Characterization of cholesteryl oleate-loaded cationic solid lipid nanoparticles for the targeted delivery of nucleic acids

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Introduction

Nanoparticles have received considerable attention as vectors for gene delivery. However, the clinical translation of nanoparticles is limited due to poor delivery, immunogenicity, toxicity, high processing costs, and production scaling problems [1]. In recent years, cationic solid lipid nanoparticles (SLNs) have gained considerable attention owing to their advantages over viral and other nanoparticle delivery systems. The use of the cell membrane component cholesterol in liposome formulations has attracted interest for successful transfection, thus opening new avenues for the synthesis of novel carriers using cholesteryl or its derivatives, which could greatly enhance the delivery and activity of nucleic acids. Here, we describe the characterization of a formulation of SLNs containing cholesteryl oleate as novel excipient. In addition to studying the physicochemical properties of the SLNs, the formation of the SLNplex was also studied. We identified a formula optimized in terms of structure, morphology, and nucleic acid binding efficiency with the possibility for scaling-up manufacturing of nanoparticles under GMP conditions to potential use in clinical applications.

Materials and methods

The composition formulation of SLNs and the manufacturing method were described in [2].

• Particle size was determined by laser diffraction according to the Mie theory on a Mastersizer 2000 (Malvern Instruments, UK).

• X-ray photoelectron spectroscopy (XPS) experiments were performed using a PHI 5500 Multitechnique System (Physical Electronics).

• The eutectic point of the dispersion of the SLNs and the SLNs:Agarose was determined by DSC.

• The DSC was performed in a DSC-822e (Mettler Toledo).

• The analysis of lipoplex formation and loading efficiency of the SLNs with plasmid DNA ([1]) was performed by electrophoretic mobility of the samples on agarose gels. Sample visualization once resolved in agarose gels takes place in a GelDoc® EZ Imageer (BioRad®, USA) system using the BioRad ImageJ 5.2.1 software.

Results and Discussion

The complete physicochemical characterization of the SLNs show a particle size of 240 nm ± 40 nm (Figure 1). The results obtained with the Mastersizer were confirmed with the use of transmission electron microscopy (TEM) (Figure 2). The SLNs showed spherical morphology and homogenous surface. Furthermore, the presence of aggregates was not observed, demonstrating that the manufacturing method avoid the presence of aggregates. Regarding the surface nitrogen percentage (Figure 3), X-ray photoelectron spectroscopy results showed the presence of this atom in the nanoparticle surface. These data confirm our previous results concerning zeta potential (data not shown), because this atom will be protonated, thereby allowing the formation of the SLNplex with the DNA and RNA.

Finally, we assessed the eutectic point of the formulation with and without cryoprotectant (trehalose). The data showed that with trehalose the eutectic point is lower than the eutectic point of fresh SLNs (Figure 4). The result showed a final eutectic point of -29.98 °C, therefore the lyophilization process should begin at temperatures of -40 °C. To note, trehalose was the only cryoprotectant capable to maintain the characteristics of the SLNs after the resuspension (data not shown).

The analysis of SLNplex formation was performed by electrophoretic mobility of the samples on agarose gels (Figure 5). The presence of unbound free DNA in the gels reflects the binding capacity of the nanoparticles. When the DNA tested was 2 µg, minimal free DNA was observed in the gel and considerable free DNA was detected in the gel upon loading 5 µg of DNA. Our results confirmed the suitability of these cSLNs for DNA binding.

Conclusions

A complete characterization of our formulation of SLNs was performed. Our results indicate that this improved formulation is suitable for gene therapy with the possibility for scaling-up manufacturing of nanoparticles, confirming the results exposed in [2].

References
