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Cationic solid lipid nanoparticles (cSLN): Structure, stability and DNA binding capacity correlation studies

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ABSTRACT

Cationic solid lipid nanoparticles (cSLN) are promising lipid nanocarriers for intracellular gene delivery based on well-known and widely accepted materials. cSLN containing single-chained cationic lipid cetyltrimethylammonium bromide were produced by high pressure homogenization and characterized in terms of (a) particle size distribution by photon correlation spectroscopy (PCS) and laser diffractometry (LD), (b) thermal behaviour using differential scanning calorimetry (DSC) and (c) the presence of various polymorphic phases was confirmed by X-ray diffraction (WAXD). SLN composed of Imwitor 900P™ (IMW) showed different pDNA stability and binding capacity in comparison to those of Compritol 888 ATO™ (COM). IMW-SLN, having *z*-ave = 138–157 nm and *d*(0.5) = 0.15–0.158 μm could maintain this size for 14 days at room temperature. COM-SLN had *z*-ave = 334 nm and *d*(0.5) = 0.42 μm on the day of production and could maintain similar size during 90 days. IMW-SLN revealed improved pDNA binding capacity. We attempted to explain these differences by different interactions between the solid lipid and the tested cationic lipid.

1. Introduction

Solid lipid nanoparticles (SLN) are versatile colloidal carriers being currently explored for administration routes (e.g. parenteral, oral, topical, dermal and transderaml) (Souto and Müller, 2007). Their advantage over colloidal carriers composed of polymers or inorganic material is the use of biocompatible lipids with well-established use in pharmaceuticals, e.g. glycerols with fatty acids, free fatty acids, fatty alcohols or waxes. This makes these systems closer to real-life use (Souto et al., 2011). Distinct advantage of SLN over polymeric nanoparticles is that SLN can be produced without use of organic solvents, using high pressure homogenization (HPH) method that is already successfully implemented in pharmaceutical industry.

Cationic SLN (cSLN), i.e. SLN containing at least one cationic lipid, have been proposed as non-viral vectors for gene delivery (Bondi and Craparo, 2010). The use of cSLN in this application is already quite well proven – it has been shown that cSLN can effectively bind nucleic acids, protect them from DNAase I degradation and deliver them into living cells (Bondi et al., 2007; Vighi et al., 2007; Xue and Wong, 2011). First proof of *in vivo* efficiency of SLN has also been reported (del Pozo-Rodríguez et al., 2010). A review of materials, production methods and *in vitro* testing has been reviewed by Bondi and Craparo (2010).

Although efficacy in DNA/RNA delivery into living cells has been satisfactorily proven (Bondi and Craparo, 2010; del Pozo-Rodríguez et al., 2010), there are still many features that need to be explained. Hardly predictable stability and polymorphic transformations of SLN have been related to their solid state (Bunjes, 2010). These features may have an impact on their use in future medicines. The same holds true for cSLN. In this study, we focused on cSLN containing one cationic lipid with a single hydrocarbon chain. We attempted to correlate physicochemical characteristics of the developed

Abbreviations: CMC, critical micelle concentration; COM, Compritol 888 ATO; CTAB, cetyltrimethylammonium bromide; HLB, hydrophilic–lipophilic balance; IMW, Imwitor 900; LD, laser diffraction; MIR, Miranol Ultra C-32; PCS, photon correlation spectroscopy; Polox, Poloxamer 188; SLN, solid lipid nanoparticles; T_m, melting temperature; *z*-ave, *z*-average (intensity weighed diameter); ZP, zeta potential.

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Table 1
Composition of three different cationic solid lipid nanoparticles formulations prepared using high pressure homogenization.

Formulation	Solid lipid %(w/w)		CTAB %(w/w)	Surfactant %(w/w)	
	IMW	COM		Polox.	MIR
SLN-A	5.0		0.5	0.25	
SLN-B	5.0		0.5		0.25
SLN-C		5.0	0.5	0.25	

systems with their stability, which is a key issue for potential medicine, and DNA-binding. HPH has been used to produce cSLN because of its high reproducibility, scale-up feasibility and suitability for use in pharmaceutical production (Shegokar et al., 2011). Differential scanning calorimetry in combination with X-ray diffraction was used for an in-depth physicochemical characterization of SLN, especially focusing on solid state.

2. Materials and methods

2.1. Materials

Cetyltrimethylammonium bromide (CTAB) was acquired from Sigma (Sintra, Portugal). Imwitor 900P™ (IMW, 40–50% glyceryl monostearate) and Lutrol F68™ (F68, poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide); Poloxamer 188) were gifts from Sasol GmbH, Germany. Compritol 888 ATO™ (COM, glyceryl tribehenate) was donated by Gattefosse, France. Miranol Ultra C-32™ (MIR, sodium cocoamphoacetate) was obtained as gift sample from Condea GmbH. Trehalose was obtained from Merck (Darmstadt, Germany). All other reagents were from Sigma (Sintra, Portugal). Ultrapure water from MilliQ system (Millipore, Schwalbach, Germany) filtered by a filter with 0.22 μm pore size was used throughout the work. All materials were used as received.

2.2. Solid lipid nanoparticles production

SLN were produced by hot high pressure homogenization method (Shegokar et al., 2011). Briefly, lipid phase, consisting of solid lipid (either IMW or COM) and CTAB, was heated up to a temperature about 10 °C higher than the melting point of the solid lipid used, i.e., 70 °C for IMW-SLN and 80 °C for COM-SLN. Aqueous solution of surfactant (MIR or F68) was simultaneously heated to the same temperature. The lipid phase was dispersed in the aqueous phase using an Ultra Turax (IKA GmbH, Germany) for 30–60 s at 8000 rpm. This pre-emulsion was then homogenized using a LAB 40 homogenizer (40 ml; APV, Germany) operating at 500 bar for 3 homogenization cycles in discontinuous mode. The samples were filled in glass vials and let to cool down at room temperature. The composition of SLN is given in Table 1.

2.3. Particle size analysis

Size distribution analysis was performed by photon correlation spectroscopy (PCS) and laser diffraction (LD). PCS measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments, United Kingdom). Each sample was diluted with MiliQ water (0.5%, v/v) to obtain a weak opalescent dispersion and measured at 25 °C. The results are presented as intensity weighted average (*z*-ave) value obtained from three measurements (10 runs each) with corresponding standard deviation. Mastersizer 2000 equipped with Hydro 2000S sample dispersion unit (Malvern Instruments, United Kingdom) was used for LD measurements. Volume weighed diameter of 50% of particle population ($d(0.5)$) is given as a mean of 5 measurements with corresponding standard deviation. Stirrer speed was set to 2000 rpm to avoid particle aggregation during the measurement.

2.4. Zeta potential

Zeta potential was determined by laser Doppler anemometry using Smoluchowski model. All measurements were performed on Zetasizer Nano ZS90 using Dispersion Technology Software 5.1 in General Purpose mode and automatic measurements settings. MiliQ water adjusted to conductivity of 50 μS cm^{-1} (by addition of few droplets of 150 mmol sodium chloride solution) was used for sample dilution to weak opalescence (0.5%, v/v).

2.5. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a Mettler DSC 821^e (Mettler Toledo) at heating rate of 5 K/min. Samples were accurately weighed into standard 40 μl aluminium pans and sealed; an empty sealed pan was used as a reference. Bulk IMW, COM, CTAB and all SLN formulations were used without any prior thermal treatment or other processing and directly weighted into the pans. The physical mixtures of IMW + CTAB and COM + CTAB were melted and allowed to solidify before the measurements. Data were processed using Star^e software 8.10. Recrystallization index (RI) was determined using the following equation (Freitas and Müller, 1999):

$$\mathrm{RI}\,[\%] = \frac{\Delta H(\mathrm{SLN})[\mathrm{J\ g^{-1}}]}{\Delta H(\mathrm{bulk})[\mathrm{J\ g^{-1}}] \cdot C(\text{lipid phase})} \times 100$$

where ΔH is the enthalpy (J g^{-1}) of SLN of bulk lipid and C is the concentration of lipid phase (%, w/w). Solid lipid (IMW or COM) was considered as lipid phase only.

2.6. X-ray diffraction

X-ray diffraction studies of bulk materials were performed on Bruker D8 Advance diffractometer with Copper anode (λ = 0.154056 nm) and LynxEye detector. Diffractograms were recorded between 2θ = 15–40° with a step of 0.04° and count time of 5 s. Samples were used without any prior thermal treatment or further processing. SLN samples were filled into sample holder with xanthan gum in an amount sufficient to form a thick paste.

2.7. Determination of DNA binding capacity

To compare the DNA binding capacity of the developed SLN formulations, SLN were mixed with model pDNA (2 μg/μl) in ratios of SLN:pDNA = 10, 20, 30, 40 and 50:1, w/w. Plasmid DNA was added into diluted SLN dispersions (0.1–0.5%, v/v, in ultrapure water), vortexed gently (3 s) immediately and allowed to form complexes at room temperature (25 °C) under mild shaking during 45 min. Size and zeta potential of resulting complexes were checked as stated above. All experiments were performed in triplicate with different pDNA stocks.

2.8. Gel-retardation assay

Immobilization of pDNA on SLN was verified by gel retardation assay (Vighi et al., 2007). 20 μl of each pDNA–SLN complex was mixed with 5 μl loading buffer (0.25%, w/w, bromophenol blue in TE buffer) and applied to 1.0% agarose gel. Electrophoresis was carried out at 80 V for 60 min at room temperature in 1× TAE buffer (40 mM Tris acetate and 1 mM EDTA). The gels were stained with 7 μg ml^{-1} ethidium bromide solution for DNA visualization.