P-094 **Development of fluorescent–loaded SLN and uptake studies in glioblastoma cell line**

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INTRODUCTION AND OBJECTIVES

Solid lipid nanoparticles (SLN) (Souto 2010) stabilised with polysorbates appear to be able to cross the blood-brain barrier and targeting drugs to the brain (Goppert 2005; Blasi 2007). Therefore, SLN have high potential in central nervous system diseases treatment, such as brain tumours (Brioschi 2007). In this context, the aim of this work was the development and characterization of stable SLN for brain drug delivery, characterized by a mean size below 200 nm and low polydispersity index (PI). Selected materials were based on GRAS status for i.v. administration. Differential scanning calorimetry (DSC) analyses were undertaken to check the crystallinity and lipid polymorphism of SLN matrices. Rhodamine 123 (R123) was loaded as a model drug into SLN to tackle particle internalisation by glioblastoma cell line (A-172). Fluorescence microscopy and MTT studies were run to ultimately assess, respectively, internalisation and particle toxicity.

MATERIALS AND METHODS

SLN composed of cetyl palmitate (CP) and stabilised with polysorbate 60 (P60) or 80 (P80) were prepared by high shear homogenisation/ultrasound technique and stored at different temperatures (4ºC, 22ºC and 40ºC) for two months. Mean particle size, PI and zeta potential (ZP) of SLN were determined by PCS using a Zetasizer Nano ZS. Mastersizer 2000 was used to discard the presence of microparticles in SLN dispersions. DSC analyses were carried out in a DSC 200 F3 Maia® from 25-85ºC at a heating rate of 5ºC/min. The recrystallization index (RI) was determined as follows:

$$RI\ [\%] = \frac{\text{Enthalpy SLN } [J/g]}{\text{Enthalpy bulk material } [J/g] \times \text{Concentration lipid phase}[\%]} \times 100$$

Viability studies were performed seeding A172 cells in a 96 well plate 24h before addition of the SLN. After 24h the MTT solution was added to the wells and measured after 2 h at 550 nm. Viability of cells in the DMEM medium was considered 100% (control). Uptake studies of R123-loaded SLN by A172 cell line were carried out by fluorescence microscopy. A172 cells ($1x10^5$) were exposed to 500 µg/ml of R123-loaded SLN diluted in DMEM medium, during 2h. The cells were stained with AF594 WGA (membrane stain) and DAPI (nuclear stain).

RESULTS AND DISCUSSION

Table 1. SLN composition

SLN	Lipid	Surfactant	Water add
CP60	CP 5%	P60 2%	100%
CP80	CP 5%	P80 2%	100%

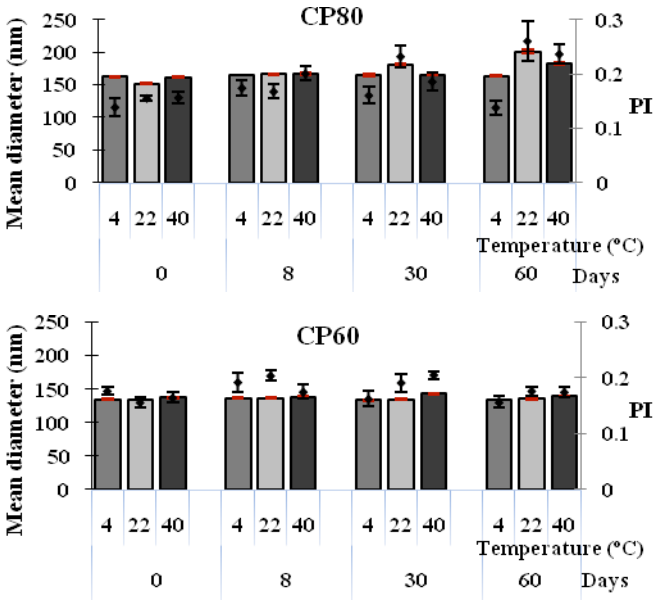

Figure 1. Mean particle size and PI of CP80 (up) and CP60 (down) stored at 4ºC, 22ºC and 40ºC, determined on the day of production and after 8, 30 and 60 days of storage.

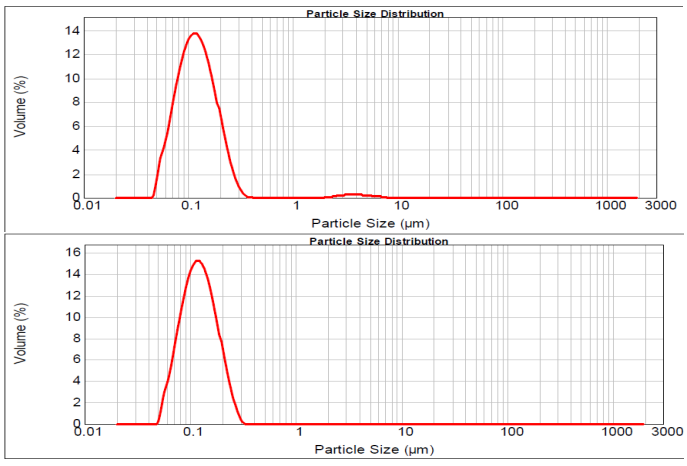

Figure 2. Particle Size Distribution of CP80 (up) and CP60 (down) stored at 25ºC during 30 days.

CP based SLN stored for 2 months at 4ºC, 22ºC and 40ºC showed mean sizes below 200 nm (PI < 0.25), were monodispersed, and ZP values in the range of -10 mV and -20 mV (data not shown). A small amount of microparticles were detected in CP80 but not in CP60. Consequently, for i.v. administration CP60 is expected to be more suitable.

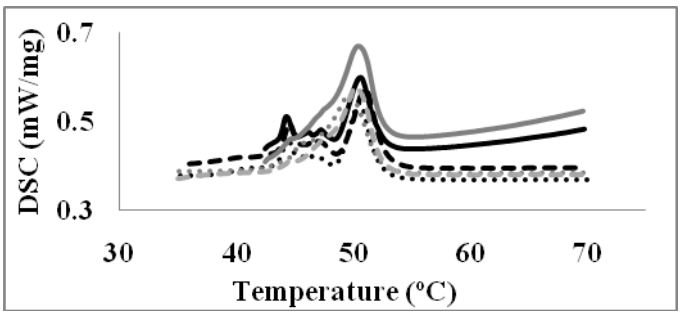

Figure 3. DSC thermograms of CP80 (black) and CP60 (grey) stored at 4ºC (. .), 22ºC (- -), and 40ºC (–).

Table I. DSC parameters of SLN: onset and melting temperatures, melting enthalpies and RI of CP60 and CP80 stored at 4ºC, 22ºC and 40ºC

Sample	Storage Temp (ºC)	Onset (ºC)	Enthalpy ΔH (J/g)	Melting point (ºC)	RI (%)
CP80	4	48.9	7.97	50.5	60
	22	49.2	9.23	51.0	69
	40	48.9	7.05	50.6	53
CP60	4	46.4	9.66	49.8	73
	22	48.3	9.22	50.2	69
	40	48.9	7.97	50.5	60
CP Bulk	22	53.2	266.5	57	100

DSC results showed that thermodynamic properties of CP based SLN, such as crystallization state and melting point, differed from those of respective pure lipid excipients. The onset and the melting point were 4 to 7ºC lower in SLN than in the bulk which is due to the colloidal size and the production inferences in the matrix. Although, the lipid matrix of SLN remained in the solid state at both room and body temperatures.

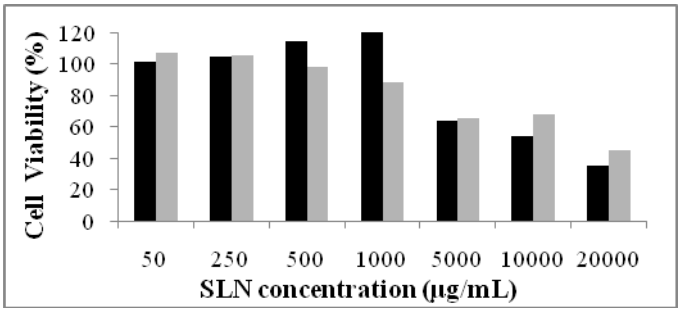

Figure 4. Viability of A-172 cells after 24h of incubation with different concentration of CP80 (■) and CP60 (■) formulations.

MTT cell viability assay showed that low concentrations of CP80 and CP60 were non toxic to A-172 cells

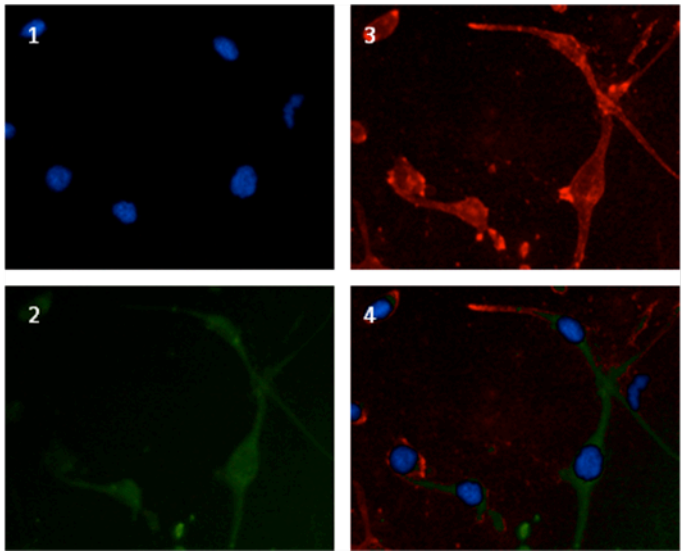

Figure 5. Fluorescence distribution in A-172 cells after exposition to R123-loaded CP80 SLN (green: 2) and stained with DAPI (blue-nucleus: 1) and with AF 594 WGA (red-cytoplasm membrane: 3). Representative images and a representative merge (4) are shown.

Under fluorescence microscopy analyses, it has been observed that SLN containing 500 µg/mL rhodamine 123 (R123) and 500 µg/mL CP80 formulations were successfully uptaken by A-172.

CONCLUSIONS

CP-based SLN appear a promising drug delivery system for brain delivery as their small hydrodynamic diameter and homogeneous size distribution contributed for the high internalisation levels and low toxicity observed in the tested glioblastoma cell line (A-172).

REFERENCES

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