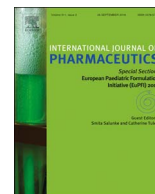




Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Full Length Article

Relationship between polarities of antibiotic and polymer matrix on nanoparticle formulations based on aliphatic polyesters

J.A.S. Ritsema^{a,*}, E.M.A. Herschberg^a, S.E. Borgos^b, C. Løvmo^b, R. Schmid^b, Y.M. te Welscher^a, G. Storm^a, C.F. van Nostrum^{a,*,1}^a Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands^b Department of Biotechnology and Nanomedicine, SINTEF Materials and Chemistry, Trondheim, Norway

ARTICLE INFO

Keywords:

PLGA
Polyesters
Nanoparticles
Antibiotics
Controlled release

ABSTRACT

In the field of nanomedicine, nanoparticles are developed to target antibiotics to sites of bacterial infection thus enabling adequate drug exposure and decrease development of resistant bacteria. In the present study, we investigated the encapsulation of two antibiotics with different polarity into different PEGylated polymeric nanoparticles based on aliphatic polyesters, to obtain a better understanding of critical factors determining encapsulation and release. The nanoparticles were prepared from diblock copolymers comprising of a poly(ethylene glycol) block attached to an aliphatic polyester block of varying polarity: poly(lactic-co-glycolic acid) (mPEG-PLGA), poly(lactic-co-hydroxymethyl glycolic acid) (mPEG-PLHMGA) and poly(lactic-co-benzylloxymethyl glycolic acid) (mPEG-PLBMGA). Hydrophobic bedaquiline and hydrophilic vancomycin were encapsulated *via* single and double-emulsion solvent evaporation techniques, respectively. Encapsulation, degradation and release studies at physiological simulating conditions were performed. Drug polarity and preparation techniques influenced encapsulation efficiency into polymer nanoparticles, giving almost complete encapsulation of bedaquiline and approx. 30% for vancomycin independent of the polymer type. The nonpolar bedaquiline showed a predominantly diffusion-controlled release independent of polymer composition. However, polar vancomycin was released by a combination of diffusion and polymer degradation, which was significantly affected by polymer composition, the most hydrophilic polymer displaying the fastest release.

1. Introduction

Since the introduction of the first effective antibiotics in 1937, development of bacterial resistance mechanisms has diminished the efficacy of antimicrobial drugs administered to patients (Aleksun and Levy, 2007). Nowadays, increases in antibiotic resistance are raising concerns worldwide. With the exception of clinical misuse and patient noncompliance, poor clinical efficacy of antimicrobial agents and development of resistance are typically correlated with inadequate drug exposure at the infection foci due to poor pharmacokinetic properties and narrow therapeutic indexes of antibiotics (DeRyke et al., 2006; Yilmaz and Özcengiz, 2017).

In the field of nanomedicine, nanosized drug delivery systems are being developed to improve the therapeutic index of antimicrobial drugs by modifying the pharmacokinetics and tissue distribution of antimicrobials to the infections foci (Huh and Kwon, 2011). The use of polymeric nanoparticles is highly attractive due to their high structural

integrity, stability, ease of preparation, functionalization and drug loading, and controlled release capabilities (Kamaly et al., 2016). Drug encapsulation and drug release from polymeric nanoparticles is influenced by the composition of the polymeric nanoparticles and by the physico-chemical properties of the loaded active pharmaceutical ingredient (Blanco and Alonso, 1998; Fredenberg et al., 2011; Fu and Kao, 2010). However, the relationship between drug polarity, polarity of polymeric nanoparticle matrix, and encapsulation and release has not yet been systematically addressed.

The aim of this study was to investigate whether polarity of the polymeric nanoparticle matrix plays a role in the loading and controlled release of a hydrophobic and a hydrophilic antibiotic drug. Three different PEGylated polymeric nanoparticles were included in this study, i.e. prepared from different diblock copolymers comprising of a ω -methoxy-poly(ethylene glycol) (mPEG) block, and an aliphatic polyesters block with varying hydrophobicity: poly(lactic-co-glycolic acid) (mPEG-PLGA), poly(lactic-co-hydroxymethyl glycolic acid) (mPEG-

* Corresponding authors.

E-mail addresses: j.a.s.ritsema@uu.nl (J.A.S. Ritsema), C.F.vanNostrum@UU.nl (C.F. van Nostrum).¹ Permanent address: Utrecht University, Utrecht Institute for Pharmaceutical Sciences, Department of Pharmaceutics, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands.<https://doi.org/10.1016/j.ijpharm.2017.11.017>

Received 12 June 2017; Received in revised form 7 November 2017; Accepted 8 November 2017

0378-5173/© 2017 Elsevier B.V. All rights reserved.

PLHMGA) and poly(lactic-co-benzyloxymethyl glycolic acid) (mPEG-PLBMGA). The use of amphiphilic diblock copolymers consisting of a polyethylene glycol (PEG) block allows the preparation of nanoparticles with long circulation in the bloodstream due to a hydrophilic shell that it creates at the particle surface (Jokerst et al., 2011; Ghassemi et al., 2010; Suk et al., 2016). This is important for targeting of drug loaded nanoparticles *via* intravenous administration, to efficiently utilize the increased permeability of the vasculature often observed within infected and other inflamed sites (a targeting mechanism often referred to as passive targeting) (Schiffelers et al., 2000; Ghassemi et al., 2010; Schiffelers et al., 2001). PLGA is a well-studied aliphatic polyester, which has been widely applied as a drug delivery system for decades (Danhier et al., 2012; Ghassemi et al., 2010; Makadia and Siegel, 2011). PLGA has favorable properties for pharmaceutical and medical application owing to its biodegradability and biocompatibility, but the tailorability of degradation and release characteristics is limited (Leemhuis et al., 2007). PLHMGA, a novel aliphatic polyester with pendant hydroxyl groups, was utilized because it allows improved tailorability of degradation and release (Ghassemi et al., 2012, 2010; Ghassemi et al., 2009; Leemhuis et al., 2007, 2006; Samadi et al., 2013, 2014). The pendant hydroxyl groups of PLHMGA enhance water absorbing capacity and hydrolysis rates, thereby increasing degradation kinetics and release rates. Its synthetic precursor, PLBMGA, an aliphatic polyester with pendant benzoyl group, was investigated here for the first time as a drug delivery system. In comparison to PLGA and PLHMGA, the relative increased hydrophobicity of PLBMGA lowers water penetrating capacity and hydrolysis, which confers to lower degradation rates and release kinetics.

Two antimicrobial compounds with different polarity, bedaquiline and vancomycin, were selected for encapsulation in order to get a deeper understanding of the critical factors determining encapsulation and release: Bedaquiline is a hydrophobic diarylquinoline that kills *Mycobacterium tuberculosis* by specifically inhibiting the ATP synthase enzyme for bacterial energy production. Bedaquiline has shown many adverse effects in tuberculosis patients after orally administration in clinical trials (Mase et al., 2013). Encapsulation and controlled release of bedaquiline could improve lung disposition and reduce (systemic) adverse effects. Vancomycin, a hydrophilic glycopeptide and inhibitor of the bacterial cell wall peptidoglycan synthesis, has exhibited poor pharmacokinetic properties in patients with methicillin-resistant *Staphylococcus aureus* (MRSA) infections, such as fast renal clearance and short half-life (Rybak et al., 2009). Increased exposure time of this concentration independent (time dependent) antibiotic *via* encapsulation of vancomycin could improve the therapy of patients with MRSA bacteremia.

As a consequence of the difference in polarity of both antibiotics, polymeric nanoparticles were formulated using single and double emulsion solvent evaporation techniques for bedaquiline and vancomycin, respectively. The encapsulation (efficiency) of both drugs into the different PEGylated polymeric nanoparticles based on aliphatic polyesters allowed us to investigate the effect of polymer composition, physico-chemical characteristics of the antibiotics and the drug concentration on the nanoparticle characteristics. The influence of polymer composition and drug characteristics on the release was studied *in vitro* by simulating physiological (sink) conditions. The differences in release behavior were supported by *in vitro* degradation studies of the nanoparticles. Results of this study provides deeper insights into the appropriate selection of physico-chemical properties of the antibiotic and composition of the polymeric matrix, to improve antimicrobial therapies targeting specific infectious disease, bacterial strains and drug delivery problems.

2. Materials and methods

2.1. Materials

Benzyloxymethyl methyl glycolide (BMMG) was synthesized and characterized as described by Leemhuis et al. (Leemhuis et al., 2006). Polyvinyl alcohol (PVA; Mw 30,00–70,000 g/mol, 88% hydrolyzed), deuterated dimethyl sulfoxide (d₆-DMSO), stannous(II) 2-ethylhexanoate, vancomycin hydrochloride, polyethylene glycol monomethyl ether (mPEG, Mw 2000 g/mol), Hyflo® Super-Cel®, lithium chloride, formic acid, potassium phosphate monobasic, potassium phosphate monobasic, sodium chloride, palladium on carbon (Pd/C, 10 wt. % loading, matrix activated carbon support), HEPES, vancomycin hydrochloride chemical standard (product number 94747) and HPLC columns for LC–MS/MS (Ascentis Express) were obtained from Sigma Aldrich (Germany). D,L-lactide and glycolide were purchased from Corbion Purac (Gorinchem, the Netherlands). Polyethylene glycol (PEG) standards were purchased from PSS Polymer Standards Service GmbH (Mainz, Germany). Chloroform, dichloromethane (DCM), methanol, tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and acetonitril were purchased from Biosolve (Valkenswaard, the Netherlands) and HoneywellFluka (USA). Bedaquiline (99%) was purchased from Advanced ChemBlocks Inc (USA). Fetal bovine serum was purchased from Lonza (USA). Formvar/Carbon on 400 Mesh Copper grids were obtained from Agar scientific (UK). Deuterated bedaquiline (bedaquiline-d₆) was purchased from Toronto Research Chemicals (Canada). Deuterated vancomycin (vancomycin-d₁₂, product number C3831) was purchased from Alsachim (France). Centrifugal ultrafiltration devices (Vivacon 500, 30 kDa Mw cut-off) were obtained from Sartorius (Germany).

2.2. Methods

2.2.1. Polymer synthesis

The random copolymer of D,L-lactide and benzyloxymethyl methyl glycolide (BMMG) was synthesized *via* ring-opening polymerization in the melt (130 °C) using polyethylene glycol monomethyl ether (mPEG, Mn 2000 Da) as initiator and stannous octoate as catalyst. In detail, D,L-lactide, BMMG and mPEG, were loaded into a schlenk tube and dried overnight under vacuum conditions. D,L-lactide and BMMG were fed in a molar ratio of 65/35. The molar feed ratio of monomers to initiator was 300:1 mol/mol. The loaded Schlenk tube was transferred to an oil bath of 130° Celsius and incubated for 30 min to remove residual water. Polymerization was initiated by the addition of the catalyst, stannous (II) 2-ethylhexanoate (monomer to catalyst molar ratio of 600:1 mol/mol). The polymerization continued overnight at 130° Celsius under nitrogen atmosphere. The crude product was cooled to room temperature, dissolved in a small volume of chloroform and precipitated into a 50-fold excess of methanol to remove unreacted monomer. Precipitation was repeated two times and the obtained diblock copolymer PEG-PLBMGA was dried under vacuum overnight. PEG-PLGA was synthesized with the identical method using D,L-lactide and glycolide at a monomer ratio of 50:50 mol/mol. PEG-PLHMGA was obtained by removal of the protecting benzyl groups of PEG-PLBMGA *via* hydrogenation (rubber balloon filled with H₂) overnight in dry THF using Pd/C as catalyst (BMMG to Pd/C ratio of 1:2 (w/w)) (Leemhuis et al., 2006). The Pd/C was removed by filtration using Hyflo® Super-Cel® and subsequent centrifugation of the filtrate at 20,000 × g. Next, the polymer solution was concentrated by partly evaporating THF under vacuum. The PEG-PLHMGA was precipitated in cold methanol and dried overnight under vacuum.

2.2.2. Polymer characterization

2.2.2.1. *NMR spectroscopy.* An Agilent 400-MR DD2 equipped with a OneNMR probe (Agilent Technologies., USA) was used to conduct ¹H-NMR measurements. All 1D NMR experiments were carried out

according to the s2pul.c standard program with a spectral width of 24,038.5 Hz and an acquisition time of 1.363 s. Polymers were dissolved in deuterated DMSO at a ~5 mg/mL concentration. Data were analyzed using Mnova NMR software (Mestrelab Research). Polymeric composition, number-average molecular weight and PEG content were determined with the following equations:

$\int_{\text{Lactic acid}}$, $\int_{\text{glycolic acid}}$, $\int_{\text{benzyloxymethyl glycolic acid}}$, $\int_{\text{hydroxymethyl glycolic acid}}$ and \int_{mPEG} are the integrals per proton at the indicated peak shifts (\int_{number} in ppm):

$$\int_{\text{Lactic acid}} = \int_{5.2-5.4}$$

$$\int_{\text{glycolic acid}} = \int_{4.7-5.0} / 2$$

$$\int_{\text{benzyloxymethyl glycolic acid}} = \int_{3.7-3.9} / 2$$

$$\int_{\text{hydroxymethyl glycolic acid}} = \int_{3.7-3.9} / 2$$

$$\%_{\text{Lactic acid}} = (\int_{\text{Lactic acid}} / \sum(\int_{\text{all polymer composing units}})) \times 100$$

$$\%_{\text{glycolic acid}} = (\int_{\text{glycolic acid}} / \sum(\int_{\text{all polymer composing units}})) \times 100$$

$$\%_{\text{benzyloxymethyl glycolic acid}} = (\int_{\text{benzyloxymethyl glycolic acid}} / \sum(\int_{\text{all polymer composing units}})) \times 100$$

$$\%_{\text{hydroxymethyl glycolic acid}} = (\int_{\text{hydroxymethyl glycolic acid}} / \sum(\int_{\text{all polymer composing units}})) \times 100$$

The number-average molecular weight (M_n) of the diblock copolymers was calculated from the NMR integrals and molecular weights (MW) of the comonomers with the following formulas:

$$\int_{\text{mPEG}} = \int_{3.5} / 182$$

$\int_{\text{Lactic acid}}$, $\int_{\text{glycolic acid}}$, $\int_{\text{benzyloxymethyl glycolic acid}}$, $\int_{\text{hydroxymethyl glycolic acid}}$ and \int_{mPEG} are the integrals per proton at the indicated peak shifts (\int_{number} in ppm):

$$M_n \text{ PEG-PLGA} = 2000 + (\int_{\text{Lactic acid}} / \int_{\text{mPEG}} \times \text{MW}_{\text{lactic acid}}) + (\int_{\text{glycolic acid}} / \int_{\text{mPEG}} \times \text{MW}_{\text{glycolic acid}})$$

$$M_n \text{ PEG-PLBMGA} = 2000 + (\int_{\text{Lactic acid}} / \int_{\text{mPEG}} \times \text{MW}_{\text{lactic acid}}) + (\int_{\text{benzyloxymethyl glycolic acid}} / \int_{\text{mPEG}} \times \text{MW}_{\text{benzyloxymethyl glycolic acid}})$$

$$M_n \text{ PEG-PLHMGA} = 2000 + (\int_{\text{Lactic acid}} / \int_{\text{mPEG}} \times \text{MW}_{\text{lactic acid}}) + (\int_{\text{hydroxymethyl glycolic acid}} / \int_{\text{mPEG}} \times \text{MW}_{\text{hydroxymethyl glycolic acid}})$$

2.2.2.2. Gel permeation chromatography (GPC). Molecular weight (number and weight average molecular weight) and molecular weight distribution (polydispersity) of the synthesized polymers were determined using a Waters Alliance System (Waters, USA). The system consisted of a Waters 2695 separating module and Waters 2414 refractive index detector. Two PL-gel 5 mM mixed-D columns (Polymer Laboratories) were fitted with a guard column and DMF containing 10 mM LiCl was used as an eluent at the flow rate of 1 mL/min. Polyethylene glycol (PEG) standards and the synthesized polymers were dissolved in DMF with 10 mM LiCl at 37 °C for 30 min and complete dissolution proceeded via overnight incubation at room temperature. Fifty μL samples were injected into the column with a total run time of 30 min. The results were analyzed using Empower software (Waters, USA).

2.2.2.3. Differential scanning calorimetry (DSC). Differential scanning calorimetry analysis was performed using Discovery DSC (TA instrument, USA). Approximately 4–10 mg polymer was loaded into an aluminum T_{zero} low mass pan and lid (TA instrument, USA), and heated from room temperature to 120 °C (5 °C/min). Afterwards, the pan was cooled down to –50 °C (2 °C/min) and heated to 120 °C (1 °C modulation, 1 °C/min). The glass transition temperature was determined in the second modulated heating run from the reversing heat flow graph.

2.2.3. Nanoparticle preparation

Vancomycin-loaded nanoparticles were prepared using a double-emulsion solvent evaporation method. In detail, a solution of vancomycin (200 μL , feed ratio of 1:10, 1:5 and 1:2% (w/w) vancomycin to polymer) was emulsified in a 5% (w/v) polymer solution in dichloromethane (DCM, 2 mL) using a Sonopuls HD 2200 ultrasonic homogenizer fitted with a MS73 micro tip (BANDELIN electronic GmbH & Co. KG, Germany) for 1 min at 10% amplitude. This primary water-in-oil (W_1/O) phase was transferred drop-wise to a 20 mL 1% PVA solution (filtered through a membrane with 0.2 μm pore size). A second sonication with a TT13 flat tip for 2 min at 40% amplitude formed the water-in-oil-in-water ($W_1/O/W_2$) emulsion. DCM was evaporated at RT under stirring conditions for three hours.

Bedaquiline-loaded nanoparticles were prepared using a single-emulsion solvent evaporation method. Briefly, bedaquiline was dissolved in a 5% (w/v) solution of polymer in 2 mL DCM (feed ratio of 1:10, 1:5 and 1:2 w/w bedaquiline to polymer) and this solution was emulsified in 20 mL 1% PVA solution (filtered through 0.2 μm membrane) using sonication with a TT13 flat tip for 2 min at 40% amplitude. DCM was evaporated at RT under stirring conditions for three hours.

Solidified nanoparticles were recovered by ultracentrifugation at 20,000 $\times g$ and washed twice with 20 mL reverse osmosis (RO) water. Empty nanoparticles were prepared according to the same methods (i.e. O/W or $W_1/O/W_2$) without the addition of vancomycin and bedaquiline.

After a last centrifugation step, the washed nanoparticles were resuspended in 5 mL RO water. The nanosuspensions were either directly used for nanoparticle characterization, release and degradation studies, or lyophilized as 1 mL aliquots in weighed Eppendorf tubes (in triplo) to establish dry particle mass of the suspension and drug load. A Chris Alpha 1–2 freeze-drier (Osterode am Harz, Germany) was used to lyophilize nanoparticles overnight at –40 °C at 1 mBar.

2.2.4. Nanoparticle characterization

2.2.4.1. Dynamic light scattering (DLS). The hydrodynamic size of the nanoparticles was measured by dynamic light scattering with an ALV-CGS-3 system (Malvern Instruments, UK) fitted with a JDS Uniphase 22 mW He-Ne laser operating at 632.8 nm and digital LV/LSE-5003 correlator. Measurements were performed at 25 °C and 90° angle at a final nanoparticle concentration of ~10 $\mu\text{g/mL}$ in 10 mM HEPES pH 7.4 buffer. Data were analyzed using DTS software.

2.2.4.2. Zeta-potential. The zeta-potential (ζ) was measured by a Zetasizer Nano-Z (Malvern Instruments, UK) at 25 °C using folded capillary cells. Nanoparticles were diluted in 10 mM HEPES pH 7.4 buffer to a final concentration of ~10 $\mu\text{g/mL}$. Data were analyzed using DTS software.

2.2.4.3. Transmission electron microscopy. Size and morphology were investigated by transmission electron microscopy (TEM) using FE Tecnai 10/12 (Philips, USA) and analyzed by MearuseIT software (Olympus). A single droplet of nanosuspension (20 μL ; ~0.1 μg NPs per mL RO water) was deposited on parafilm. Glow discharged Formvar carbon film on copper grids were placed on top of the droplets. The grids were removed after 2 min incubation and any residual water was removed from the grids by filter paper. Subsequently, the grid was placed onto a droplet of 20 μL 2% uranyl acetate. After 2 min, any residual uranyl acetate was removed by filter paper and the grids were air-dried for 5 min.

2.2.5. Encapsulation efficiency and drug load

Lyophilized nanoparticles were dissolved in acetonitrile and incubated for 1 h. Bedaquiline and vancomycin were extracted by the addition of a 10-fold excess of methanol or potassium phosphate monobasic (pH 2.8), respectively. The obtained samples were centrifuged at 20,000 $\times g$ for 5 min to remove precipitated polymer or any

possible impurities. The concentration of vancomycin and bedaquiline was determined by UPLC. The UPLC system comprised an Acquity UPLC System equipped with a photodiode array detector (Waters, USA) fitted with an Acquity UPLC BEH C18 (2.1 × 50 mm, 1.7 μm) column.

The isocratic method for vancomycin analysis was modified following a reported study and run at a flow rate of 0.8 mL/min in a mobile phase containing 50 mM KH₂PO₄ pH 4/ACN (92:8 v/v) at 35 °C (Shah et al., 2014). A gradient method was run for bedaquiline. Eluent A: ACN/ 25 mM ammonium acetate pH 6.6 10/90% (v/v) with Eluent B: 100% ACN. The gradient was run at a flow rate of 0.8 mL/min from 100% eluent A to 90% Eluent B from 0.2–1 min followed by equilibration with 100% eluent A. Absorbance of vancomycin and bedaquiline were monitored at a wavelength of 274 and 333 nm, respectively.

Both methods were evaluated by analysing drug recovery of samples consisting of a mixture of accurately weighed polymer and antibiotic powders (recovery ≥ 98%). The results were analysed using Empower software (Waters, USA). The concentration of bedaquiline and vancomycin was calculated with the standard concentrations (10–120 ppm). Encapsulation efficiency and drug load of bedaquiline and vancomycin was calculated with the following formulas:

$$\text{Drug encapsulation efficiency} = \frac{\text{actual weight antibiotic per weight nanoparticles}}{\text{theoretical weight of antibiotic per weight nanoparticles}} \times 100\%$$

$$\text{Drug Loading} = \frac{\text{weight of antibiotic in the nanoparticles}}{\text{gross weight of the nanoparticles}} \times 100\%$$

2.2.6. In vitro drug release

2.2.6.1. Cumulative release study.

Bedaquiline-loaded and vancomycin-loaded nanoparticles were resuspended in PBS buffer pH 7.4 (0.033 M NaH₂PO₄, 0.066 M Na₂HPO₄, 0.056 M NaCl) containing 10% fetal bovine serum at a final bedaquiline concentration of 1–15 μg/mL and vancomycin concentration of 100–150 μg/mL. The final drug concentrations were chosen differently to ensure sink-conditions in order to effect proper dissolution of both drugs into the medium. Sodium azide (0.06%) was added to all media to prevent antimicrobial growth. The resuspended nanosuspensions were aliquoted (1 mL volume) in triplo and incubated at 37 °C under mild agitation conditions. At appropriate intervals, nanosuspensions were centrifuged at 20,000 × for 25 min. Three-quarter of supernatant was collected and analysed for drug content. Fresh medium was added in return, the particles were resuspended by vortex and incubated for another time interval.

For bedaquiline analysis, protein from the release medium was precipitated with acetone (90% acetone in final dilution) prior to analysis. Deuterated bedaquiline was used as internal standard for quantification. The amount of bedaquiline and vancomycin in the supernatants was quantitatively analysed by mass spectrometry using LC-QqQ-MS/MS, equipped with an Agilent 1290 HPLC system coupled to an Agilent 6490 triple quadrupole mass spectrometer. The HPLC column was an Ascentis Express C8 column (75 × 2.1 mm, 2.7 μm particles size with a 5 × 2.1 mm guard column of the same material (both Sigma). Eluent A was 25 mM formic acid and eluent B was methanol. The mobile phase was run at 0.4 mL/min and comprised an isocratic loading in 100% eluent A from 0–1 min, then a gradient of eluent B from 0% to 75% from 1–2 min, followed by equilibration back to 100% eluent A. Mass detection was in positive ESI mode (Agilent Jetstream) quantified in multiple reaction monitoring (MRM) mode with transitions *m/z* 556.5–58.1 and 556.5–42. The deuterated standard was quantified in MRM mode with transitions *m/z* 563.2–64.1.

For vancomycin analysis, samples were 100 × diluted in water and serum protein was removed by ultrafiltration prior to analysis (Vivacon centrifugal filters, 14,000 × for 15 min). Deuterated vancomycin was

used as internal standard for quantification. The mobile phase was run at 0.3 mL/min and comprised an isocratic loading in 100% eluent A from 0 to 2 min, then a gradient of eluent B from 0% to 50% from 2 to 2.2 min, then an isocratic elution at 50% eluent B until 3 min, followed by equilibration back to 100% eluent A. Mass detection was in positive ESI mode (Agilent Jetstream) quantified in MRM mode with transitions *m/z* 725.5 to 144.2 and 725.5 to 100. The deuterated standard was quantified in MRM mode with transitions *m/z* 731.5–144.2 and 731.5–100 (all doubly charged precursor ions). The cumulative release of bedaquiline and vancomycin is reported relative to the determined loading% and dry particle mass of the nanosuspensions.

2.2.7. In vitro degradation of nanoparticles

Empty nanoparticles were dispersed in PBS buffer pH 7.4 (final composition: 0.033 M NaH₂PO₄, 0.066 M Na₂HPO₄, 0.056 M NaCl) at a concentration of 5–15 mg/mL, aliquoted (1 mL) into weighed Eppendorf tubes and incubated at 37 °C under mild agitation conditions. At appropriate time points, three samples were collected by centrifugation at 20,000 × at 4 °C and washed twice with RO water. After lyophilization of the samples, the dry weight of the lyophilized

nanoparticles or insoluble residues was determined. The molecular weight of the lyophilized samples was measured with GPC, as previously described in Section 2.2.2.2.

2.3. Statistical analysis

The two sample *t*-test and one-way analysis of variance (ANOVA) were performed using IBM SPSS Statistics 24. *T*-test was utilized comparison between two groups. One-way analysis of variance (ANOVA) (followed by a modified *t*-test) was used when more than 2 groups were compared. *P* < 0.05 was regarded as statistically significant. Diagrams and figures show means and SDs unless indicated otherwise.

3. Results and discussion

3.1. Polymer synthesis and characterization

In this study, three different poly(ethylene glycol)-polyester diblock copolymers were synthesized with varying hydrophobicity of the aliphatic polyester block by different copolymer compositions, i.e. the relatively hydrophobic PLBMGA, intermediate PLGA, and the relatively hydrophilic PLHMGA (Fig. 1). First, random copolymers of *D,L*-lactide and benzyloxymethyl methyl glycolide (BMMG) (molar feed ratio of 65:35) were synthesized via ring-opening polymerization using poly(ethylene glycol)-monomethyl ether (mPEG, 2000 Da) as initiator and stannous octoate (Sn(Oct)₂) in the melt at 130 °C to obtain PEG-PLBMGA (Fig. 1). Catalytic removal of the pendent benzyl groups from mPEG-PLBMGA yielded mPEG-PLHMGA with pendent hydroxyl groups. mPEG-PLGA diblock copolymer was synthesized by the same method using *D,L*-lactide and glycolide as monomer units. These diblock copolymers, mPEG-PLGA, mPEG-PLBMGA and mPEG-PLHMGA, were obtained in high yields of 93, 95 and 87%, respectively. Similar yields were reported before for similar polymers. (Leemhuis et al., 2006; Rahimian et al., 2015a, b).

The monomer composition was confirmed by ¹H-NMR spectroscopy (Supporting Information (S.I.), Supplementary Fig. 1). Peak integrals at peak shifts are representative of the protons of the composing units, mPEG, *D,L*-lactide, glycolide and BMMG. ¹H-NMR analysis (S.I., Supplementary Table 2) showed that mPEG-PLGA was composed of *D,L*-lactic acid and glycolic acid with a molar ratio of 50:50, which was

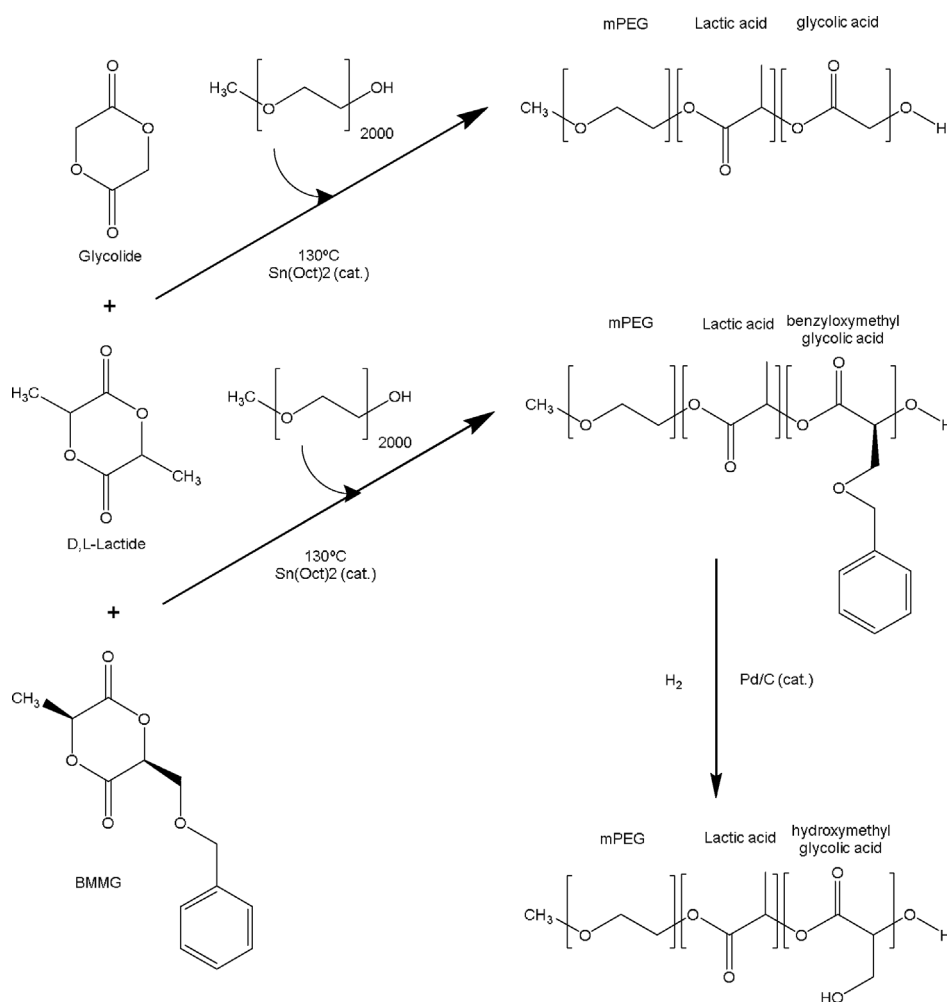


Fig. 1. Synthesis of mPEG-PLGA, mPEG-PLBMGA and mPEG-PLHMGA.

similar to the feed ratio (50:50). Since the BMMG monomer is a cyclic dimer of D,L-lactic acid (LA) and benzyloxymethyl glycolic acid (BMGA), the corresponding monomer feed ratio of 65:35 should result in a ratio of 82.5:17.5 LA/BMGA in the polymer mPEG-PLBMGA. We found a molar ratio of 82:18 – the equivalent to the lactide and BMMG molar ratio of 65:36 –, which is in good agreement with the feed ratio. This ratio of monomers (LA/HMGA) remained similar after removal of the benzoyl groups. Similar results were previously reported (Leemhuis et al., 2006; Rahimian et al., 2015a,b).

¹H-NMR was not only used to calculate the composition of the polymers, but also the total number of monomer units attached to each mPEG chain, giving the total number average molecular weight, by relating the monomer integrals to the PEG integral per proton of each unit. The number average molecular weight of mPEG-PLGA and mPEG-PLBMGA were in good agreement with the theoretical molecular weight, while the molecular weights of mPEG-PLHMGA was significantly higher than expected. After hydrogenation and extensive purification of the polymer *via* precipitation, filtration or centrifugation, unreacted PEG chains or diblock copolymers with relatively small and hydrophilic polyester blocks may have been removed, thus increasing the polyester content and providing higher than expected molecular weights.

Number and weight average molecular weights and the polydispersity were also determined with gel permeation chromatography (GPC) using PEG reference standards and 10 mM LiCl in DMF as eluent. GPC analyses (S.I., Supplementary Table 2) showed similar polydispersities of 1.4–1.5 for all polymers, but apparent differences between molecular weights (M_w and M_n) are observed in comparison to

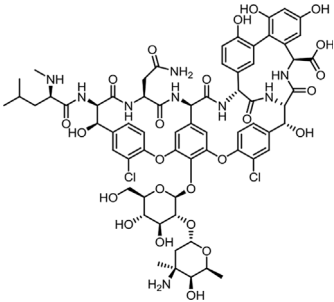
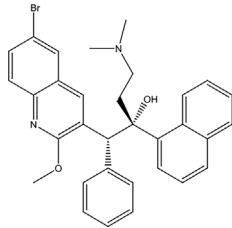
¹H-NMR and theoretical values. These differences are obtained, since the hydrodynamic size of polymer chains, as determined by GPC, depend on the extent of solvation of the entire polymer. Different degrees of solvation of PEG standard and diblock copolymers result in differences between theoretical and analyzed molecular weights (Rahimian, Samadi (Rahimian et al., 2015a,b; Samadi et al., 2013, 2014)).

DSC analysis showed that the obtained diblock copolymers were fully amorphous (S.I., Supplementary Fig. 3). The glass transition temperatures (T_g) are reported in S.I., Supplementary Table 2. The increase of the glass transition after removal of the protected benzyl group of mPEG-PLBMGA is in agreement with previous findings (Rahimian et al., 2015a,b; Samadi et al., 2014). Polar groups such as –OH tend to raise the glass transition (more than non-polar groups), because of polar interactions (Cowie and McEwen, 1982; Pizzirani et al., 1971).

3.2. Nanoparticle preparation and characterization

To study the influence of polymer composition and drug characteristics on the encapsulation of antibiotics, two model antibiotics of different hydrophobicity/hydrophilicity were encapsulated utilizing the synthesized diblock polymers: the polar vancomycin, a complex relatively large hydrophilic molecule with negative calculated logD, and apolar bedaquiline, a hydrophobic antibiotic with a highly positive calculated LogD (Table 1). These differences in physico-chemical properties required the use of different encapsulation approaches. Vancomycin-loaded PEG-polyester nanoparticles were prepared using a double-emulsion solvent evaporation method, whereas bedaquiline was

Table 1
Physico-chemical properties of vancomycin and bedaquiline.

Property	Vancomycin	Bedaquiline
Structural formula		
MW (g/mol)	1449	555
ACD logD (pH 7.4)	-4.8	6.17
Est. solubility in water at 25 °C (ALOGPS)	Freely soluble, 225 g/L	Insoluble, 192 µg/L

encapsulated with the single-emulsion solvent evaporation method. The nanoparticles were obtained in good yields (~65–70%).

To determine the maximum drug load for each antibiotic and the effect of drug to polymer ratio (w/w) on the preparation and encapsulation, the hydrodynamic size, size distribution (PDI), zeta-potential and drug encapsulation efficiency were studied for mPEG-PLGA nanoparticles prepared with increasing drug to polymer ratio (1:10, 1:5 to 1:2% (w/w)).

The nanoparticle properties of the antibiotic-loaded mPEG-PLGA NPs are summarized in S.I., Supplementary Table 4. Increasing the bedaquiline to polymer feed (from 1:10 to 1:2), yielded nanoparticles with a slightly higher mean hydrodynamic size and polydispersity: 241 ± 5 nm (PDI = 0.134) to 290 ± 12 nm (PDI = 0.251). The bedaquiline load of the NPs increased with increasing drug to polymer ratio ($p \ll 0.05$, one-way ANOVA). The increase in particle size can be attributed to the increased drug content in the emulsion nanodroplets or increased viscosity of the organic phase (Mao et al., 2008). The encapsulation efficiency was almost complete (99%) when the drug to polymer feed was increased from 1:10 to 1:5% (w/w) ($p \gg 0.05$, two sample *t*-test). A slight decrease of the encapsulation efficiency (to 81%) was observed upon a further feed increase from 1:5 to 1:2% (w/w) ($p \ll 0.05$, two sample *t*-test). The loss of bedaquiline was visually evident due to the presence of floating, crystalline-like aggregates during the collection of nanoparticles by centrifugation of the NPs. Apparently, a maximum amount of bedaquiline can be incorporated with a given amount of polymer.

The zeta-potential of bedaquiline-loaded nanoparticles slightly increased from -5.8 ± 0.7 to -2.8 ± 1.5 mV with increasing drug feed ($p \ll 0.05$, two sample *t*-test). The increase in zeta-potential compared to empty nanoparticles suggest that cationic bedaquiline is (partially) located on the surface of the nanoparticles. Surface-bound bedaquiline cannot be removed *via* washing steps, as bedaquiline is insoluble in

water and organic solvents destroy the integrity of the formed nanoparticles.

S.I., Supplementary Table 5 shows the results obtained with vancomycin-loaded mPEG-PLGA nanoparticles prepared *via* the double-emulsion solvent evaporation method. In this case, the hydrodynamic particle size, polydispersity index and zeta-potential of vancomycin-loaded nanoparticles did not differ with different vancomycin feed. The relative amount of loaded vancomycin increased with increasing drug to polymer feed from 3.5% to 8.6%, although the encapsulation efficiencies slightly reduced from 38% to 26% (both $p \ll 0.05$, one-way ANOVA). Non-loaded vancomycin is removed during the washing procedures, as the compound is well soluble in water. Therefore, the measured vancomycin is likely entrapped inside the nanoparticles or perhaps partially bound at the interface *via* electrostatic interactions. Encapsulation efficiencies are relatively low as compared to bedaquiline. Indeed, polar substances have a tendency of migrating towards the external aqueous phase during preparation of the nanoparticles (Astete and Sabliov, 2006).

Nanoparticles with the other two diblock copolymers (mPEG-PLBMGA and mPEG-PLHMGA) were prepared with comparable drug load (~9% wt%) of bedaquiline and vancomycin (using 1:10% (w/w) and 1:2% (w/w) drug to polymer feed, respectively), to rule out the influence of significant differences in drug load on the nanoparticle characteristics. The nanoparticles were obtained in good yields (approx. 65–70%). The characteristics of the loaded nanoparticles based on these different aliphatic polyesters are shown and compared with the corresponding ones from PEG-PLGA in Table 2 and Fig. 2.

Bedaquiline-loaded nanoparticles based on mPEG-PLGA, mPEG-PLBMGA and mPEG-PLHMGA diblock copolymers had similar hydrodynamic size (241–264 nm range) and high encapsulation efficiencies of 95.0–99.1%. Hydrophobic small molecules owing to their inherently high logP, usually have a high encapsulation efficiency due to drug

Table 2
Characteristics of PEG-PLGA, PEG-PLBMGA and PEG-PLHMGA nanoparticles loaded with bedaquiline and vancomycin. (n = 3).

Antibiotic	Polymer	Drug to Polymer ratio (w/w)	Size (nm)	PDI	Zeta potential (mV)	Drug load (wt%)	Encapsulation efficiency (%)
Bedaquiline	PEG-PLHMGA	1:10	254 ± 8	0.140 ± 0.20	-20.6 ± 2.3	8.9 ± 0.9	98.6 ± 1.0
	PEG-PLGA		241 ± 5	0.134 ± 0.01	-5.8 ± 0.7	9.0 ± 0.2	99.1 ± 1.1
	PEG-PLBMGA		265 ± 10	0.211 ± 0.04	-11.0 ± 2.6	8.6 ± 0.4	95.0 ± 2.4
Vancomycin	PEG-PLHMGA	1:2	261 ± 11	0.105 ± 0.03	-11.0 ± 4.9	11 ± 1.0	32.0 ± 3.1
	PEG-PLGA		242 ± 12	0.148 ± 0.07	-6.2 ± 2.0	8.9 ± 1.6	26.8 ± 4.8
	PEG-PLBMGA		250 ± 7	0.090 ± 0.02	-12.9 ± 1.3	9.1 ± 0.4	27.4 ± 1.2

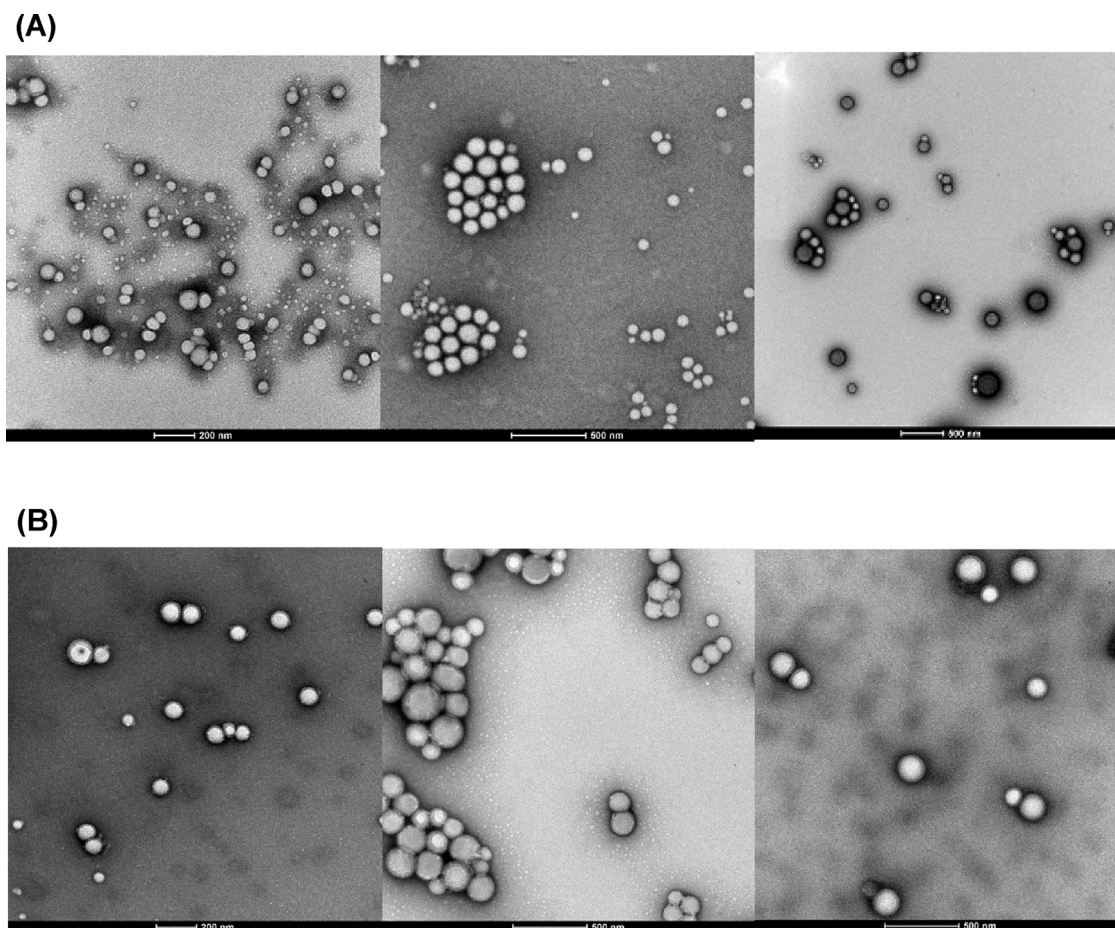


Fig. 2. TEM Pictures of (A) bedaquiline-loaded and (B) vancomycin-loaded mPEG-PLGA (left), mPEG-PLBMGA (middle) and mPEG-PLHMGA (right) nanoparticles.

partitioning towards the organic phase using the single emulsion solvent evaporation method (Mittal et al., 2007). Although drug load can also be affected by the drug-polymer interactions and the drug miscibility with the polymer, our findings show that polymer composition had essentially no effect on the drug partitioning and miscibility during formation of the o/w nanoemulsion and consolidation of the nanoparticles via extraction and evaporation of DCM (Blanco and Alonso, 1998; Desai et al., 2010; Katou et al., 2008; Panyam et al., 2004).

Fig. 2a depicts transmission electron microscopy (TEM) pictures of all nanoparticles, showing spherical particles for all polymers. The particles appear smaller compared to the hydrodynamic size obtained by DLS analysis. Such artifacts are caused due nanoparticle shrinkage through drying of the TEM samples. Loaded polymeric nanoparticles (Table 2) have slightly less negative zeta-potentials (differences of $\sim +4$ mV; $p \ll 0.05$, two-tailed t -test) as compared to the corresponding empty ones (-25 , -9 and -15 mV for empty PEG-PLHMGA, PEG-PLGA and PEG-PLBMGA, respectively), which again suggests partly located (resorbed) bedaquiline on the surface of the nanoparticles.

Preparation of vancomycin-loaded nanoparticles also yielded spherical nanoparticles of comparable hydrodynamic size and drug load for all three polymer matrixes (Table 2 and Fig. 2b). Apparently, the polymer composition of the different nanoparticle matrices does not influence encapsulation of vancomycin, and polar vancomycin is likely incorporated in the internal hydrophilic water pockets of the W/O/W emulsion. The drug load into the most hydrophilic mPEG-PLHMGA NPs could be considered slightly higher compared to the other polymeric nanoparticles. The zeta-potential of mPEG-PLHMGA increased by ~ 11 mV compared to the empty counterpart, while the zeta-potential of vancomycin-loaded mPEG-PLGA and mPEG-PLBMGA NPs only

increased by ~ 3 mV. These findings suggest that vancomycin is probably located at the surface to a higher extent for the mPEG-PLHMGA nanoparticles which could correspond to the higher drug load.

3.3. Nanoparticle degradation

Chemical stability of polymeric nanoparticles was investigated at physiological conditions. Therefore, empty nanoparticles prepared from the different diblock copolymers were incubated in PBS at pH 7.4 and 37 °C. The degradation behavior of all nanoparticles was studied by analyzing the dry weight of the nanoparticles and number average molecular weight of the constituting polymers (and insoluble residues) over time (Fig. 3).

In a timeframe of approx. 1 month, nanoparticles of mPEG-PLGA, mPEG-PLBMGA and mPEG-PLHMGA showed a continuous loss of relative dry mass (Fig. 3a) and decrease in relative number average molecular weights (Fig. 3b). mPEG-PLGA, mPEG-PLHMGA and mPEG-PLBMGA nanoparticles showed decay patterns with mass loss of 70 and 90 and 30% over 35 d, respectively. Previously, nanoparticles from polymers that are similar to mPEG-PLHMGA have been prepared, i.e. mPEG-PLGHMGA block copolymers that contain additional glycolic acid units (Samadi et al., 2013). Those showed similar degradation behavior compared to the present mPEG-PLHMGA NPs. Also, the degradation kinetics of mPEG-PLGA nanoparticles is similar to previously published data (Zweers et al., 2004).

Loss in relative dry mass coincided with the decrease in relative number average molecular weights for all polymeric nanoparticles, which indicates hydrolysis of the ester bonds throughout the nanoparticle matrix and release of water-soluble degradation products by diffusion (Gopferich, 1996). The decrease in relative number average

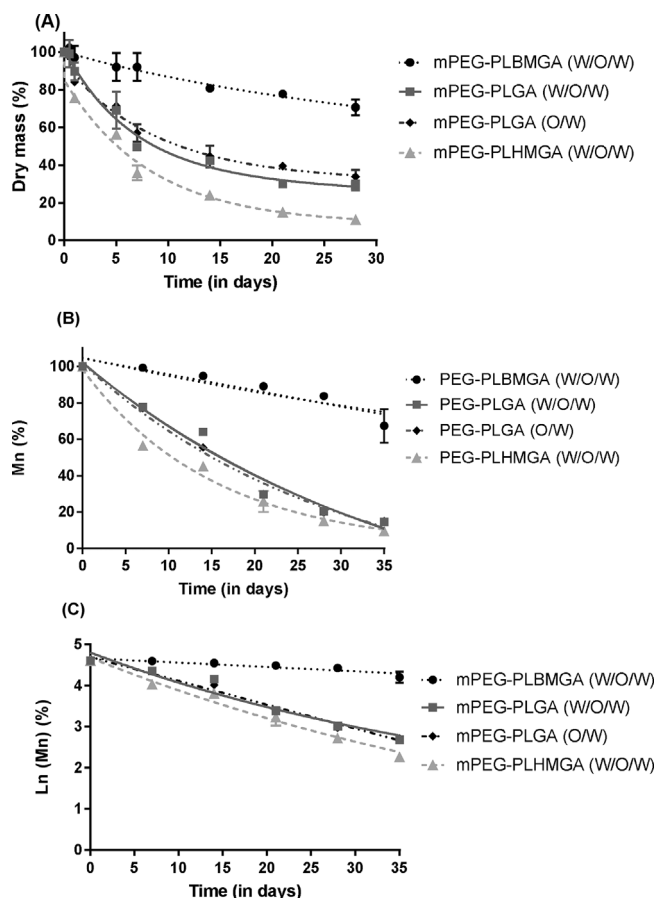


Fig. 3. The degradation kinetics at physiological conditions (PBS, pH 7.4, 37 °C) of empty nanoparticles prepared by single (O/W) and double (W/O/W) emulsion solvent evaporation methods: (A) The relative dry weight, (B) the relative number average molecular weight (M_n , GPC analysis), and (C) semi-logarithmic plot of the relative number average molecular weight ($\ln(M_n)$, GPC analysis) of mPEG-PLGA, mPEG-PLBMGA and mPEG-PLHMGA. $n = 3$.

molecular weights for all polymeric nanoparticles follows pseudo-first-order kinetics with the following rate equation (Siepmann et al., 2005):

$$Mn_t(\%) = Mn_0(\%) \cdot e^{-k_{\text{degr}} \cdot t} \quad (1)$$

where $Mn_t(\%)$ and $Mn_0(\%)$ denote the relative number-averaged molecular weight (in %) of the polymer at time t and $t = 0$, respectively, and k_{degr} denotes the degradation rate constant of the polymer.

Using a semi-logarithmic plot (Fig. 3c), the degradation rate constants were obtained from the slopes. The linear relationship ($R^2 \gg 0.98$) indicates degradation mechanism *via* bulk erosion for all nanoparticles (Siepmann et al., 2005). During bulk degradation, water can penetrate the polymeric matrix readily and all polyester chains are reached for degradation by hydrolytic scission. The degradation rate constants were equal to 0.010, 0.059 and 0.066 d^{-1} for mPEG-PLBMGA, mPEG-PLGA and mPEG-PLHMGA NPs, respectively, and illustrate that polarity of the polymer matrix and thus the degradation rate of nanoparticles based on aliphatic polyesters can be tailored by functionalization of the random copolymer with pendant hydrophobic or hydrophilic groups. The benzyl pendant groups reduces degradation (i.e. hydrolysis) *via* decreased polymer hydration and/or decreased water solubility of monomeric or oligomeric degradation products, whereas the hydroxyl group of mPEG-PLHMGA enhances degradation by enhanced water retention and solubility of degradation products as compared to PEG-PLGA. Molecular weight of polymers may also affect the degradation rate, with higher molecular weight polymers generally exhibiting lower degradation rates (Park, 1995). However, Supplementary Fig. 1 and Table 2 in Supporting information showed that

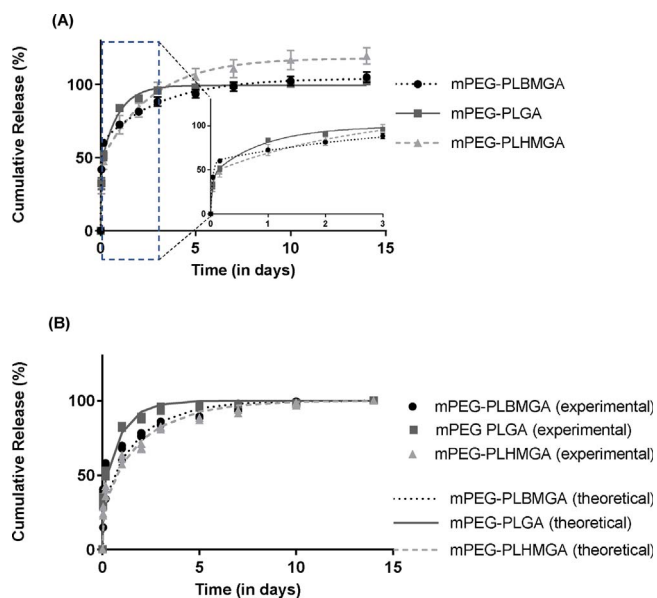


Fig. 4. Cumulative release of bedaquiline from PEG-PLGA, PEG-PLBMGA and PEG-PLHMGA nanoparticles: (A) Experimental cumulative release over time, (B) Normalized mean experimental cumulative release (circles, squares, triangles) and theoretical mean release (open and closed lines) over time. Release medium was 10% FBS in PBS, pH 7.4 at 37 °C. $n = 3$.

mPEG-PLBMGA, mPEG-PLGA and mPEG-PLHMGA had similar number-average molecular weights based on GPC and 1H NMR. The significant differences in degradation rates of mPEG-PLBMGA and mPEG-PLHMGA are too large to exclusively be attributed to the relatively small differences in the molecular weights.

The influence of the preparation method (O/W or W/O/W method) on the degradation behavior was checked with mPEG-PLGA nanoparticles: The degradation constant for the mPEG-PLGA NPs prepared with the O/W method (0.058 d^{-1}) was comparable to NPs prepared with the W/O/W method (0.059 d^{-1}). This suggest that bulk erosion is mainly induced by water penetration from the environment into nanoparticle matrices, while the contribution of the internal aqueous pockets of nanoparticles prepared with W/O/W method is insignificant.

3.4. Antibiotic release from polymeric nanoparticles

The release of bedaquiline and vancomycin from mPEG-PLGA, mPEG-PLBMGA and mPEG-PLHMGA nanoparticles was investigated in PBS (pH 7.4) containing 10% fetal bovine serum (FBS) at 37 °C. FBS was used to bind released drug and thus acquire full dissolution into the release medium, which was especially required for the poorly water soluble bedaquiline. Saturation solubility of bedaquiline in 10% FBS was determined and reached at least 5 times higher than the concentrations that were obtained after full release from the nanoparticles,

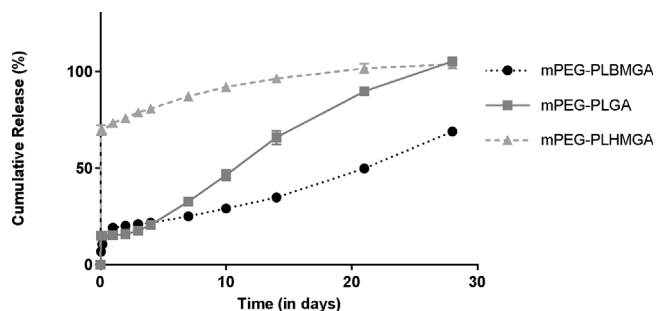


Fig. 5. Cumulative release of vancomycin from PEG-PLGA, PEG-PLBMGA and PEG-PLHMGA nanoparticles. Release medium was 10% FBS in PBS, pH 7.4 at 37 °C. $n = 3$.

which guaranteed sink conditions. During release experiments, nanoparticles from the samples taken at different time points were separated from the medium by ultracentrifugation, and released drug in the medium was quantitatively analyzed by LC-QqQ-MS/MS including a stable isotope labelled internal standard for optimal accuracy. The release of bedaquiline and vancomycin from different formulations are presented in Figs. 4 and 5 and are expressed in terms of cumulative release versus time.

The release of bedaquiline from all formulations consisted of an initial, fast release (30–42%) within the first hour, which is probably partially caused by non-encapsulated or surface-bound bedaquiline (Fig. 4a). After the initial (burst) release, all bedaquiline-loaded nanoparticles showed similar trends of a fast, continuous release up to 100% within ~3–7 days. The (complete) release is governed by the presence of proteins in the media that can bind the otherwise insoluble bedaquiline. The contribution of degradation/erosion mechanisms is most likely very low, since the release of bedaquiline does primarily not coincide with the degradation rates of the nanoparticles and polymers (Fig. 3).

Fick's second law of diffusion can be used to analyze the release of bedaquiline from the different polymeric nanoparticles (Crank, 1956). Crank's model for spherical geometry was applied on the notion that release is governed by diffusion with constant diffusivities (Arifin et al., 2006; D'Aurizio et al., 2011):Crank, 1956; D'Aurizio et al., 2011):

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \cdot \sum_{n=1}^{\infty} \frac{1}{n^2} \cdot e^{-D_{\text{eff}} n^2 \pi^2 t / r^2} \quad (2)$$

where M_∞ and M_t represent the absolute drug release at infinite and specific time t , respectively; r denotes the radius of the nanoparticles and D the diffusion coefficient of the drug. Crank's model assumes that drug is released from a monolithic system and drug is uniformly dispersed or dissolved in the polymer.

The theoretical curves were fitted to the cumulative release data with the least-square method, in which M_∞ (of Fig. 4A) was normalized to 100%, thereby obtaining the diffusion-coefficients (Table 3). Fig. 4B shows the theoretical release curves of the mean theoretical diffusion coefficients. Table 3 shows close agreements ($R^2 = 0.97–0.98$) between the theoretical and experimental cumulative release patterns, thus supporting the principal release mechanism of diffusion. In the case of diffusion-controlled release, it was expected that the benzoyl groups of mPEG-PLBMGA would enhance drug retention within the nanoparticles and reduce release/diffusion rates via hydrophobic interactions (i.e. pi-pi stacking) (Shi et al., 2013), as bedaquiline is encapsulated within the polymeric nanoparticle via the single-emulsion evaporation technique. However, drug release patterns and diffusion coefficients of bedaquiline from all polymeric nanoparticles are around $1 \times 10^{-20} \text{ m}^2/\text{s}$. Release of bedaquiline via diffusion through the polymer matrix thus seems to be independent of the polymeric structure.

The release of vancomycin from all polymeric nanoparticles is shown in Fig. 5. In contrary to bedaquiline loaded nanoparticles,

Table 3

Effective diffusion coefficients (D_{eff}) of bedaquiline in PEG-PLGA, PEG-PLBMGA and PEG-PLHMGA nanoparticles of radius r , obtained from fitted release curves via least-squares regression. Drug to polymer ratio in the feed was 1:10 (w/w). R^2 represents the correlation coefficient, $n = 3$.

Antibiotic	Polymer	Average hydrodynamic radius (r ; in nm)	D_{eff} ($10^{-20} \text{ m}^2/\text{s}$)	R^2
Bedaquiline	PEG-PLHMGA	127	0.80 ± 0.08	0.98 ± 0.01
	PEG-PLGA	120	1.02 ± 0.13	0.98 ± 0.01
	PEG-PLBMGA	132	1.26 ± 0.22	0.97 ± 0.02

differences in the polymeric composition (i.e. polarity of the polymer matrix) have an influence on the burst release and release rate of vancomycin. The initial (burst) release of vancomycin measured within the first hour was 20% from mPEG-PLBMGA NPs, 18% from PEG-PLGA nanoparticles, and 65% from PEG-PLHMGA NPs. After the initial release, vancomycin-loaded PEG-PLHMGA nanoparticles showed a continuous release up to 100% within 8–10 days, while the burst release of vancomycin from PEG-PLBMGA and PEG-PLGA NPs was followed by a lag phase of approx. 5 d. This triphasic release pattern of vancomycin resulted in 100% release of vancomycin from PEG-PLGA NPs in 28 days, whereas only 65% of vancomycin was released from mPEG-PLBMGA NPs within the same time frame.

The release patterns of vancomycin indicate a combination of a diffusion and degradation controlled release: After the initial (burst) release and evident lag phase, a second phase of increased release rates correlates with the mass loss and polymer degradation. In contrary to bedaquiline, which is incorporated into the polymer matrix, encapsulated vancomycin being a hydrophilic molecule is retained within the internal aqueous volume of the nanoparticles formed via the double-emulsion evaporation technique. Therefore, release of vancomycin is probably predominantly dependent on the presence of hydrophilic pores, absorption of water, and/or degradation of the polymer (Kang and Schwendeman, 2007; Kim et al., 2006; Mochizuki et al., 2008; Shah et al., 1992). The high initial release from mPEG-PLHMGA nanoparticles could be explained by the relatively high degradation rate, the presence of hydrophilic pores due to the hydrophilic polymer composition, and the relative high amount of vancomycin present on the surface of the mPEG-PLHMGA NPs or their pores through electrostatic interactions as suggested by the zeta-potential data presented above. Previously, microspheres prepared from non-PEGylated PLHMGA polymer but loaded with another hydrophilic antibiotic (gentamycin) showed a burst release depending on their porosity, followed by a lag phase of 35 days with hardly any gentamycin release and subsequent complete sustained release in 25 days (Chaisri et al., 2011). The differences between the release patterns of the microspheres (relatively low burst and lag phase) and the PEGylated nanoparticles (high burst, no lag phase) could be ascribed to the differences in size, i.e. diffusion path length, and the presence of PEG in the nanoparticle which make them even more hydrophilic than the non-PEGylated PLHMGA microspheres (Crank, 1956; Samadi et al., 2014).

4. Conclusion

This study provides an understanding of the relationship between polymer nanoparticle composition and the encapsulation and release of antibiotics of different polarity. Drug polarity and preparation techniques affected absolute drug load into polymer nanoparticles. Independent of the polymer composition, the hydrophobic antibiotic, bedaquiline, could be encapsulated completely, while a hydrophilic antibiotic, vancomycin, could only be entrapped moderately. Nonpolar bedaquiline was predominantly released rapidly by predominant mechanism of diffusion, which was independent of the polymer matrix. On the contrary, the release of vancomycin was governed by a combination of diffusion and degradation and was thus dependent on the composition of the polymer matrix. Findings show that selection of drug polarity and polymer composition is pivotal to obtain desirable encapsulation and release characteristics.

Acknowledgements & funding sources

This work is part of the NAREB European research network (Collaborative Project) supported by the European Union's 7th Framework Programme for research, technological development and demonstration under grant agreement no 604237.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ijpharm.2017.11.017>

References

- Alekshun, M.N., Levy, S.B., 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell* 128, 1037–1050.
- Arifin, D.Y., Lee, L.Y., Wang, C.-H., 2006. Mathematical modeling and simulation of drug release from microspheres: implications to drug delivery systems. *Adv. Drug Deliv. Rev.* 58, 1274–1325.
- Astete, C.E., Sabliov, C.M., 2006. Synthesis and characterization of PLGA nanoparticles. *J. Biomater. Sci. Polym. Ed.* 17, 247–289.
- Blanco, D., Alonso, M.J., 1998. Protein encapsulation and release from poly(lactide-co-glycolide) microspheres: effect of the protein and polymer properties and of the encapsulation of surfactants. *Eur. J. Pharm. Biopharm.* 45, 285–294.
- Chaisri, W., Ghassemi, A.H., Hennink, W.E., Okonogi, S., 2011. Enhanced gentamicin loading and release of PLGA and PLHMGGA microspheres by varying the formulation parameters. *Colloids Surf. B Biointerfaces* 84, 508–514.
- Cowie, J.M.G., McEwen, I.J., 1982. Glass and sub-glass transitions in methylphenyl and chlorophenyl polyitaconic acid esters. *Eur. Polym. J.* 18, 555–558.
- Crank, J., 1956. *The Mathematics of Diffusion*. Clarendon Press.
- D'Aurizio, E., van Nostrum, C.F., van Steenberg, M.J., Sozio, P., Siepmann, F., Siepmann, J., Hennink, W.E., Di Stefano, A., 2011. Preparation and characterization of poly(lactide-co-glycolic acid) microspheres loaded with a labile antiparkinson prodrug. *Int. J. Pharm.* 409, 289–296.
- Danhier, F., Ansorena, E., Silva, J.M., Coco, R., Le Breton, A., Pr at, V., 2012. PLGA-based nanoparticles: an overview of biomedical applications. *J. Controlled Release* 161, 505–522.
- DeRyke, C.A., Lee, S.Y., Kuti, J.L., Nicolau, D.P., 2006. Optimising dosing strategies of antibacterials utilising pharmacodynamic principles: impact on the development of resistance. *Drugs* 66, 1–14.
- Desai, K.G., Olsen, K.F., Mallery, S.R., Stoner, G.D., Schwendeman, S.P., 2010. Formulation and in vitro-in vivo evaluation of black raspberry extract-loaded PLGA/PLA injectable millicylindrical implants for sustained delivery of chemopreventive anthocyanins. *Pharm. Res.* 27, 628–643.
- Fredenberg, S., Wahlgren, M., Reslow, M., Axelsson, A., 2011. The mechanisms of drug release in poly(lactide-co-glycolic acid)-based drug delivery systems—a review. *Int. J. Pharm.* 415, 34–52.
- Fu, Y., Kao, W.J., 2010. Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. *Expert Opin. Drug Deliv.* 7, 429–444.
- Ghassemi, A.H., van Steenberg, M.J., Barendregt, A., Talsma, H., Kok, R.J., van Nostrum, C.F., Crommelin, D.J., Hennink, W.E., 2012. Controlled Release of octreotide and assessment of peptide acylation from poly(D,L-lactide-co-hydroxymethyl glycolide) compared to PLGA microspheres. *Pharm. Res.* 29, 110–120.
- Ghassemi, A.H., van Steenberg, M.J., Talsma, H., van Nostrum, C.F., Crommelin, D.J., Hennink, W.E., 2010. Hydrophilic polyester microspheres: effect of molecular weight and copolymer composition on release of BSA. *Pharm. Res.* 27, 2008–2017.
- Ghassemi, A.H., van Steenberg, M.J., Talsma, H., van Nostrum, C.F., Jiskoot, W., Crommelin, D.J., Hennink, W.E., 2009. Preparation and characterization of protein loaded microspheres based on a hydroxylated aliphatic polyester, poly(lactide-co-hydroxymethyl glycolic acid). *J. Controlled Release* 138, 57–63.
- Gopferich, A., 1996. Mechanisms of polymer degradation and erosion. *Biomaterials* 17, 103–114.
- Huh, A.J., Kwon, Y.J., 2011. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J. Controlled Release* 156, 128–145.
- Jokerst, J.V., Lobovkina, T., Zare, R.N., Gambhir, S.S., 2011. Nanoparticle pegylation for imaging and therapy. *Nanomed. (Lond., Engl.)* 6, 715–728.
- Kamaly, N., Yameen, B., Wu, J., Farokhzad, O.C., 2016. Degradable controlled-release polymers and polymeric Nanoparticles: mechanisms of controlling drug release. *Chem. Rev.* 116, 2602–2663.
- Kang, J., Schwendeman, S.P., 2007. Pore closing and opening in biodegradable polymers and their effect on the controlled release of proteins. *Mol. Pharm.* 4, 104–118.
- Katou, H., Wandrey, A.J., Gander, B., 2008. Kinetics of solvent extraction/evaporation process for PLGA microparticle fabrication. *Int. J. Pharm.* 364, 45–53.
- Kim, H.K., Chung, H.J., Park, T.G., 2006. Biodegradable polymeric microspheres with "open/closed" pores for sustained release of human growth hormone. *J. Controlled Release* 112, 167–174.
- Leemhuis, M., Kruijtz, J.A., Nostrum, C.F., Hennink, W.E., 2007. In vitro hydrolytic degradation of hydroxyl-functionalized poly(alpha-hydroxy acid)s. *Biomacromolecules* 8, 2943–2949.
- Leemhuis, M., van Nostrum, C.F., Kruijtz, J.A.W., Zhong, Z.Y., ten Breter, M.R., Dijkstra, P.J., Feijen, J., Hennink, W.E., 2006. Functionalized poly(alpha-hydroxy acid)s via ring-opening polymerization: toward hydrophilic polyesters with pendant hydroxyl groups. *Macromolecules* 39, 3500–3508.
- Makadia, H.K., Siegel, S.J., 2011. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers* 3, 1377–1397.
- Mao, S., Shi, Y., Li, L., Xu, J., Schaper, A., Kissel, T., 2008. Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method. *Eur. J. Pharm. Biopharm.* 68, 214–223.
- Mase, S., Chorba, T., Lobue, P., Castro, K., 2013. Provisional CDC guidelines for the use and safety monitoring of bedaquiline fumarate (sirturo) for the treatment of multidrug-resistant tuberculosis. *MMWR. Recommendations and reports: morbidity and mortality weekly report. Recomm. Rep.* 62, 1–12.
- Mittal, G., Sahana, D.K., Bhardwaj, V., Ravi Kumar, M.N., 2007. Estradiol loaded PLGA nanoparticles for oral administration: effect of polymer molecular weight and copolymer composition on release behavior in vitro and in vivo. *J. Controlled Release* 119, 77–85.
- Mochizuki, A., Niikawa, T., Omura, I., Yamashita, S., 2008. Controlled release of argatroban from PLA film—effect of hydroxylesters as additives on enhancement of drug release. *J. Appl. Polym. Sci.* 108, 3353–3360.
- Panyam, J., Williams, D., Dash, A., Leslie-Pelecky, D., Labhasetwar, V., 2004. Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. *J. Pharm. Sci.* 93, 1804–1814.
- Park, T.G., 1995. Degradation of poly(lactide-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials* 16, 1123–1130.
- Pizzirani, G., Magagnini, P., Giusti, P., 1971. Glass transition temperatures of some linear polymers containing phenyl side groups. *J. Polym. Sci. Part. A-2: Polym. Phys.* 9, 1133–1145.
- Rahimian, S., Fransen, M.F., Kleinovink, J.W., Amidi, M., Ossendorp, F., Hennink, W.E., 2015a. Polymeric microparticles for sustained and local delivery of antiCD40 and antiCTLA-4 in immunotherapy of cancer. *Biomaterials* 61, 33–40.
- Rahimian, S., Fransen, M.F., Kleinovink, J.W., Christensen, J.R., Amidi, M., Hennink, W.E., Ossendorp, F., 2015b. Polymeric nanoparticles for co-delivery of synthetic long peptide antigen and poly IC as therapeutic cancer vaccine formulation. *J. Controlled Release* 203, 16–22.
- Rybak, M.J., Lomaestro, B.M., Rotschafner, J.C., Moellering, J.R.C., Craig, W.A., Billeter, M., Daloviso, J.R., Levine, D.P., 2009. Vancomycin therapeutic guidelines: a summary of consensus recommendations from the Infectious Diseases Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists. *Clin. Infect. Dis.* 49, 325–327.
- Samadi, N., van Nostrum, C.F., Vermonden, T., Amidi, M., Hennink, W.E., 2013. Mechanistic studies on the degradation and protein release characteristics of poly(lactide-co-glycolic-co-hydroxymethylglycolic acid) nanospheres. *Biomacromolecules* 14, 1044–1053.
- Samadi, N., van Steenberg, M.J., van den Dikkenberg, J.B., Vermonden, T., van Nostrum, C.F., Amidi, M., Hennink, W.E., 2014. Nanoparticles based on a hydrophilic polyester with a sheddable PEG coating for protein delivery. *Pharm. Res.* 31, 2593–2604.
- Schiffelers, R.M., Bakker-Woudenberg, I.A., Storm, G., 2000. Localization of sterically stabilized liposomes in experimental rat Klebsiella pneumoniae pneumonia: dependence on circulation kinetics and presence of poly(ethylene)glycol coating. *Biochim. Biophys. Acta* 1468, 253–261.
- Schiffelers, R.M., Storm, G., Bakker-Woudenberg, I.A., 2001. Host factors influencing the preferential localization of sterically stabilized liposomes in Klebsiella pneumoniae-infected rat lung tissue. *Pharm. Res.* 18, 780–787.
- Shah, S.R., Henslee, A.M., Spicer, P.P., Yokota, S., Petrichenko, S., Allahabadi, S., Bennett, G.N., Wong, M.E., Kasper, F.K., Mikos, A.G., 2014. Effects of antibiotic physico-chemical properties on their release kinetics from biodegradable polymer microparticles. *Pharm. Res.* 31, 3379–3389.
- Shah, S.S., Cha, Y., Pitt, C.G., 1992. Poly (glycolic acid-co-dl-lactic acid): diffusion or degradation controlled drug delivery? *J. Controlled Release* 18, 261–270.
- Shi, Y., van Steenberg, M.J., Teunissen, E.A., Novo, L.S., Gradmann, S., Baldus, M., van Nostrum, C.F., Hennink, W.E., 2013. II-II Stacking increases the stability and loading capacity of thermosensitive polymeric micelles for chemotherapeutic drugs. *Biomacromolecules* 14, 1826–1837.
- Siepmann, J., Elkharraz, K., Siepmann, F., Klose, D., 2005. How autocatalysis accelerates drug release from PLGA-based microparticles: a quantitative treatment. *Biomacromolecules* 6, 2312–2319.
- Suk, J.S., Xu, Q., Kim, N., Hanes, J., Ensign, L.M., 2016. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv. Drug Deliv. Rev.* 99, 28–51.
- Yilmaz, C.,  zcengiz, G., 2017. Antibiotics: pharmacokinetics, toxicity, resistance and multidrug efflux pumps. *Biochem. Pharmacol.* 133, 43–62.
- Zweers, M.L., Engbers, G.H., Grijpma, D.W., Feijen, J., 2004. In vitro degradation of nanoparticles prepared from polymers based on DL-lactide, glycolide and poly(ethylene oxide). *J. Controlled Release* 100, 347–356.