ANALYTICAL INVESTIGATION TECHNIQUES IN THE SERVICE OF LIPOSOME DEVELOPMENT

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Abstract
The quality of the liposome formulations differs based on the features of the compositions. Several investigation methods help with their results to make the right decision to achieve an optimised preparation. During these sets of experiments to prepare liposomes via the thin-film hydration technique, dynamic light scattering (DLS) and zeta potential measurements, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) investigations, and Fourier-transformed infrared (FT-IR) studies were used to select the more optimal compositions. The formulations differed in the phospholipid-cholesterol ratio, the type of the applied PEGylated phospholipid, the quality of the solvent, the hydration media and the cryoprotectant.

Introduction
The investigation of nanocarrier systems, such as the liposomes, is one of the core points of pharmaceutical research and development fields [1]. Liposomes are described as artificially prepared vesicles composed of one or more concentric lipid bilayers that are enclosing one or more aqueous compartments by the European Medicine Agency [2]. Due to their bilayer structure, liposomes provide a sufficient opportunity for targeted drug delivery in case of both hydrophobic and hydrophilic compounds over the body. As the number of information and novelties increases, the options of developments raise as well to get a high-quality product.
The goal of this study was to find the optimal phospholipid-cholesterol ratio, solvent, hydration media and cryoprotectant for liposomal formulations made from different types of PEGylated phospholipids (DPPE-PEG2000, DSPE-PEG3000).

Experimental
The liposomes were prepared via the thin-film hydration method [3] using different combinations of phosphatidylcholine, cholesterol, and PEGylated phospholipids. The particle size and size distribution values were determined via dynamic light scattering (DLS) technique and the zeta potentials of the vesicle surfaces measured with a zetasizer. Additionally, thermogravimetric analyses (TGA) and differential scanning calorimetry (DSC) investigations were done. The interactions between the compounds of the liposomes were studied via Fourier-transform infrared (FT-IR) spectroscopy measurements.

Results and discussion
Liposome samples with size under 200 nm and polydispersity index lower than 0.30 proving homogeneity were prepared while the zeta potential values were slightly negative. The particular TG and DSC curves showed that the weight of the samples does not change above 250°C. Alternations between the formulations prepared with different phospholipid combinations were detected. The measured spectra were identical in case of the samples prepared from compositions with the same ratios but different PEGylated phospholipids. The traces of the FT-IR curves were consistent.

The results showed that the usage of 60:40 phospholipid-cholesterol weight ratios led to more stable formulations than that of the 80:20. The properties of the liposomes made with ethanol 96% were better than samples prepared with the chloroform-methanol mixture. The higher ionic strength of the hydration media maintained more negative zeta potential. Liposomes made from formulations prepared with trehalose as cryoprotectant were smaller than those with inulin; however, the inulin-containing formulations were less polydisperse. Samples containing DPPE-PEG2000 had smaller liposome with more negative surface charge than the ones with DSPE-PEG3000.

Conclusion

It has been shown that the usage of different PEGylated phospholipid compositions influences the quality of the liposomes and that those factors should be considered when the formulation is optimised. The results of the study set examples for analytical monitoring during the decision-making process of a liposomal development procedure.

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