Molecular Mixing of Lipids by Dual Centrifugation – a DSC Study

Jonas Koehler [a], Johannes Schnur [a], Heiko Heerklotz [a], Ulrich Massing [ab]

[a] Department of Pharmaceutical Technology and Biopharmacy, Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität, Freiburg i.Br., Germany

[b] Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany

Dual Centrifugation (DC) is a novel in-vial homogenization technique for the fast preparation of liposomes. DC causes highly frequent and very strong sample movements in closed sample vials. The homogenization process is supported by zirconium oxide beads. Formation of liposomes consisting of more than one lipid usually requires the previous formation of a molecular dispersed lipid blend, especially when the poorly water-soluble cholesterol is part of the mixture. To get a lipid-film, the lipids are dissolved in organic solvents followed by a time-consuming and careful removal of the solvents, which involves the risk that lipid is lost by foaming. In order to ease the process of liposome preparation, we investigated whether the shear forces caused by DC are strong enough to already form molecular dispersed lipid mixtures without the cumbersome and error-prone process of lipid-film formation.

To investigate if DC-homogenization is able to prepare liposomes without the previous formation of lipid-films, the process of molecular mixing of phosphatidylcholine-species (DSPC and DPPC) during DC was investigated by differential scanning calorimetry (DSC). To investigate whether lipid-film formation can also be avoided if the desired liposomes also contain cholesterol, mixtures of hydrogenated Egg PC and increasing ratios of cholesterol used in the DC-homogenization process and investigated by DSC.

We could clearly show that the shear forces provided during the DC-process are high enough to mix the phospholipids as well as cholesterol in a molecularly dispersed manner. The finding that DC allows the formation of molecularly dispersed lipid membranes (and finally liposomes) without the prior formation of a lipid-film is a significant progress in the field. While the molecular mixing of different PC-species could be expected during homogenization, the transfer of poorly water-soluble cholesterol from its crystalline form into the PC-bilayer was surprising and could be explained by simultaneous nano-milling of cholesterol crystals during DC.