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Short communication

Influence of organic to aqueous phase solvent volume ratio on the physicochemical characteristics of rosuvastatin and ezetimibe loaded lipid-polymer hybrid nanoparticles prepared by nanoprecipitation method

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Introduction

Lipid-lowering drugs rosuvastatin (ROS) and ezetimibe (EZE) have been used as a fixed-dose combination in conventional dosage forms due to their complementary mechanism of action and ability to reduce low-density lipoprotein cholesterol levels. In the actual study an attempt was made to incorporate ROS and EZE in lipid-polymer hybrid nanoparticles (LPHNPs). These NPs have been reported as advantageous over lipid or polymer type NPs and liposomes due to their superior *in vivo* cellular delivery (Sivadasan et al., 2021). One expects that with incorporation of ROS and EZE in these advanced delivery systems, therapeutic response will be improved due the possibility for selective hepatocellular targeting and avoiding possible side effects of nonselective cholesterol synthesis impairment. For ROS and EZE loading in LPHNPs, the nanoprecipitation method was used as the most simple and efficacious one, where the solvent phase is added drop-wise in the "non-solvent" aqueous phase. In this paper the potential of the nanoprecipitation method for successful encapsulation of fixed-combination of drugs having hydrophilic (ROS) and lipophilic (EZE) characteristics was evaluated. Specifically, the influence of phase volume ratios on the encapsulation efficacy (EE), particle size distribution (PSD), polidispersity index (PDI) and zeta potential (ZP) was determined.

Materials and methods

Materials

Ester terminated poly(D,L-lactide/glycolide) with Mw 45000-80000 (50:50) was obtained as a gift from Corbion (Netherlands), hydrogenated soybean phosphatidylcholine and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[metoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}) from Lipoid GmbH (Germany), Poloxamer 188 was purchased from BASF (Germany), all used for the LPHNPs preparation. ROS as calcium salt and EZE as a free base were purchased from DSM (India) 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 ROS and EZE loaded LPHNPs

ACN solution of EZE (0.8 mg/mL) and ROS (0.5 mg/mL), and PLGA (7.5 mg/mL) was 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 (ACN solution to 4% w/w hydroethanolic lipid dispersion) ratio was varied in a range 1:1; 1:2; 1:4 and 1:6, while keeping the other variables constant. Poloxamer 188 in 1:1 weight

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ratio to polymer was used as a NPs stabiliser present in hydroethanolic phase. LPHNPs separation was performed through initial vacuum mechanical filtration on 2-3 µm pore size cellulose filter and subsequent purification and concentration with ultracentrifugation of the pre-filtered LPHNs suspension on 3 kDa cut off filters (Amicon®, Merck, Germany) for non-encapsulated drugs removal.

Physicochemical characterization of ROS and EZE loaded LPHNPs

PSD, PDI and ZP of particles were determined using Zetasizer Nano ZS-100 (Malvern Instruments Ltd., UK) in purified water and 10 mM phosphate buffers of pH 6.8 and 7.4. EE % was calculated by indirect method through analysis of drugs quantity difference in pre-filtered nanoparticle suspension and 3 kDa resulting filtrate using HPLC method (Zorbax Eclipse XDB-C18, 80Å, 4.6 x 150 mm, 5 µm column; Agilent, USA).

Results and discussion

EEs of 1.78-50% for ROS and 9.8-69.12% for EZE were obtained. PSD ranged from 123.8-275 nm and PDIs were within 0.261-0.581. Slightly negative to neutral values for ZP were obtained (-3.38 to 0.24 *mV*) when measured in purified water as well as in both phosphate buffers having pH 6.8 and 7.4. Non-significant difference in ZPs between the different trials was observed when measured in the same medium, while significant difference between the formulations was observed ($p=0.013$) when measured in different media. Water phase volume increase led to increase of EE of hydrophilic drug ROS up to 50%, reaching the maximum value at 1:4 ratio, while the EE of the lipophilic drug EZE decreased to 9.8%, reaching its minimal value at 1:6 ratio. For comparison, the EE of only 1.78% was obtained for ROS and 42.75% for EZE when the LPHNPs were prepared by a phase volume ratio of 1:1. Having this in regard, one can assume that the precipitation potential of the lipophilic drug at the initial contact with the higher water content media increased and by that, the chances for spontaneous precipitation within the assembled particles through evaporation of ACN decreased. These results are slightly different from those of Tahir et al. (2019), who obtained better EE of the lipophilic drug and lower values for hydrophilic drug when using nanoprecipitation method and same lipid and polymer coatings. Optimal EE of both drugs was achieved when 30 w/w lipid to polymer, 1:15 ROS and 1:10 EZE to polymer weight ratio and 1:4 ACN to hydroethanolic phase were utilized, being 50% and 15.6% for ROS and EZE, respectively. For this formulation, an average PSD of 124 nm, PDI of 0.285 nm and ZP of –1.14 *mV* were obtained, being an ideal candidate for hepatocellular targeting. Change in the volume ratio led to statistically significant difference in the PSD among trials ($p=0.012$), without statistically significant difference in the PDIs.

Although volume ratios of 1:1 are mentioned in the literature as a worst condition in nanoprecipitation method (Lepeltier et al., 2015), influence on double increase of polymer concentration while keeping this volume ratio as 1:1 was also evaluated. The increase of polymer concentration resulted in reverse case of the EE in favour of hydrophobic drug where EE of 69.12% was observed, while obtaining 17.6% EE for the hydrophilic drug ROS. The particle size increased as well to 275.9 nm, with PDI being 0.581 and ZP –0.466 *mV*. In order to exclude/confirm the hypothesis that the EE is influenced only by the capacity of the NPs to entrap the drugs, in the series prepared with 1:1 volume ratio, the concentrations of APIs were decreased for one half. No increase in EE for both drugs was observed, but with higher API concentrations drug loading was increased.

Conclusion

In this study, the synergistic fixed-drug combination of lipid-lowering drugs ROS and EZE in LPHNPs was efficaciously incorporated using nanoprecipitation method. With the phase volumes ratio variation, LPHNPs with optimal physicochemical characteristics were obtained suitable for hepatocellular targeting. Further studies for their biopharmaceutical characterization are needed in order to confirm their potential for hepatocellular targeting.

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