

**P-047 Preliminary approach for the mathematical study of the production of Lipid Nanoparticles by High Pressure Homogenization**

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**INTRODUCTION AND OBJECTIVES**

Lipid nanoparticles (LN) consist of carrier systems made from lipids, in which active compounds can be incorporated. Its mean particle size is in the submicron range (40 nm - 1000 nm). Firstly, Solid Lipid Nanoparticles (SLN) appeared as the first generation of LN. Later, Nanostructured Lipid Carriers (NLC) were developed as the second generation of LN to overcome some limitations associated with SLN, showing a higher loading capacity for a number of active compounds (Menhert 2001). LN appeared as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. They were proved to improve physical stability of compounds and formulations and control occlusion and skin hydration (Müller 2007). Furthermore, compounds of GRAS status (Generally Recognized as Safe, by the FDA) can be employed to manufacture lipid nanoparticles. This supports the safety character of these nanosystems, which has been ascertained in toxicological studies (Müller 2000).

For the production of LN, high pressure homogenization technique (HPH) stands out because of its easy scale up, avoidance of organic solvents and short production times (Liedtke 2000). This production method consists of applying pressure over liquids in order to subdivide particles or drops in the nanometer range. The homogenization process has been theoretically and experimentally studied as a combination of turbulence, shear, cavitation and impact mechanisms. In the first stage, parameters influencing the process were theoretically considerate as the following:

$$\begin{aligned} d_{\max} &= x_{\max} = C \epsilon^{-2/5} \gamma^{3/5} \rho^{-1/5} \\ d_{\max} &= C \gamma \epsilon^{-1/2} \eta_c^{-1/2} \end{aligned}$$

Where C is a constant (non dimensional), d droplet diameter (m),  $\epsilon$  energy density ( $W m^{-3}$ ),  $\gamma$  interfacial tension,  $\rho$  mass density ( $kg m^{-3}$ ) and  $\eta_c$  the viscosity of the continuous phase ( $Pa s$ ) (Walstra 1993). More recently, the effect of homogenization on droplet size distributions has been intensely studied on micro- and nanoemulsions from food industry. All the experiments support initial mechanical theories, although show differences in mathematical relations. However, all of them are focused on homogenized nanoemulsions characteristics, but not lipid nanoparticles. Although hot homogenization pressure technique comprises a nanoemulsion treatment, that is not the case when applying cold HPH technique. Furthermore, the sizes of nanoparticles produced by cold homogenization with different equipment are much more reproducible than in the case of hot homogenization technique (Liedtke 2000).

The aim of this work is a practical approach to the production of LN by cold high pressure homogenization technique. The effect of homogenization parameters and formulation composition on nanoparticles is analyzed. This study is intended as a first step for the optimization and prediction of nanoparticles characteristics prior to production.

**MATERIALS AND METHODS**

**SLN production**

Nanoparticles were produced by cold High Pressure Homogenization. O/W emulsions were prepared at 75 °C with Monostearin (glyceril monostearate) as solid lipid (0,5 % - 10 % w/w) and Span® 60 as surfactant (0,25 % - 5 %). After cooling down of emulsions and therefore obtention of microparticles suspensions, they were processed at high pressure homogenization at a pressure of 300 to 1500 for 1 to 8 passes. The resultant suspensions of lipid nanoparticles were stored at 4°C.

**Nanoparticles characterization**

Mean size and size distribution were studied by laser diffraction (LA-950V2 Horiba, Japan). Morphology, aspect and surface were determined by scanning electron microscopy (SEM) (Jeol 6460LV). Zeta potential was evaluated by electrophoretic mobility measures with a Malvern Zetasizer 2000 device (Malvern Instruments Ltd., United Kingdom).

**RESULTS AND DISCUSSION**

Mean particle size ranged between 1.5  $\mu m$  and 79 nm (CV 156.22 % - 14.74 %). In reference to parameters influencing homogenization process, a progressive increase in applied pressure (from 300 bar to 1500 bar) produced a decreased in particle size (2.99  $\mu m$  to 93 nm), which is in agreement with energy density parameter  $\epsilon$  from equations. Size distribution evolved from a bimodal distribution with a higher concentration of biggest particles towards a one-mode distribution by means of increasing smaller particles concentration. Mean size evolution followed the equation  $y = 3E-06x^2 - 0,007x + 4,869$  with a regression coefficient of  $R^2 = 0,98$  (fig. 1).

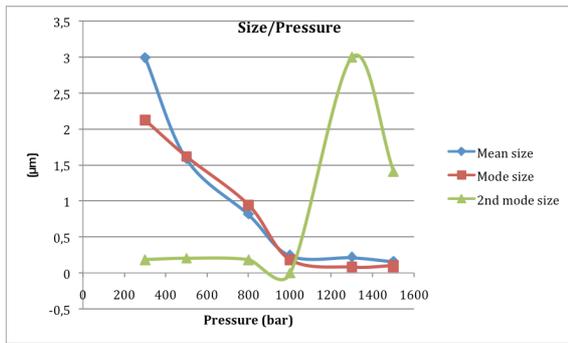


Figure 1: Particle size evolution with increasing pressure

Regarding modifications in formulation compositions, not significant differences were observed between samples elaborated with different oil phase concentration in the studied range. Changes in surfactant concentration did not lead to modifications in particles produced at 1500 bar, but did in those produced at 500 bar, with a decreased in particle size from 1.53 µm to 89 nm 0.25% a 1.5% w/w (fig.1). This can be attributed to a decreased in the interfacial tension from the equations exposed (fig. 2).

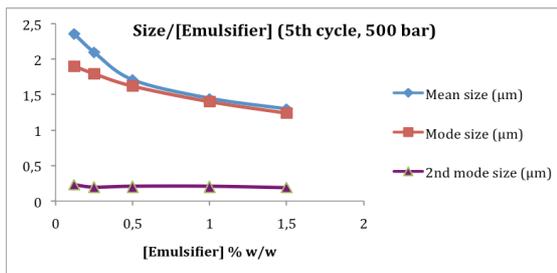


Figure 2: Particle size and emulsifier concentration

Besides, an increase in the number of successive homogenization cycles applied to the same sample led to a reduction of particle size and a strong decreased in size distribution (CV 136.47 % a 17.11 %). Therefore, narrow distributions seem to be obtained from higher number of passes (fig. 3).

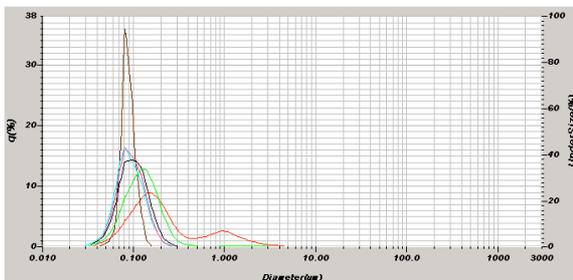


Figure 3: Particle size after application of different number of cycles

Microphotographs obtained by SEM allow observing the produced particles. Despite this technique not being much suitable (applied voltage at high augments caused melting of particles), the images show clearly spherical particles and higher sized particles can be observed even during melting process (fig. 4 and fig. 5).

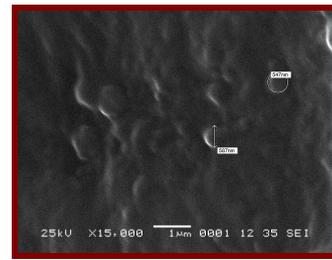


Figure 4: Microphotograph of lipid nanoparticles (x1500)

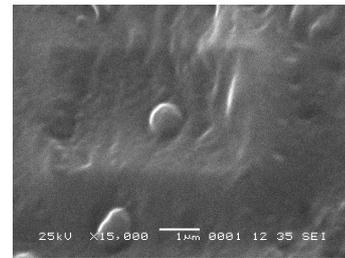


Figure 5: Microphotograph of lipid nanoparticles (x1500)

Mean potential values were of approximately  $-33.5$  mV with DS 0.2 mV, which indicates that produced suspensions can be considered stable, *a priori*.

## CONCLUSIONS

From the obtained data, it can be concluded that HPH is a highly suitable method for the production of LN under a wide range of conditions, allowing to obtain particle sizes lower than 90 nm with desired characteristics from the modification of parameters according to necessity. Furthermore, there are promising perspectives for the mathematical control of the process prior to production.

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