

DSPE-PEG-PLGA nanoparticles as carriers of fixed dose drug combination; effect of polymer type and concentration on their physical characteristics

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Introduction

Lipid-polymer hybrid nanoparticles (LPHNPs) are relatively new drug delivery systems that can help in overcoming several challenges in drug delivery such as insufficient drug loading, dual-drug loading, undefined drug release kinetics, instability, limited drug circulation time and cellular uptake, systemic metabolism, and bio-incompatibility. Modulated polymeric core of natural, semi-synthetic or synthetic polymer provides relatively high loading of drugs and prevents their systemic metabolism, while lipid coating enhances the biocompatibility and stability of NPs, prevents the escape of encapsulated fluid, delays the polymer degradation by preventing inward water diffusion and in this way sustains drug release. Cationic lipids could be further used to enhance cytotoxicity and mucoadhesion of NPs as well, while PEGylated outer lipid shell to diminish NPs aggregation, improve their stability in serum, biocompatibility, prolong systemic circulation and enhance drug release along with improved activity. Along with the polymeric core, the lipid coating has also drug entrapping potential and these characteristics offer loading of combination therapies. On the surfaces of LPHNPs an adequate ligand(s) can be also bonded for providing targeted delivery (Shah et al., 2022).

Various methods have been used for preparation of LPHNPs, each having different challenges (Sivadasan et al., 2021). Among them, single-step nanoprecipitation method followed by particles self-assembly is listed as a

preferred one being less time consuming and adequate for designing this kind of NPs. Within the formulation variables defining the physical properties of LPHNPs, the polymer type, viscosity and concentration play one of the key roles. In a previous study, fixed combination of hydrophilic drug Rosuvastatin (ROS) and a hydrophobic one Ezetimibe (EZE) was incorporated in LPHNPs using one step nanoprecipitation method (Nakjinova et al., 2022). The aim of this study was to test the influence of the polymer type and concentration on the physical characteristics of these dual-drug loaded LPHNPs. Ester or acid terminated PLGA was used for forming the polymer core and the encapsulation efficacy (EE), drugs content (DC), hydrodynamic particle diameter (z-average), polydispersity index (PDI) and zeta potential (ZP) of NPs were determined as responses to the formulation variables.

Materials and methods

Ester terminated poly (D,L-lactide/glycolide) (PLGA 50:50, inherent viscosity 0.4 dL/g) and acid terminated PLGA (50:50, inherent viscosity 0.2 dL/g) were obtained as gifts from Corbion (Netherlands). Soybean phosphatidylcholine and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] from Lipoid GmbH (Germany). Poloxamer 188 was purchased from BASF (Germany). ROS (calcium salt) and EZE (free base) were purchased from DSM and Lupin Ltd. (India), respectively. As organic phase solvent, acetonitrile

(ACN) was used, supplied by Merck (Germany). All other chemicals used were of analytical grade.

Preparation of drugs loaded LPHNPs: ACN solutions of EZE (0.8 mg/mL) and ROS (0.5 mg/mL), and ester terminated PLGA (5, 7.5 and 10 mg/mL) were prepared and added drop-wise into the 4% w/w hydroethanolic lipid dispersion pre-heated at 65 °C under constant mixing, allowing self-assembly of nanoparticles to occur with subsequent ACN evaporation. The phase volume ratio (ACN solution to 4% w/w hydroethanolic lipid dispersion) was 1:4. Poloxamer 188 (7.5 mg/mL) was used as a bi-functional block copolymer surfactant and NPs stabilizer present in hydroethanolic phase. LPHNPs separation was performed through initial vacuum filtration on cellulose filters of 2-3 µm pore size and subsequent ultracentrifugation of the initially filtrated LPHNPs suspension on 3 kDa cut off filters (Amicon®, Merck, Germany) for removal of non-encapsulated drugs.

Physical characterization of drugs loaded LPHNPs: Z-average, PDI and ZP of ROS/EZE loaded LPHNPs were determined using Zetasizer Nano ZS-100 (Malvern Instruments Ltd., UK). EE was calculated by indirect method, analyzing the difference between the quantity of drugs in pre-filtered NP suspension and in 3 kDa resulting filtrate, using HPLC method (Zorbax Eclipse XDB-C18, 80Å, 4.6 x 150 mm, 5 µm column; Agilent, USA). DC was calculated based on amount of encapsulated drugs divided by total LPHNPs weight.

Results and discussion

With ester terminated PLGA (7.5 mg/mL) and described synthesis method, dual-drug loaded LPHNPs were prepared, with z-average 124 nm, PDI 0.285 and ZP -1.14 mV, and EE of ROS and EZE up to 50% and 16% respectively, with DC 2.5% (theoretical 4.4%) for ROS and 1.2% (theoretical 7.1%) for EZE. When lowering the concentration of ester terminated PLGA from 7.5 mg/mL to 5 mg/mL while keeping other variables constant, the EE decreased for both drugs, with a decrease being higher for EZE (from 16% to 9%) compared to ROS (from 50% to 35%). Unexpectedly, increase in the concentration of PLGA (ester terminated) up to 10 mg/mL led to higher EEs compared to 5 mg/mL (39% and 12% for ROS and EZE, respectively), but this trend was not observed when comparing with the concentration of 7.5 mg/mL, where a decrease in EE from 50% to 39% for ROS and from 16% to 12% for EZE was determined. DC followed the trend of decrease with increase in the PLGA concentration, being within 2.5-1.8% for ROS and 1.1-0.8% for EZE. Irrespective of the concentration of PLGA (ester terminated), the ZP remained neutral to slightly negative [0.09 mV (5 mg/mL) and -0.07 mV (10 mg/mL)]. Increase in the concentration of ester terminated PLGA led to

increase in z-average and decrease in the PDI, ranging between 114 nm (5 mg/mL) and 160 nm (10 mg/mL) and 0.382 and 0.200, respectively, suggesting that the increased viscosity of organic phase slows the diffusion of the polymer into the hydroethanolic phase thereby provoking increased polymer precipitation. When ester terminated PLGA was replaced by acid terminated (at 7.5 mg/mL), EE of ROS and EZE decreased from 50% to 19% and from 16% to 14%, respectively, which points to significant influence of polymer type on the EE of hydrophilic drug. DC was 1.2% (theoretical 4.4%) for ROS and 1.4% (theoretical 7.1%) for EZE. Expectedly, more negative ZP (-14.1 mV) and lower z-average (79 nm; PDI 0.471) of LPHNPs were obtained, which can be explained by a higher attraction between the PLGA molecules than with water of the more hydrophobic ester terminated PLGA, which ended up in larger NPs.

Conclusion

Fixed combination of hydrophilic and hydrophobic drugs was successfully incorporated in LPHNPs. Type and concentration of polymer used significantly affected the physical characteristics of the NPs and should be considered as formulation variables in the optimization study.

References

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