Manufacturing of Lipid-based nanoparticles by microfluidics for chemotherapy

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Background: Nanoparticles (NPs) have been used for decades as drug delivery systems (DDSs) due to their unique properties, including the ability to adsorb and encapsulate molecules. Controlling the delivery of cancer drugs can overcome the lack of chemotherapy selectivity and increase the concentration of the drug in specific tissues, which enhances the efficacy and reduces side effects. Among the different platforms of NPs that can be used for DDS, liposomes have the ability to carry hydrophobic and hydrophilic molecules and reported as one of the lowest toxicity NPs *in vivo*. Liposomal formulations usually prepared by conventional methods such as film hydration. Conventional methods have many limitations restricting their use for drug delivery systems, such as large particle size, high polydispersity index (PDI), and batch-to-batch variability. Microfluidics (MF) is a novel technology used to produce liposomal formulations by offering high control of different parameters, including total volumetric flow rate (TFR) and flow rate ratio (FRR). The technology uses microscopic channels that provide the laminar flow of fluids. This type of flow enhances the mixing quality and provides the same mixing conditions over time, which reduced batch to batch variation. The current work aims to produce liposomes with dimensions < 200 nm, low PDI, good stability, and high encapsulation efficiency (EE) of anticancer drugs.

Methods: Variety of Phospholipids (e.g., DMPC, DPPC, DOPC, and DSPC) are combined with cholesterol in different ratios to investigate the effect of the lipid type and phospholipid to cholesterol ratio on the liposomes size, PDI, and stability. Lipid type and ratio investigated using different MF parameters (e.g., TFR 1, 2, and 3 ml min^{-1} and FRR 1:2, 1:3, 1:4) in order to investigate the effect of the flow on liposomes characteristics. The physiochemical characteristics of the empty liposomes were tested by DLS, FTIR, and stability study (e.g., at 4°C, 25°C, and 37°C). In order to investigate the formulated liposomes for chemotherapy, paclitaxel encapsulated and NPs formulated by MFs. Physiochemical characteristics of the encapsulated liposomes are tested by DLS and the EE and release tested by HPLC. Drug release studies were then performed using dialysis membrane method.

Results: DPPC and DMPC with a 2:1 phospholipid to cholesterol ratio produced the most suitable liposomes at TFR 1 ml min^{-1} and 1:4 FRR. The stability study reported that most formulations showed a significant increase in particle size and SD at 37 °C. In addition, the particle size kept relatively constant with most of the formulations at 4°C and 25°C. Encapsulated DMPC liposomes show a slight increase in particle size compared to DPPC liposomes, which show a decrease in particle size after encapsulation. The EE was 91% for DPPC formulation and 88% for DMPC formulation. The release percentage of the DPPC formulation was higher than the DMPC formulation.

Conclusions: The microfluidic system, as a computerized, flexible, and highly controlled system, allows the modifying of parameters that affect liposomes properties. Based on the results, the most optimum FTR, FRR, and phospholipid type and ratios are determined to encapsulate and release paclitaxel. The EE of DMPC and DPPC formulations was high and the drug release seems slow and extended.