Investigation Of Buffer, pH And Ionic Strength Effects On Sponge Phase Lipid Nanoparticles (L_3LNPs) For Myoglobin And Polyadenylic Acid (PolyA) Encapsulation Through Microfluidic Pump

Giorgia Tanca[1*], Marshall R. Machingauta[2], Tommy Nylander[2], Cristina Carucci[1], Andrea Salis[1]

[1] Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria, S.S. 554 bivio Sestu, Monserrato (CA) 09042, Italy & CSGI; [*]g.tanca6studenti.unica.it.
[2]Division of Physical Chemistry, Department of Chemistry, Lund University, Lund, Sweden.

Non-lamellar liquid crystalline with lipidic matrix represent an emerging technology of high value in the drug delivery field and food processing industry. The structures of these systems depend on nature of lipids, mixture composition and external conditions [1][2]. Different phases like cubic, hexagonal and sponge phases could be achieved modulating these three factors. The non-ionic nature of the components leads to versatility due to the possibility of encapsulating or solubilize hydrophobic, hydrophilic and amphiphilic molecules [1]. The co-polymer polysorbate-80 (P80) covers an important role as stabilizer, specifically in leading a narrow and controllable size distribution, and all the components are biocompatible [2]. The sponge phase, defined also as melted cubic phase, is characterized by a short-range structure as a cubic phase, but no long-range order. It shows interconnected water channels delimited by curved bilayers towards the aqueous phase, large enough to encapsulate macromolecules and biomolecules such as proteins and enzymes [3]. Lipid nanoparticles were prepared with a composition of 28:42:30 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC); Campul GMO-50 (GMO-50); Polyoxyethelene (20) sorbitan monooleate (P80), resulting in 40/60 DOPC/GMO-50 and 30% of P80. Bulk phase samples and dispersed phase were prepared following the procedure described by Machingauta et all. [4]. This work is focused on lipid sponge phase characterized with light scattering analysis such as dynamic light scattering (DLS), electrophoretic light scattering (ELS), small angle X-ray scattering (SAXS) and cryogenic transmission electron microscopy (cryo-TEM) analysis. With the aim of investigate the effects of different buffers, buffer concentrations and ionic strength of mono- and di- valent ions such as NaCl, KCl and $CaCl_2$, on size and structure of sponge phase lipid nanoparticles (L_3LNPs). Innovative procedure adopting a microfluidic pump provided with a herringbone chip was tested and compared to the classical preparation method. The microfluidic pump presents advantage on the size control and allow to obtain lower PDI and narrow size distribution. Encapsulation of myoglobin and polyadenylic acid (polyA) were deeply investigated with DLS, ELS and SAXS measuraments. Bulk phase L_3LNPs show heterogeneity on the size154-437 nm and PDI values in a broader range of 0.14-0.6. Vice versa, microfluidic samples present hydrodynamic diameter into a range of 175-238 nm with a PDI values lower than 0.27. Zeta potential of microfluidic samples depends on the buffers which generally increase negatively with increasing pH, except for TAPS buffer that shows an opposite trend. Furthermore, dissolved NaCl, KCl and $CaCl_2$ salts in Tris buffer resulted in lower zeta potential. The Cryo-Tem analysis confirms the presence of L_3LNPs in the samples prepared utilizing the microfluidic pump. On the basis of these results, the microfluidic device appears to be a more feasible and faster way to prepare L_3LNPs, considering the high control on the size that it is possible to achieve, and lower PDI values.

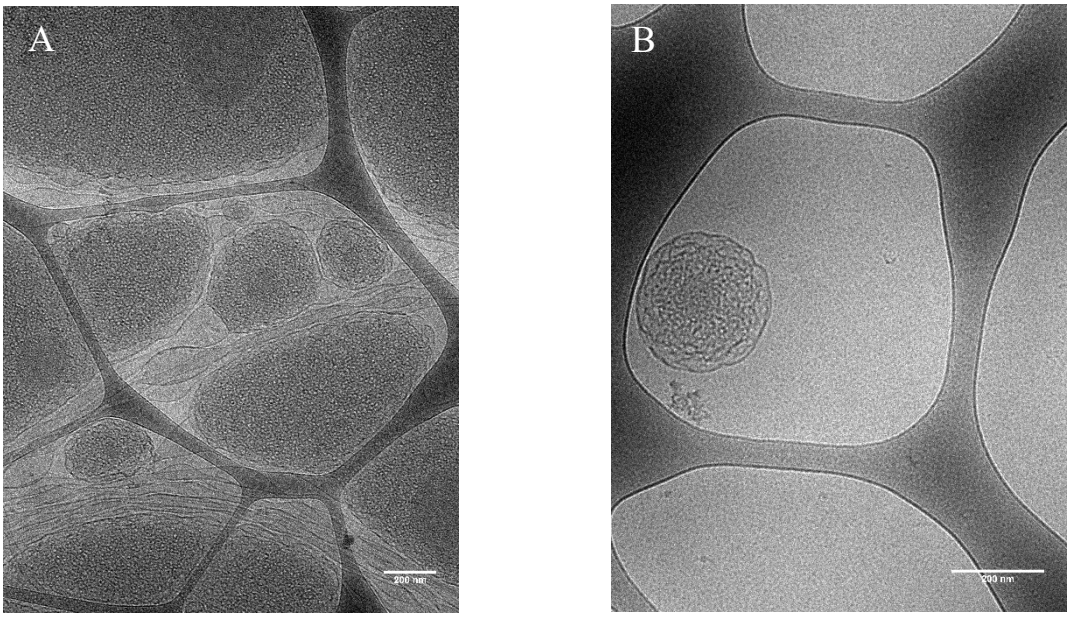

Figure 1 – L_3LNPs in tris 20 mM pH 8 (A) and L_3LNPs prepared with a solution of 0.1 mg/mL of myoglobin dissolved in Tris 20 mM pH 8 (B). Samples prepared adopting the microfluidic pump.

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