Solid lipid nanoparticles entrapping curcumin by supercritical fluid technology

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INTRODUCTION

Curcumin has been considered as an important alternative for a wide range of biomedical applications, mainly antiinflammatory, analgesic and antioxidant (Aggarwal 2009). However this natural curcuminoid obtained from rhizomes of Curcuma longa, is apolar and prone to photo and thermal degradation which limit its formulation (Shen 2012). Nanoencapsulation techniques have been studied in order to improve bioavailability properties of curcumin and for providing safer and more effective formulations. Several works have been using solid lipid nanoparticles (SLN) as pharmaceutical form of choice (Kakkar et al. 2013).

The supercritical fluid technology has been studied as an option for SLN production in order to reduce or even eliminate the use of toxic organic solvents, obtainment of more stable particles, as well as, with narrow size distribution. Among various methods, the Particles Generated from Saturated Solution (PGSS) has presented interesting results (Salmaso 2009). This work aimed to formulate and characterize curcumin-entrapped SLN samples by PGSS method.

MATERIALS AND METHODS

Materials

Tristearin (99% pure), DMSO and citric acid were purchased from Sigma-Aldrich (St. Louis, MO). Epikuron 200 was a kind gift from Cargill Inc. (Minneapolis, MN). Curcumin (98% pure), HPLC grade methanol, ethanol and acetonitrile were acquired from Merck (Darmstadt, Germany). The CO_2, synthetic air and N_2 were purchased from Rivoira (Padova, Italy).

Particle production

1.5g of the homogenous lipid mixture composed of tristearin, epikuron 200, DMSO and curcumin was loaded into the mixer of PGSS plant depicted on Figure 1, at an initial temperature of 75°C. The system was pressurized with CO_2 to 150bar and the temperature decreased to 55°C. A nozzle of 100µm was tested. After 30min, the valve connecting the melting chamber with the collecting vessel was opened and the melted mass was sprayed into the precipitation chamber. Simultaneously, a N_2 flow at 140bar and an air flow at room pressure were co-axially injected. At the end of the run, the dry material was collected from the bottom of the precipitation vessel where a circular stainless microfilter set was placed.

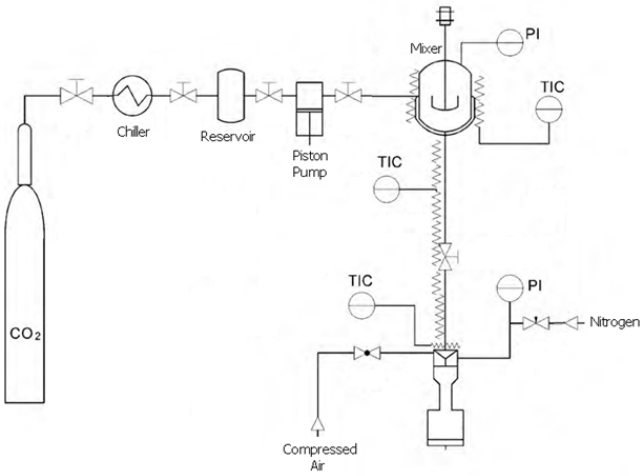

Figure 1 : PGSS plant.

Size measurement and morphology

Size measurements were carried out by static light scattering analysis by a mastersizer 2000 equipment (Malvern, UK) and by dynamic light scattering method on zeta sizer nano ZS (Malvern, UK).The morphology of obtained particles was observed by scanning electron microscopy directly on powder samples after gold-coating treatment.

Entrapment efficiency (EE%) of curcumin

The EE% was determined by a RP-HPLC method using a C18 column isocratically eluted at 1mL/min with 0.5% citric acid (pH3.0)/ acetonitrile 48:52 ratio, with detection at 429nm. Particles were disrupted in 1mL of methanol by centrifuge at 14,462xg for 15min. The clear supernatant was diluted in mobile phase and analysed by HPLC. All the samples were analysed in triplicate.

Cell Viability Test

C2C12 myoblasts cells were seeded in a 96-well plate in a density of $2x10^3$ cells/well and incubated for 24h. The medium was replaced with increasing concentrations of curcumin and drug-loaded lipid particles. Incubation was continued as above for 72h. All media were removed and MTT was added to the wells. After 3h incubation, MTT was removed and DMSO was added to dissolve formazan crystals. The optical density at 570nm was determined. Untreated cells were taken as control with 100% viability and Triton X-100 at 1% (v/v) was used as positive control

of cytotoxicity. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The Table 1 lists the different results obtained with different ratios of curcumin. This result is probably linked to the tendency of aggregation due to presence of DMSO on formulation. The larger the DMSO amount, the greater aggregation tendency. On the other hand, at higher curcumin contents, a decrease of EE% is noticed. It can be related to a probable phase separation on the mixer of PGSS plant which was evidenced by residues of curcumin solution inside the chamber after expansion leading to a decrease on EE%

Table 1: Particle size and EE% of different formulations

Composition (%)				Size (µm)	EE%
Tri	PC	DMSO	Cur		
57.6	36.5	4.0	1.9	2.7	36.06
58.7	38.2	2.1	1.0	0.229	54.52
59.4	39.1	1.0	0.5	0.098	80.51

Tri – tristearin; PC – epikuron 200; Cur – Curcumin.

The figure 2 shows the SEM micrographs of two samples of lipid particles at different compositions. The prior size measurements showed the sample of Figure 2.a as microparticles, while the one from Figure 2.b as nanoparticles. However, the microscopy indicates aggregates on microscale for both samples. The electrostatic surface charge natural to powdered particles constituted of lipids lead to an aggregation of the solid material. As the size measurements are carried out in liquid media, the particles are dispersed during the analysis.

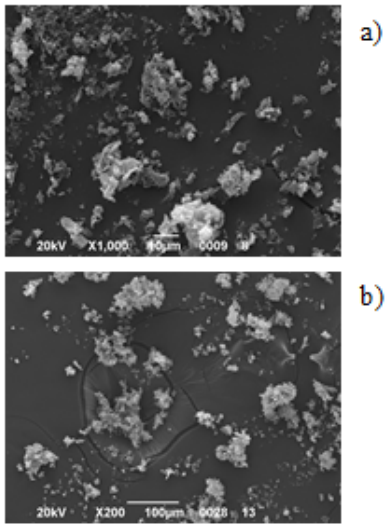

Figure 2 : SEM micrographs. a) 1.0% curcumin; b) 1.9% curcumin.

The MTT test depicted on Figure 3 revealed that the toxicity is clearly reduced by its association with solid lipid nanoparticles.

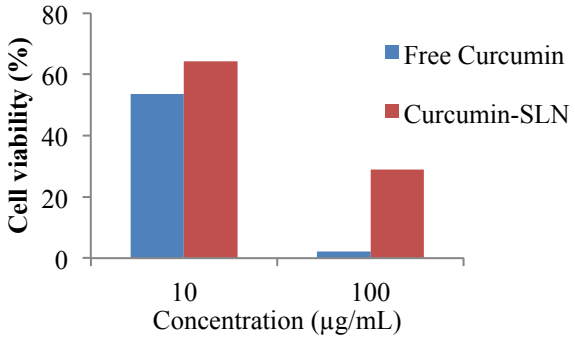

Figure 3 : Cell viability test.

CONCLUSIONS

SLN produced by PGSS demonstrated to be an interesting option for tailoring of encapsulated curcumin in order to increase its safety and also probable effectiveness**.**

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