ABSTRACT

Micronutrients like vitamin D_3 (VD_3) and omega-3 polyunsaturated fatty acids (ω-3 PUFAs) in particular, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) play very important role in development, reproduction and functioning of the various organs in the human body. The endogenous production of these nutrients depends on the precursors available in the foods and the genetic makeup of the individual. The westernized diets and lifestyle modifications are promoting deficiencies that are observed in majority of the population. Supplementing these nutrients in the recommended dietary levels is critical. Despite their supplementation benefits, the stability to light, oxygen, pro-oxidants and poor bioavailability are major concerns in their fortification.

In order to address these issues, in the present study, we have opted for nanotechnology in particular oil-in-water nanoemulsions to encapsulate VD_3, ω-3 PUFAs individually and in combination by low energy emulsion phase inversion method using edible oils as the carriers. A systematic screening of edible oils, surfactants and co-surfactants has been carried out by supersaturation method and the appropriate ratios of the surfactant mixture and the oil phase has been selected by constructing a ternary plot. The ingredients and ratios of oil phase selected based on ternary phase diagram are 2% of sunflower oil, 1:4 ratio of oil:S_{mix} (1:1 ratio of Tween 85 and Isopropanol) that gave stable O/W nanoemulsions withstanding physical stress conditions. Further, these nanoemulsions were used to incorporate various concentrations of the VD_3 i.e., 0.1 to 0.4% and ω-3 PUFAs (EPA and DHA) individually and in combinations and were monitored for their physico-chemical stability at different temperatures like 4°C,25°C and 40°C by measuring their particle size, homogeneity and stability using dynamic light scattering and their compound stability using High Performance Liquid Chromatography (HPLC) and Gas Chromatography–Flame Ionization Detector (GC-FID) for VD_3 and ω-3 PUFAs respectively. The fate of these micronutrients has been studied by *in vitro* simulated gastrointestinal tract model, followed by VD_3 cellular uptake studies both in presence and absence of ω-3 PUFAs using Caco-2 cell line models.

Mean droplet size of the emulsions encapsulating 0.1-0.4% (w/v) of cholecalciferol ranged from 57.7±1.9 to 158.13±1.8 nm, and zeta potential (ζ) remained above -30mV. Encapsulation efficiency was found to be in the range of 94.1% to 95.7%. These nanoemulsions were stable for at least 28 days upon storage at 4°C, 25°C and

21days at 40°C with negligible loss of VD_3. A simulated *in vitro* digestion model showed ~98% of VD_3 was bioaccessible.

The droplet size of ω-3 PUFAs nanoemulsions was within the range of 100-200 nm, with ζ-potential above -30 mV, and polydispersity index less than 0.3 upon storage for 28 days. ω-3 fatty acid content of the nanoemulsions decreased by ~15% upon storage for 4 weeks at either 4 or 25 °C, but suffered 49% loss at 40 °C within 2 weeks. Bioaccessibility of ω-3 fatty acids from the prepared nanoemulsions was ~53% as compared to 34% of release from plain oils blended with ω-3 fatty acids.

The co-encapsulation of VD_3 (0.4%) and ω-3 PUFAs (500mg) combinatorial formulations were also successfully fabricated by emulsion phase inversion method. These emulsions were found to be stable at 4°C and 25°C for 28 days and 21 days at 40°C without phase separation. The stability of combinational nanoemulsions was extended for another seven days and no phase separation was observed upto 21 days. The co-encapsulated VD_3-ω-3 PUFAs showed increased stability of EPA: DHA and only 23.17% of loss was seen compared to 49% loss in ω-3 PUFAs nanoemulsions at 40°C. Further, the emulsions structure was found to be intact with the addition of $VD_3$with improved oxidative stability. The levels of FFAs released for the co-encapsulated VD_3-ω-3PUFAs was 75-80 %, which is high compared to single ω-3PUFAs nanoemulsions. The bioaccessibility of the co-encapsulated compounds was 95% for VD_3 and 60% for ω-3PUFAs, where the EPA and DHA bioaccessibility was found to be increased whereas, slight decrease in VD_3 bioaccessibility was observed. The Caco-2 cell uptake studies suggest that undigested nanoemulsions showed higher bioavailability (40%) in comparison to digested micellar form of nanoemulsions (30%). On the other hand the combination of VD_3-ω-3PUFA nanoemulsions showed slight decrease (38%) in VD_3 uptake compared to VD_3 nanoemulsions. The bioaccessibility and cellular uptake of VD_3 is better when they are given in the encapsulated form rather than in the free form in plain oils.

This study is the first of its kind that fabricated an efficient nanovehicle using a low energy method with cooking oil as a carrier for the delivery of long chain polyunsaturated fatty acids. These nanoemulsions prepared by simple formulations and inexpensive methods can be used for supportive therapies and food based approaches to improve vitamin D and ω-3 PUFAs nutritional status.