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Research paper

Chitosan-coupled solid lipid nanoparticles: Tuning nanostructure and mucoadhesion

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ABSTRACT

Solid Lipid Nanoparticles (SLNs) composed of biodegradable physiological lipids have been widely proposed as efficient drug delivery systems, also for ophthalmic administration. Recently, chitosan-associated-SLNs have been developed to further improve the residence time of these colloidal systems in the precorneal area by means of mucoadhesive interaction. In the present study, a one-step preparation protocol was used aiming both at scale-up ease and at stronger coupling between chitosan and SLNs. The resulting particles were chitosan associated-SLNs (CS-SLNs). These nanoparticles were characterized, as compared to both the chitosan-free and the usual chitosan-coated ones, by applying a multi-technique approach: light, neutron and X-ray scattering, Zeta-potential, AFM, calorimetry. It was assessed that, while keeping the features of nano-size and surface-charge required for an efficient vector, these new nanoparticles display a strong and intimate interaction between chitosan and SLNs, far more settled than the usual simple coverage. Moreover, this one-step preparation method allows to obtain a strong and intimate interaction between chitosan and SLNs, firmer than the usual simple coating. This confers to the CS-SLNs an improved mucoadhesion, opening the way for a high-performing ophthalmic formulation.

1. Introduction

Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers composed of biodegradable physiological lipids with GRAS (Generally Recognized As Safe) regulatory status. Therefore SLNs are commonly considered to be less toxic than polymer-based nanoparticles. Moreover SLNs combine the advantages of colloidal systems (higher surface-to-volume ratio, protection of incorporated labile drugs from degradation, controlled release properties and improved pharmacokinetics and biodistribution) with a physical stability almost similar to that of conventional drug delivery systems.

SLN formulations have been developed for various application routes (parenteral, oral, dermal, ocular, pulmonary, rectal) and thoroughly characterized *in vitro* and in vivo. As for the ophthalmic administration route, SLNs have been widely proposed as efficient drug delivery systems due to small particle size (less than 1 μm), narrow size and low polydispersity, adequate bioavailability, good tolerability and compatibility with ocular tissue and absence of blurred vision [1–3]. SLNs administered in the precorneal district demonstrated to be able to increase bioavailability [4]. This is likely due to the small size of the SLN, allowing for increased entrapment and retention in the mucin layer covering the corneal epithelium [4]. These features have emerged to be central and challenging issues for ocular drug delivery. In fact, the retention time of common formulations in the precorneal district is normally short, being subjected to eyelid movement (blinking) and removal by tears via the naso-lachrymal duct. In addition eye's barriers (epithelial, aqueous–vitreous, blood–aqueous barrier) limit the entry of topically administered drugs. Drug penetration into the anterior and posterior chambers is however necessary to treat some ophthalmic diseases such as glaucoma or uveitis [5].

Recently, chitosan-associated-SLNs (CS-SLNs) have been developed to further improve the residence time of SLNs colloidal systems in the precorneal area, via mucoadhesive interaction [6]. The peculiar properties of chitosan, a cationic polysaccharide, are exploited. In fact, chitosan shows good mucoadhesion properties and enhances drug penetration across various epithelia [7,8]. These features would further prevent removal of nanoparticles from the eye while simultaneously favouring internalization in the corneal

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cells. CS-SLNs demonstrated to be biocompatible and to enhance permeation/penetration of a loaded drug, namely cyclosporin A, in both *in vitro* (RCE cell substrate) and *ex vivo* (excised cornea) models. CS-SLNs interaction with the corneal surface, required for internalization/uptake, is favoured by the presence of positively-charged chitosan. This aspect could be exploited in the treatment of eye pathologies, the cornea acting as a reservoir, maintaining a constant drug level and thus decreasing the number of required instillations.

In order to maximise the potentiality of chitosan, a one-step protocol has been here proposed aimed to closer coupling with SLNs, avoiding simple coating. CS-SLNs were prepared by means of high-shear homogenization and ultrasound method [9] optimized previously [6,10]. The preparation process was based on one-step emulsion: the lipid phase (Compritol® 888 ATO) was emulsified with an aqueous phase containing chitosan and surfactants (Pluronic® F68 and Tween® 80).

In the present work, the structure of chitosan-based SLNs has been studied, in order to identify the peculiar features induced by the type of chitosan association, either as simple coverage of preformed SLNs or early participating to the nanoparticle formation [6]. For a complete structural characterization, different techniques were applied on naked-SLNs (prepared without chitosan, hereafter SLNs), on chitosan-coated-SLNs (hereafter CS-c-SLNs) and on new CS-SLNs. On the whole particle length-scale, the particles size, the surface charge and the particles morphology were assessed by Dynamic Light Scattering (DLS) and Zeta-Potential (ZP) measurements and Atomic Force Microscopy (AFM), respectively. The shape and the internal structure of the nanoparticles were studied by Small-Angle X-ray and Neutron Scattering (SAXS and SANS). Wide-Angle X-ray Scattering (WAXS) was applied to get hints on the very local structure (Ångstrom lenghtscale). Parallel Differential Scanning Calorimetry (DSC) measurements were performed to assess the thermotropic behaviour of the nanoparticles. This multi-technique approach was essential to deeply investigate the differences in the nanoparticles structure that could result in significant differences in their mucoadhesive properties.

Finally, mucoadhesion properties were evaluated by Turbidimetry (T) and compared for the three different systems.

2. Materials and methods

2.1. Materials

Glyceryl Behenate, Compritol®888 ATO, a kind gift from Gattefossè (Milan, Italy), was chosen as lipid. Poloxamer188 (Plu) (Pluronic®F68, BASF, Cesano Maderno, Italy) and Tween®80 (T80, Fluka, Milan, Italy) were used as surfactants. Chitosan (CS) (MW 251 kDa, assessed by viscosimetric evaluation [11]; deacetylation degree: 98%) (Giusto Faravelli, Milan, Italy) was hydrated in distilled water by adding a stoichiometric amount of HCl and then freeze-dried.

2.2. Methods

2.2.1. SLNs preparation

Chitosan-associated-SLNs (CS-SLNs) were prepared by means of high shear homogenization and ultrasound method [9] optimized previously [6]. The lipid phase, Compritol®888 ATO (C888) (100 mg), was melted at 85 °C. The aqueous phase was based on Pluronic®F68 (50 mg) and Tween®80 (50 mg) dissolved in 12.5 ml of a 1% chitosan hydrochloride solution (125 mg of chitosan as base hydrated by using hydrochloric acid aqueous solution 0.18 N), and heated to 85 °C. The aqueous phase (12.5 ml) was poured into the lipid phase under homogenization at 24,000 rpm for 5 min with UltraTurrax (T25, IKA-Werke GmbH, Germany). The solution was then diluted with 12.5 ml bidistilled water at 4 °C and kept at −20 °C for 10 min. CS-SLNs were submitted to ultrasound treatment (ultrasound frequency 37 kHz) (Elmasonic S80 H, Elma Hans Schmidbauer GmbH & Co, Singen, Germany) for 10 min to avoid agglomeration during recrystallization. They were kept at 4 °C until measurement, at least for one day. All samples were measured within two weeks from preparation.

Chitosan-coated-SLNs (CS-c-SLNs) were prepared using an analogous procedure, using the same lipid phase. The aqueous phase, containing Pluronic®F68 and Tween®80, was chitosan free. Chitosan was added afterwards, after hot emulsification of SLNs, upon dilution with a 1% w/w chitosan solution at 4 °C, instead of water.

Naked-SLNs (SLNs) were prepared according to the previously described procedure [10], including high-shear homogenization and ultrasound treatment, with the same components except for chitosan.

2.2.2. Particle size measurements

Laser light scattering measurements were carried out on a standard apparatus (SM200, Brookhaven Instruments Co, Holtsville, New York, USA) equipped with an Argon ion laser operating on the 514 nm green line (Lexel, Fremont, CA, USA) and a thermostat. Samples were properly diluted, typically 1:15 with respect to the original solutions, in order to avoid multiple scattering, and inserted into the measuring cell. Water was accurately filtered (on 0.2 μm pore size polycarbonate membranes) in order to avoid dust contamination.

DLS experiments were performed on each sample.

The correlation function of the scattered light, was collected at 90° scattering angle, at T = 25 °C. At least three measurements were performed for each sample.

The correlation function analysis was performed both with the Cumulant Method [12], giving the particle average size and polydispersity index, and with NNLS (Non-Negatively constrained Least Squares) [13], estimating the particle size distribution.

2.2.3. Zeta Potential (ZP) measurements

The ZP of nanoparticles was calculated (as described in the instruction manual for ZeraPALS, Zeta Potential Analyzer) measuring the electrophoretic mobility of dispersed particles at 25 °C, by using a standard Zeta PALS instrument (Brookhaven Instruments Co, Holtsville, New York, USA). Measurements were performed on each system at three concentrations: (a) as from the preparation protocol (b) after 1:2 dilution and (c) after 1:15 dilution with deionized and filtered (0.2 μm) water (pH 6.5). Ten repetitions were made on each sample.

2.2.4. Atomic Force Microscopy (AFM) measurements

Samples were mounted onto a Multimode AFM with a NanoScope V system (Veeco/Digital Instruments) operating in Tapping Mode, using standard phosphorus-doped silicon probes (T: 3.5–4.5 μm, L: 115–135 μm, W: 30–40 μm, f_0: 311–364 kHz, K: 20–80 N/m) (Veeco). The original samples were diluted 1:200 with MilliQ filtered (0.2 μm) water. 60 μl of diluted sample were deposited on a freshly-stripped mica surface, with circular shape (diameter 10 mm) and left for 10 s. Then, the surface was thoroughly rinsed with deionized filtered water and subsequently dried under a gentle flux of nitrogen. Two-to-five different fields of the same deposited sample were observed. The processing of the AFM images was performed with the instrument built-in program (Nanoscope Veeco).

2.2.5. Differential Scanning Calorimetry (DSC) Measurements

Calorimetric measurements were performed on an updated version of the MASC instrument, operated in the DSC mode [14].