**Supercritical CO2 assisted process for the production of nano-niosomes**

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**1. Introduction**

Niosomes are a kind of drug nanocarrier formed by non-ionic compounds, with a lipophilic tail and a hydrophilic head that tend to self-assemble in aqueous dispersions, producing vesicles [1,2]. They are characterized by a high biocompatibility and good physico-chemical stability at room temperature; moreover, the raw materials for their preparation are cheap and readily available [3]. The traditional techniques proposed for niosomes production, such as thin-film hydration, reverse phase evaporation, emulsion, microfluidization and freeze-drying [2,4] suffer from some limits: i.e., they are batch and time consuming [5,6], use organic solvents [7], and have a reduced control on size and shape of the obtained vesicles [8,9]. Supercritical CO2 (SC-CO2) assisted processes have been proposed as a green alternative to produce micro- and nanocapsules [10], liposomes [11] and, in some cases, niosomes [12-14]. The advantages of using a SC-CO2 assisted process are mainly related to SC-CO2 gas-like diffusivity and liquid-like density [12-14] that allow to obtain solvent-free nanometric particles, at high drug encapsulation efficiency and in a faster way [11]. Therefore, the aim of this work is the production of nanometric and stable niosomes, using a SC-CO2 assisted process. Different Span® 80 and Tween® 80 formulations were tested and analyzed by dynamic light scattering and field emission scanning electron microscope, in order to determine the optimal ones to be used for a following loading of active pharmaceutical ingredients.

**2. Methods**

100 mL of ethanolic solution containing the surfactants (20 mg/mL total surfactant concentration) were prepared by magnetic stirring at 250 rpm, for 1 h and at room temperature. The formulations tested in this work were obtained at a Span® 80 to Tween® 80 weight ratio of: 100/0 (N01), 90/10 (N02), 80/20 (N03) and 70/30 (N04). The hydrophilic to lipophilic balance (HLB) of the surfactants’ mixture was 4.30, 5.37, 6.44 and 7.51, respectively.

The apparatus used for niosomes production was a lab-scale high-pressure plant, formed by three feeding lines for the delivery, in the formation vessel, of CO2, water and the ethanolic solution of surfactants. At the end of the experiment, the system was slowly depressurized and the mixture ethanol+CO2 was removed using a separator downstream of the formation vessel; whereas the niosomal suspension was collected in a reservoir located at the bottom of this vessel. More details about this high-pressure plant and the experimental procedure are published elsewhere [14].

Niosomes suspension was characterized by dynamic light scattering (DLS, mod. Zetasizer Nano S, Worcestershire, United Kingdom) and field emission scanning electron microscope (FE-SEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany).

**3. Results and discussion**

This work is aimed at the identification of an optimal Span® 80 to Tween® 80 ratio for the preparation of niosomal formulations that produce nanometric and stable vesicles. The operative conditions adopted for the SC-CO2 assisted process were: 100 bar pressure, 40 °C temperature, 7 mL/min water flow rate, 3.5 mL/min ethanolic solution flow rate and 6.5 g/min CO2 flow rate.

DLS results showed that niosomes mean diameter varied from 120 ± 51 nm for N01 to 215 ± 80 nm for N04, increasing the amount of Tween® 80 from 0 to 30% in the starting ethanolic solution. The different Span® 80 to Tween® 80 ratio determined a variation of HLB parameter from 4.30 to 7.51, and the literature demonstrated that, when HLB value increases, a larger number of hydrophilic groups are present in the vesicle structure [15] that increases the surface-free energy of the system, producing larger niosomes [16]. In all cases, PDI lower than 0.4 and ζ-potential larger than 20, in modulus, were measured. Figure 1 shows an example of the niosomes morphology observed by FE-SEM: spherical and regular niosomes were produced, with a mean dimension consistent with DLS results.



**Figure 1.** SEM images of N02 niosomes, produced at 100 bar and 40 °C.

N01 to N04 samples were analyzed by DLS after 2 and 4 months storage in Falcon tubes at 4 °C. These samples were stable over time since preserved the mean size: i.e., an increase in size of around 12% was detected only for N01 samples after 4 months from the production; whereas the other samples showed an increase in size lower than 8%, after the same time. This result is a consequence of the ζ-potential values detected up to 4 months from the niosomes production that ranged from a minimum of -37.0 mV for N02 after production to a maximum of -20 mV for N04 after 4 months storage.

**4. Conclusions**

The systematic study performed in this work on the niosomes mean dimension, morphology and stability over time demonstrated that the optimal formulations for the following encapsulation of active pharmaceutical ingredients were the vesicles prepared at a Span® 80 to Tween® 80 ratio of 90/10. Indeed, they were characterized by a mean diameter lower than 200 nm, spherical morphology and good stability up to 4 months storage.

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