

Microencapsulated solid lipid nanoparticles for pulmonary delivery of biopharmaceutical agents

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Introduction: Biopharmaceuticals agents are highly vulnerable molecules, usually presenting short *in vivo* half-lives and poor bioavailability in the action site [1, 2]. Solid lipid nanoparticles (LNPs) have attracted wide attention of researchers in order to overcome these limitations [2], once they can avoid some problems associated to the traditional carrier systems [3, 4]. Lung delivery of LNPs represents an interesting goal [5]. In this work, we propose a new delivery system based on microencapsulated LNPs for pulmonary delivery of therapeutic molecules. The microencapsulation of LNPs using spraying techniques without the use of organic solvents is an interesting challenge, which requires rigorous optimization of the technique employed. Two types of LNPs were formulated based on Compritol[®] 888 ATO and dynasan[®] 118. Afterwards, these LNPs were microencapsulated by spray-drying in mannitol microspheres which were characterized according to their size, morphology, structure and capacity to release the encapsulated nanoparticles in aqueous media.

Methods: The preparation of LNPs was formulated by a hot high shear homogenization method using Compritol[®] 888 ATO and dynasan[®] 118 as the lipid component and tween[®] 80 as surfactant. The physicochemical properties (mean particle size, polydispersity index and surface charge) were performed by Photon Correlation Spectroscopy (PCS) and Laser Doppler Anemometry using a Zetasizer[®] Nano-ZS (Malvern instruments, Malvern, UK) on freshly prepared and recovered samples. The morphological appearance of nanoparticles was examined by transmission electron microscopy (TEM) (CM 12 Philips, Eindhoven, Netherlands) on samples previously stained with 2% phosphotungstic acid and placed on copper grids with Formvar[®] films for viewing. The physical stability of the LNPs was analysed after storage at 4°C. To prepare the LNPs-loaded mannitol dry powders, a suspension of LNPs in mannitol was spray-dried using a laboratory-scale spray-dryer (Büchi[®] Mini spray-dryer, B-290, Switzerland) [5]. The size and morphology of the LNPs loaded microspheres were determined by scanning electron microscopy (SEM, EVO LS15 Zeiss). Surface and structural analysis of microencapsulated LNPs samples was conducted by a confocal laser scanning microscopy (CLSM) using an AOBS SP5X (Leica GmbH, Germany), for that LNPs were previously labeled with the fluorescent dye coumarin-6 and mannitol solution with Bodipy[®]. Release studies of LNPs from mannitol microspheres were performed following their incubation in water Milli-Q plus and in phosphate buffered saline (PBS, pH 7.4) during 3h.

Results and Discussion: LNPs showed a size in nano-range scale (figure 1) and a negative surface charge (table 1) due to their lipid composition. The stability studies indicated that, as the physicochemical properties remained almost constant for up to 60 days, the LNPs do not have

tendency to form aggregates and have a good stability. SEM microphotograph, depicted in figure 2, shows that microspheres obtained by spray-drying are spherical and they further present a smooth surface. CLSM results suggested that LNPs are efficiently encapsulated in the microspheres and showed that they are homogenously distributed throughout the mannitol matrix. LNPs recovered from microspheres maintained the nano-range size (figure 3) and the negative surface charge when released in either water Milli-Q plus or in PBS pH 7.4 (table 1). We suppose that once administered by pulmonary route, the mannitol microspheres will dissolve rapidly, releasing the LNPs and, hence, the nanoencapsulated therapeutic molecule. Next, the LNPs will be loaded with drugs, peptides and genes with interest for pulmonary delivery.

Conclusions: The present work demonstrates that the incorporation of LNPs into mannitol based micrometer-sized carrier particles through a carefully adapted spray-drying technique is possible. This micro-nanoparticulate delivery platform opens up a wide range of treatment applications of pulmonary diseases through delivery strategies via LNPs. It also constitutes a promising alternative to systemically deliver therapeutic macromolecules like insulin to the lungs.

References:

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Figures:

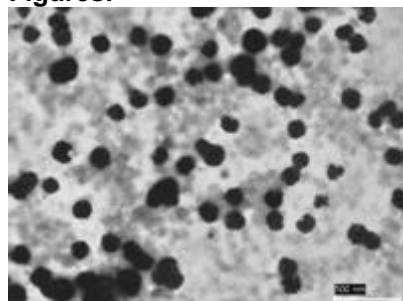


Figure 1: TEM micrograph of LNPs based on dynasan® 118 without microencapsulation process.

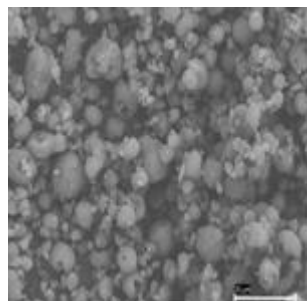


Figure 2: SEM micrograph of mannitol microspheres loaded with dynasan® 118 LNPs.

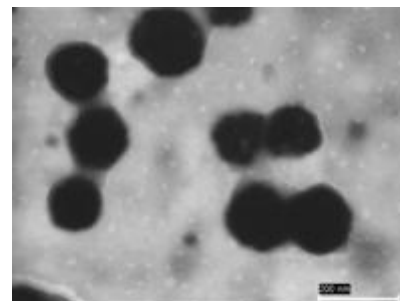


Figure 3: TEM micrograph of recovered dynasan® 118 LNPs delivered from mannitol microspheres in water.

Table 1: Physicochemical properties (mean particle diameter, \varnothing ; polydispersity index, PI and zeta potential, ξ) of LNPs without microencapsulation and after recovery from mannitol microspheres. The data are presented as mean \pm SD (n=3).

	$\varnothing \pm SD$ (nm)	PI \pm SD	$\xi \pm SD$ (mV)
Compritol® 888 ATO LNPs (without microencapsulation process)	97.9 \pm 4.0	0.121 \pm 0.013	-18.2 \pm 0.9
Dynasan®118 LNPs (without microencapsulation process)	188.2 \pm 8.0	0.145 \pm 0.007	-17.8 \pm 0.7
Recovered Compritol® 888 ATO LNPs from microspheres	486.9 \pm 3.32	0.628 \pm 0.004	-11.8 \pm 1.34
Recovered Dynasan®118 LNPs from microspheres	459.0 \pm 4.67	0.616 \pm 0.044	-8.50 \pm 4.81

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