P-011 **Factorial design - a tool to optimize the composition of solid lipid nanoparticles**

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INTRODUCTION AND OBJECTIVES

Solid lipid nanoparticles (SLN) have emerged as a promising colloidal carrier system for drug delivery. The biodegradable and biocompatible nature of these particulate systems confers them distinct advantages over traditional lipid carriers such as an excellent tolerability, physical stability, protection of labile drugs, and the possibility to modulate drug release (Müller 2000, Wissing 2004).
SLN can be produced resorting to several methods and techniques, such as solvent emulsification/evaporation, ultrasound, via microemulsion, and high shear homogenization (Mehnert 2001). The combination of these methods is performed in some cases, in order to obtain stable particles, with the best properties in what concerns e.g. particle size and size distribution. These characteristics are crucial in the production of SLN, and they are the result both of the production method and, to a large extent, the respective composition (Anton 2008).
In this work, SLN were prepared resorting to a modified solvent emulsification/evaporation method, in which high shear homogenization and ultrasound were also employed. The aim is to optimize and systematically assess how different composition parameters, such as lipid concentration, amount of solvent, and emulsifier concentration influence the size of SLN. The individual effect of each variable and their interaction is assessed resorting to factorial planning.

MATERIALS AND METHODS

Glyceryl tripalmitate (tripalmitin) was purchased from Sigma, Polyvinyl alcohol 87-89% hydrolyzed (PVA, typical MW 13.000-23.000) was purchased from Sigma-Aldrich. All other chemicals are from analytical grade.

SLN were prepared by the emulsification-solvent evaporation method (Mehnert 2001). Briefly, the lipid was dissolved in dichloromethane (DCM) and then added dropwise to the emulsifier solution in a high shear homogenizer (Silverson, UK) at 12 300 rpm for 7 min. In the final, optimized, method of preparation, the inner lipid phase was added dropwise to the external phase of PVA in a sonifier (40W, 5 min) (Branson, Sonifier 250). The pre-dispersion obtained was subsequently high-shear homogenized (12 300 rpm, 7 min). The dispersion obtained in both cases was then magnetically stirred at 200 rpm for 4 h, in order to allow for solvent evaporation.

Two factorial designs (two-level, three-variable), including distinct composition levels (see Table 1), were performed for the optimization procedure, resorting to the following mathematical model: $D = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{23} x_2 x_3 + \beta_{13} x_1 x_3 + \varepsilon$ (Equation 1). Three variables were selected, namely lipid concentration, solvent:lipid ratio and emulsifier concentration, denoted respectively as x_1, x_2, and x_3 in Equation 1. The effect of each variable in the mean particle size (D) is indicated by the magnitude and signal of the respective coefficient (β_1–β_3) and their interaction by the combined terms (β_{12}, β_{23} and β_{13}). The plannings included formulations (F) in a low (L), a medium (M) and a high (H) level of the lipid phase concentration that are combined in a low/medium (L1 to M6) and a medium/high (M1 to L4) designs.
The effect of variables, and production method in particle size was assessed resorting to photon correlation spectroscopy (PCS) and zeta potential (ZP). Differential scanning calorimetry (DSC) and attenuated total reflectance infrared (ATR-IR) were performed so as to obtain information about the crystal (inferred by thermal behaviour) and molecular structure. Additional physicochemical characterization relied on laser diffractometry (LD), fluorescence, atomic-force and scanning electron microscopy (SEM).

RESULTS AND DISCUSSION

Preliminary formulation studies included the selection of the appropriate lipid phase and emulsifier type: tripalmitin and PVA, respectively. Table 1 describes the composition of SLN, prepared according to a double 2^3 factorial planning. Note that M2 and M5 formulations (F) were not included in the design, but in a further optimization process.

Table 1: SLN composition for the different levels considered in the two 2^3 factorial designs (n=3).

F	Composition W_{lipid}:V_{DCM}:C_{PVA}	Particle Size (nm)	PI	ZP (mV)
L1	100:1:0.5	788.6±79.8	0.618	-17.9±0.5
L2	100:1:1.5	891.9±63.8	0.707	-16.1±0.4
L3	100:2:0.5	286.5±33.6	0.330	-17.5±0.6
L4	100:2:1.5	333.9±13.0	0.367	-16.0±0.5
M1	250:2.5:0.5	260.8±4.8	0.240	-26.2±0.2
M2	250:2.5:1	300.2±14.4	0.385	-35.3±0.6
M3	250:2.5:1.5	437.2±7.4	0.506	-26.0±0.6
M4	250:5:0.5	263.9±3.8	0.145	-28.6±0.1
M5	250:5:1	226.3±2.5	0.187	-36.2±0.4
M6	250:5:1.5	274.9±2.7	0.295	-24.6±0.9
H1	500:5:0.5	300.3±2.5	0.175	-19.3±0.1
H2	500:5:1.5	296.4±0.8	0.307	-16.0±0.3
H3	500:10:0.5	380.9±1.3	0.268	-18.3±0.4
H4	500:10:1.5	268.8±2.7	0.226	-15.4±0.6

Table 2: Parameters of the response surfaces.

F	β_0	β_1	β_2	β_3	β_{12}	β_{23}	β_{13}
L1-M6	442.2	-133.0	-152.4	42.3	112.6	-27.7	4.6
M1-H4	310.4	1.2	-13.3	8.9	26.5	-34.2	-37.9

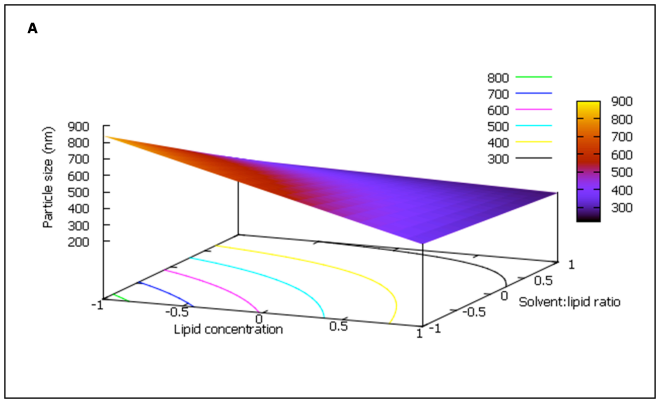
According to the values of the coefficients obtained for the L/M and M/H designs (Table 2), it can be observed that the impact not only of each variable but also of their interaction varied significantly from level to level, being generally more marked for the L/M ones. This effect is illustrated in detail by the surface responses (Figure 1), in which the two more significant variables for each fitted mathematical model are plotted.

Overall, it was observed that the solvent:lipid ratio constitutes the main factor influencing particle size. An increase in the amount of solvent tends to decrease this size, since a lower lipid content in the initial DCM droplet results in a less viscous inner phase. The amount of lipid has a limited influence upon particle size, being more relevant relatively to the other parameters, for lower lipid concentrations. The highest size obtained corresponds to a formulation with the lowest amount of inner phase. If this amount is increased, either by augmenting the lipid content, the organic solvent content or both, the size markedly diminishes, which may be ascribed to a deficient dispersion if the size of inner phase is too small. The amount of emulsifier has a non-trivial impact upon size, depending on whether systems are located below (H), above (L) or close (M, specifically for M4-M6) to the optimal surface coverage. Thus, where the system is above this optimal value, a direct correlation is observed between particle size and emulsifier concentration. Conversely, if the system is below this optimal value, particle size decreases with emulsifier concentration.

An optimal formulation, in terms of size and stability, M5, was selected for intermediate levels of the three variables. The sonication step added to the initial method promoted a reduction in both particle size (209.1±2.722 nm) and polydispersity (0.094), and has little impact upon particle stability (-35.5 mV). LD has also confirmed PCS results.

DSC and FTIR have provided some insight in the molecular structure and arrangements, compatible with rationales obtained from the experimental design.

Microscopy techniques allowed direct visualization of particle size and morphology, corroborating size measurements by PCS (Figure 2).



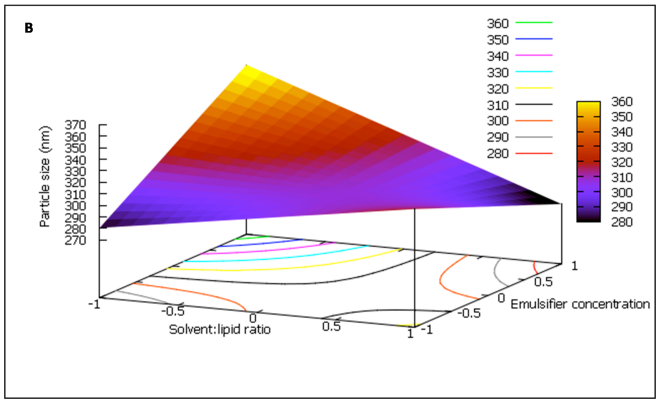


Figure 1: Particle size response surfaces: A) lower/medium design; B) medium/higher design.

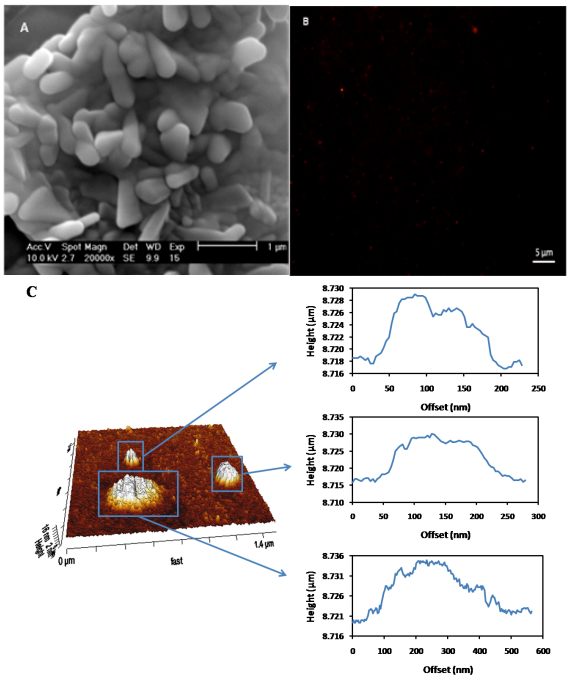


Figure 2: Microscopic images of the optimized formulation. A) SEM; B) Fluorescence; C) AFM.

CONCLUSIONS

Factorial design allowed to rationalize the effect of variables under study and enabled to successfully formulate SLN with an optimized nanometric particle size, and good stability.

REFERENCES

- Müller R. H. et al. (2000) *Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art*. European Journal of Pharmaceutics and Biopharmaceutics. 50:161-177.
- Wissing S.A. et al. (2004) *Solid lipid nanoparticles for parenteral drug delivery*. Advanced Drug Delivery Reviews. 56:1257-1272.
- Mehnert W. et al. (2001) *Solid lipid nanoparticles: Production, characterization and applications*. Advanced Drug Delivery Reviews. 47:165-196.
- N. Anton et al. (2008) *Design and production of nanoparticles formulated from nano-emulsion templates-A review*. Journal of Controlled Release. 128:185-199.