Manufacturing & characterisation of lipid nanoparticles by microfluidics

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Background: Applying nanoparticles (NPs) technology for therapeutic purposes, particularly for targeting delivery as Drug Delivery System (DDS), makes a significant development in the drug administration systems for a number of medicinal purposes, including overcoming the limitations of chemotherapy. Between the different platforms of NPs, lipid-based NPs (e.g., liposomes) reported as the less toxic formulation *in vivo*, beside the ability to carry hydrophobic or hydrophilic molecules, and the prolonged half-life. Among the multiple methods of preparing liposomes, microfluidic technology is a promising method for the manufacturing of liposomes. Microfluidics offering a high-level control of the process's various parameters, which support controlling particle size, distribution, and physiochemical properties. Using microfluidic technology to encapsulate hydrophobic drugs (e.g., cancer drugs), has shown a better uptake and potency in several cell lines in comparison to other carriers prepared by non-microfluidic methods.

Methods: Multiple phospholipids (e.g., DMPC, DPPC, DSPC, and DOPC) have been combined with cholesterol, using microfluidic apparatus, obtained empty liposomes as a standard formulation. Different lipids to cholesterol ratios (e.g., 2:1, 3:1) have been investigated independently. All of experiments took place at total flow ratio (TFR) 1, 2, and 3 ml min^{-1}. In addition, every TFR has experimented at three different flow rate ratios (FRR) 1:2, 1:3, and 1:4. Particle size, polydispersity PDI, and zeta potential was investigated in the formulated NPs. Moreover, FTIR and DSC studies were also performed for further analysis of the obtained NPs.

Results: By comparing the phospholipid structure, it can be determined that DPPC, DMPC, and DOPC provide the most relevant result based on the particle size, PDI, and SD values**.** As the increase of the phospholipid acyl length and transition temperature (DSPC > DPPC > DMPC), display increasing of the liposome diameter. The phospholipid to cholesterol composition ratio shows an effect on produced liposomes, as DMPC and DPPC 2:1 phospholipid to cholesterol ratio shows a smaller average of liposome size, lower PDI, and more stable particles comparing to 3:1 phospholipid to cholesterol ratio. DMPC produced the smallest liposomes diameter among the whole samples (147 ± 19 nm). The PDI average of each phospholipid was 0.22 for DPPC, 0.25 for DMPC, and 0.27 for DOPC, which indicates a homogenous formulation, and especially for DMPC and DPPC. From the presented data, it can be shown that DMPC and DPPC 2:1 phospholipid to cholesterol formulation specifically with TFR 1 and 1:4 FFR provide the most suitable liposomes with confirmed size range and homogenous formulation.

Conclusions: The microfluidic system, as a computerized, flexible, and highly controlled system, allows modifying variety of parameters that affect the manufacturing process, such as the TFR and FFR. The change of FFR and TFR impacts the liposome dimensions, uniformity and assists in achieving the most optimum liposomes with desired dimension (< 200 nm) and PDI (<0.25). Most of the results show that increasing the FFR at a given TFR decreased the size of the liposomes. Optimal liposomes with preferred dimension (< 200 nm) could be an excellent carrier beside the capacity to cross tissues and cell barriers and act as an "ideal" a drug delivery system.