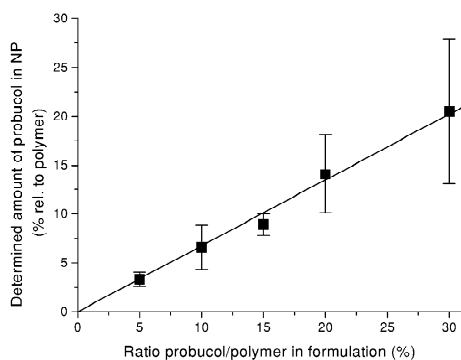


Fig. 2. Probucol loading as a function of the starting amount of probucol relative to the PEO-PLGA ($n=2$).

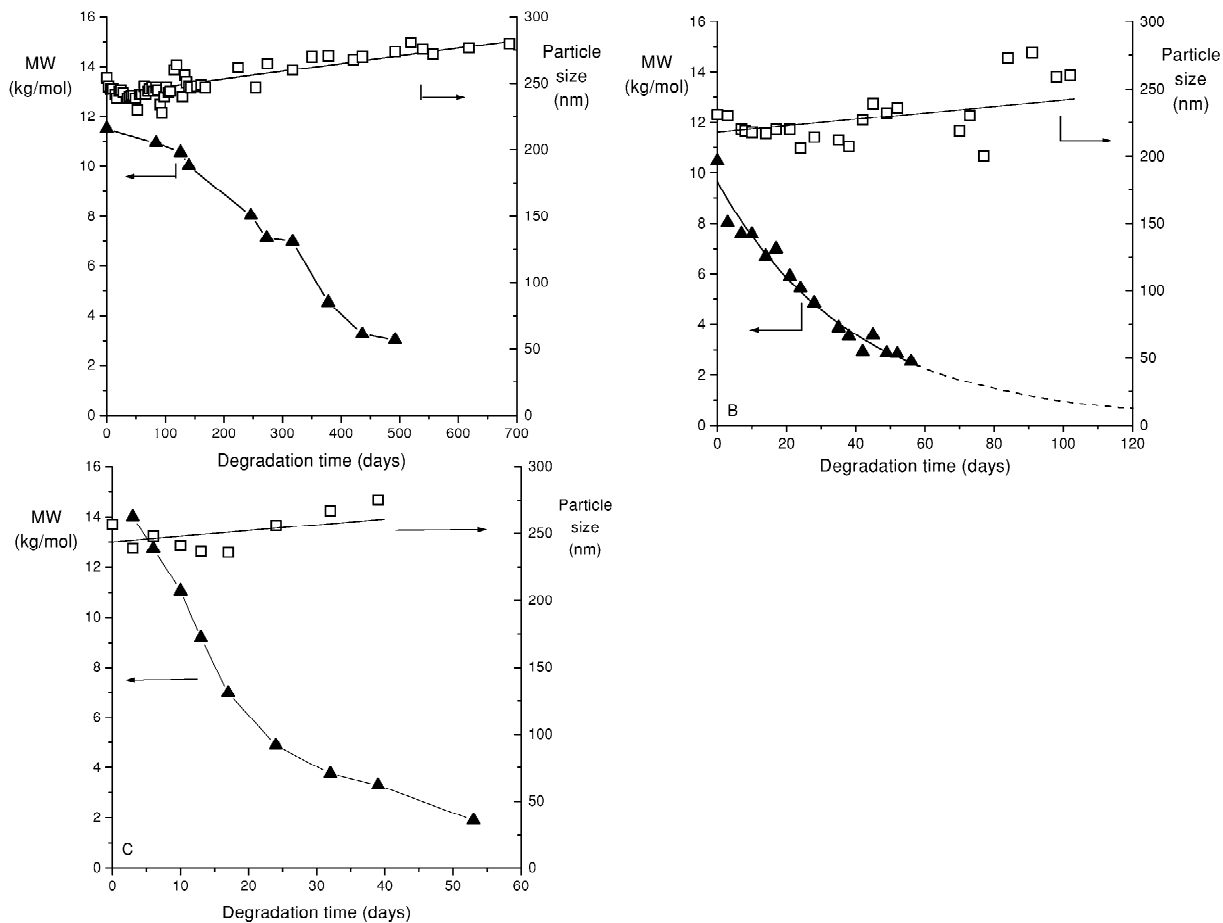


Fig. 3. Particle size and molecular weight (MW) as a function of degradation time for PDLLA (A), PLGA (B) and PEO-PLGA (C) nanoparticles.

The particle size was determined by dynamic light scattering (DLS; 25 °C, 90° angle), the drug loading by UV-spectroscopy and the particle morphology and size by transmission electron microscopy (TEM).

To determine the influence of PEO-content on the stability of nanoparticles without stabilizer, nanoparticles of various ratios of PEO-PLGA to PLGA were prepared. The size and zeta-potential of these particles were determined.

The in vitro degradation at 37 °C was studied after redispersing the particles in phosphate buffered saline (NPBI, Emmer-Compascuum, The Netherlands; pH=7.4) containing 0.02 wt% sodium azide (Merck).

Results

Monodisperse, spherical (drug-loaded) nanoparticles were prepared (Fig. 1). Nanoparticles without drug were smaller than drug-containing nanoparticles. The average hydrodynamic diameter as determined by DLS was larger than the diameter in the dry state (TEM), due to swelling of the particles. It was determined that the stability of the particles of PEO-PLGA without stabilizer was dependent on the composition of the copolymer: the lower the PEO-content of the copolymer, the broader the size distribution became, and even partial aggregation was observed at PEO-percentages less than 15 wt%. Increasing particle stability was associated with a higher surface coverage with increasing PEO-content, as indicated by a less negative zeta-potential. The relative drug content in the nanoparticles increased linearly with the percentage of drug in the polymer solution (Fig. 2), with an efficiency of approximately 65%.

The in vitro degradation of PDLLA, PLGA and PEO-PLGA particles was followed in time (Fig. 3). The degradation appeared to be in the order PDLLA < PLGA < PEO-PLGA as seen in the molecular weight (MW). The higher degradation rate of PLGA when compared to PDLLA is caused by the higher hydrophilicity of PLGA. The reason for the higher degradation of PEO-PLGA when compared to PLGA is twofold. First of all, the water-solubility of the formed oligomers is increased when PEO is covalently bound.

Secondly, the water-uptake due to the hydrophilic character of the PEO is higher, which increases the diffusion of water-soluble oligomers into the medium.

Throughout the degradation, the particle size remained constant in time for the PDLLA and PLGA particles. However, the PEO-PLGA nanoparticles tended to aggregate upon degradation, especially after day 35, probably caused by the loss of PEO, leading to less stable particles.

Conclusions

It is shown that monodisperse, spherical nanoparticles from PEO-PLGA block copolymers can be prepared without stabilizer using the salting-out method. To obtain stable nanoparticle preparations, a minimum PEO-content of 15 wt% is necessary. It is shown that drug-loaded nanoparticles can be prepared with up to 20% of drug and a loading efficiency of 65%. The in vitro degradation time of the nanoparticles can be controlled by the copolymer composition.

Acknowledgements

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