Cationic Lipid Nanoparticles for ocular delivery of epigallocatechin gallate

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Abstract

Epigallocatechin gallate (EGCG) is a natural product and the major polyphenolic constituent found in green tea. This molecule has been reported for several biomedical purposes such as anticancer, antinflammatory, antidiabetic, antibacterial and antiaging [1,2]. The antioxidant activity of EGCG is useful for several ocular diseases, including retinopathy that is mainly caused by an antiagiogenesis process. The purpose of this work is the encapsulation of EGCG in lipid nanoparticles (LN) to improve the poor bioavailability and high susceptibility to degradation [3]. The use of LN could be a safe and biocompatible carrier for ocular drug delivery [4]. Since ocular mucosa has an anionic nature, the use of cationic LN is important to promote LN adhesion to ocular mucosa and prolong drug retention time. Two cationic lipids, namely quarternary salts of ammonium (CTAB and DDAB) were used to develop LN dispersions and their physicochemical parameters were analysed.

EGCG-loaded LN were prepared using a novel double emulsion technique [5]. The drug was dissolved in the inner aqueous phase composed of ultra-purified water. This phase was added to the fat phase based on solid lipid, cationic lipid, glycerol and Lipoid S PC-3 at 5ºC above the melting point of the solid lipid. The resulting mixture was homogenized for 30s with a sonication probe (6mm diameter) by means of an Ultrasonic processor VCX500 (Sonics, Switzerland). A power output with amplitude of 70% was applied. A poloxamer 188 solution was added and homogenized for additional 1min. This pre-emulsion was poured in the total volume of the poloxamer 188 cooled solution under magnetic stirring for 15min to allow the formation of the LN.

Characterization of lipid nanoparticles, namely the mean particle size (Z-ave), polydispersity index (PI) and zeta potential (ZP) were assessed by photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern, UK). The dispersions were diluted with purified water with a conductivity adjusted to 50 µS/cm. DSC measurements were performed using a Mettler DSC 823e (Mettler Toledo, Spain). Approximately 1–2mg of bulk lipid and/or LN formulations, were weighted in 40µl aluminium pan and cold sealed. The reference pan was left empty. Indium with purity ≥99.95% (Fluka, Switzerland) was used to calibrate the system. Heating curves for the bulk lipid and the lipid formulations were recorded with a scan rate of 5°C/min from 25°C to 90°C. Data were obtained from the peak areas using the Mettler STARe V 9.01 DB software (Mettler Toledo, Spain). The recrystallization indices (RI) of LN dispersions were calculated according to the following equation.

$$RI(\ \%) = \frac{Enthalpy_{LNs}}{Enthalpy_{bulklipid} x Concentration_{lipidphase}} x100 \quad \text{(Equation 1)}$$

The physical stability of prepared LN dispersions was assessed with an optical analyser TurbiscanLab® (Formulation, France). This technique allows the preliminary detection of destabilization phenomena of concentrated colloidal formulations before their appearance at a macroscopic scale. The dispersions were placed in a cylindrical glass cell, at room temperature (25 ◦C). The detection head scans the entire height of the sample cell (20mm longitude), acquiring Transmission and Backscattering each 40µm, 3 times during 10 min at different days after production.

The results showed the LN dispersions with submicron size (<150nm) (Table I) for both different cationic lipids formulations. The results of DCS analysis for the bulk Softisan®100 were compared to those obtained for LN dispersions (Table II and Figure I). These results indicate that incorporation of EGCG accelerated the polymorphic transitions of bulk lipid upon crystallization due the decrease of the recrystallization index (Table II). This emphasizes the fact that EGCG affects the thermal properties of LNs. The evaluation of physical stability of prepared LN dispersions could predict a good physical stability of developed LN dispersions. From the graphics (Figure II) a small destabilization phenomena

(little fluctuations in BS signal) in the end of the cell is observed. However, this deviation does not mean an instable formulation, because is lower than 2% in the two LN dispersions
In conclusion, the cationic LN dispersions developed could be a promising carrier for EGCG and more studies including toxicity, pharmacokinetics and efficiency delivery of EGCG in the eye will be carried out.

References

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Figures and Tables

Table I. Physicochemical parameters of EGCG-loaded LN dispersions.

Formulation	Z-Ave (nm)	PI	ZP (mV)
CTAB_EGCG	149.10±1.779	0.24±0.008	+20.80±0.896
DDAB_EGCG	143.70±0.450	0.16±0.015	+25.40±1.420

Table II. DSC parameters for the LN dispersions analyzed.

Formulation	Onset temperature (°C)	Melting point (°C)	Integral (mJ)	Enthalpy (Jg^{-1})	Recrystallization index (RI)
Softisan100 bulk	36.68	39.12	-3111.15	-44.76	----------
CTAB_EGCG	28.25	33.50	- 39.05	- 0.55	0.25%
DDAB_EGCG	28.13	34.83	- 56.05	- 0.73	0.36%

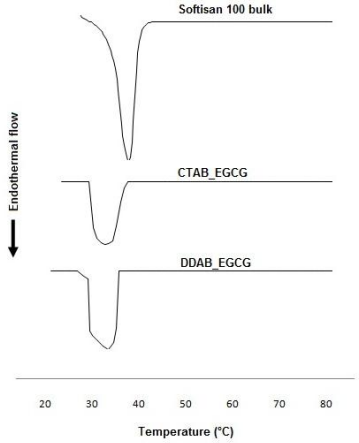

Figure I. DSC thermograms for bulk lipid Softisan®100 and the cationic LN dispersions developed.

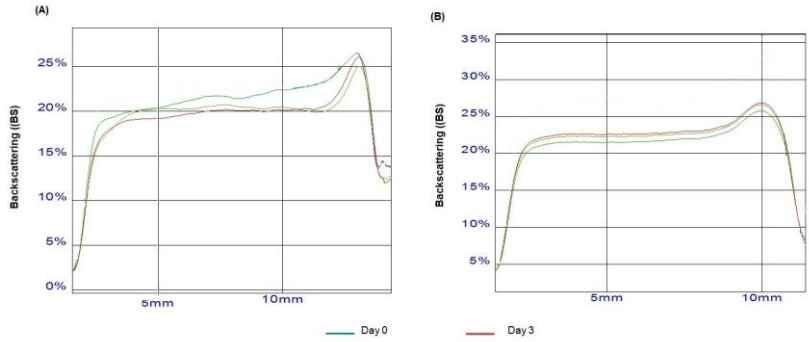

Figure II. BS profiles of LN dispersions, measured across the height of the sample cell during 10 min, on day 0 (n = 3) and day 3 (n=3). LN Dispersions prepared with CTAB (A) and DDAB (B). For all LN dispersions, the data acquisition was repeated 3 times for a period of 10 min at day 0 and day 3 after production for each sample, which characterizes its stability/instability condition.