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Preparation, characterization and optimization of liposomes containing eicosapentaenoic and docosahexaenoic acids; A methothodology approach

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Background and Aims: Epidemiological, clinical and biochemical studies have evidenced a protective effect of omega-3 fatty acids against some common diseases. Omega-3 fatty acids are sensitive to oxidative changes which limit their shelflife. The goal of this study was to prepare and evaluate liposomes containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by different methods to prevent this problem, it was decided to encapsulate these fatty acids in liposomes which the effect of preparation methods on liposomes characteristics are evaluated.
Methods: Liposomes was prepared by thin film hydration fallowed by either extraction, probe sonication or both sonication. A thin lipid film was formed by dissolving a known amount of dipalmitoyl phosphatidylcholine in chloroform and mixed with DHA and EPA in a flask and solvent removal by evaporation under reduced pressure. The film was then hydrated by Tris buffer. Multilamellar vesicles (MLVs) suspension were extruded through polycarbonate membrane filters using Avestin Liposo-Fast™ device. In probe and bath sonication methods, MLVs were sonicated by using Hielscher UP200H and Tecno Gaz 3 respectively. Liposomes encapsulating efficiency was determined by GC after separation of DHA and EPA by gel filteration chromatography technique. The physical morphology of liposomes was determined by Transmission Electron Microscopy. All sizing and zeta potential measurements were made on a Zetasizer Nano ZS at 25°C. Volatile compounds including propanal, pentanal, hexanal and heptanal were determined in liposomes samples by GC/MS. GC/MS analysis was carried out using an Agilent technology 7890A gas chromatograph interfaced to an Agilent 5975C inner MSD mass spectrometer in electron-impact mode (EI) at 70 eV, equipped with a HP-1MS capillary column.
Results: Results indicated that average size of liposomes by extrusion and probe sonication methods were 100 nm and 89.3 nm respectively and the amount of DHA and EPA encapsulated by the liposomes were about 13.5%, 5.9% and 38%, 87% respectively. The zeta potential of liposomes by extrusion and probe sonication methods were -42 mV and -34.5 mV respectively. TEM images of the liposomes, stained with uranyl acetate, showed that the liposomes are unilamellar, spherical in shape, and maintain high structural integrity.
Conclusions: Present data show that probe sonication technique provide a better method for preparation of liposomes containing DHA and EPA in term of both size and encapsulation efficiency. TEM studies revealed that the liposomes were essentially spherical and hollow structures.

Keywords: Nano-liposome, DHA, EPA, Extrusion, Sonication.