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Rapamycin-loaded solid lipid nanoparticles: Morphology and impact of the drug loading on the phase transition between lipid polymorphs

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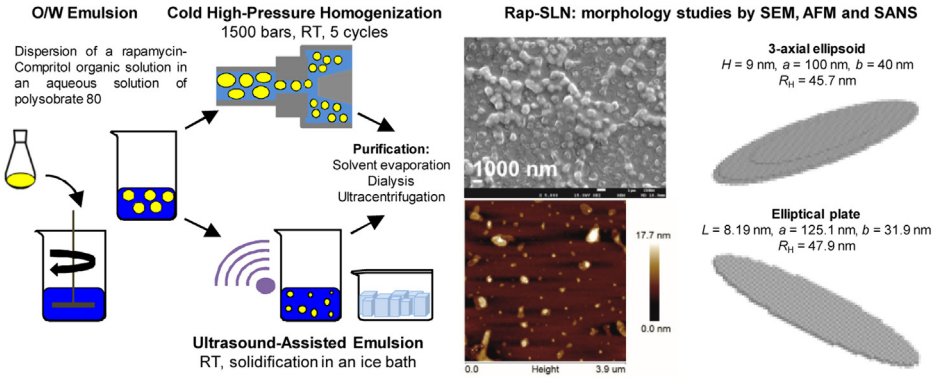
HIGHLIGHTS

- Cold high-pressure homogenization and ultrasound-assisted emulsion were used.
- The stability and drug content varied with regard to the method applied.
- Modeling of the elliptical plate and flat ellipsoid SLN shapes.
- The reduction of the phase transition point in the lipid matrix by the drug.

GRAPHICAL ABSTRACT



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ABSTRACT

In recent decades solid lipid nanoparticles (SLN) have become a well-performing tool for the site-targeted delivery of water-insoluble drugs. In this study, Compritol® 888 ATO-based SLN, coated with polysorbate 80, were loaded with rapamycin (Rap), a lipophilic immunomodulator, broadly-used in therapies of cancer and neurodegenerative diseases. Rap-SLN were formulated using cold high-pressure homogenization and ultrasound-assisted emulsion. The exploitation of these methods yielded the nanoparticles of various values of zeta-potential (from −1 mV to −20 mV) and efficacies of the Rap entrapment (from 37.5 ± 2.3% to 77.0 ± 5.4%). The SEM and AFM imaging and shape-modeling by the combined DLS-SANS analysis revealed that the Rap-SLN of the hydrodynamic radius of ~46 nm preserve the platelet-like or flat ellipsoidal structure with a thickness as large as 8–9 nm. These dimensions correspond to a single lipid bilayer, organized in a triclinic L_β polymorph, and covered with a 1–2-nm shell of the surfactant. Consistently, FT-IR spectra acquired in the range 52–75 °C, showed that the Rap incorporation within the lipid matrix decreases the point of the gel-liquid crystalline (L_β-L_α) phase transition. These outcomes imply a thermodynamically-driven mechanism of the Rap release from SLN.

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1. Introduction

In recent decades great attention has been drawn to the preparation of solid lipid nanoparticles (SLN) as a drug delivery system. SLN are formulated from a lipid matrix that preserves the solid state at room and physiological temperatures. Particularly, SLN refer to colloidal dispersions made of various lipids (waxes, fatty acids, glycerides), stabilized with surfactant on the surface, and are usually produced by using high pressure homogenization, microencapsulation and emulsions methods [1]. In comparison to widely-used polymeric nanovehicles, SLN display a series of advantages, such as: improvement of biocompatibility and bioavailability of lipophilic drugs, or fast, inexpensive and scalable manufacture [2]. Specifically, SLN based on Compritol® 888 ATO, which constitutes a physical mixture of mono-, di-, and triglycerides of behenic acid, have been reported to facilitate both transportation and therapeutic activity of a number of water-insoluble or lipophilic drugs, dedicated to various diseases of affluence, targeted for instance to central nervous system [3], cancer [4], inflammation [5], or gene therapy [6]. Such formulations can be delivered to the body in a form of oral [7], topical [8], parenteral [9], ocular [10] and intestinal lymphatic [11] administration. Up to now, these nanocarriers have been extensively studied in terms of the drug incorporation influence on the lipid matrix, thermal stability, cytotoxicity, biodistribution and biodegradation in both *in vitro* and *in vivo* models.

Compritol® 888 ATO (thereafter named Compritol) displays an evident hydrophobic nature, expressed by a hydrophilic-lipophilic balance value of 2 and relatively high melting temperature in the range of 64–74 °C [12,13]. Compritol is soluble in chloroform and dichloromethane, when heated, slightly soluble in hot (but not cold) 96% ethanol, and insoluble in hexane, mineral oils and water. The feasibility of the industrial application of this non-toxic lipid composite, belonging to GRAS specimens and FDA Inactive Ingredients Database, has been widely applied to cosmetics, foods and pharmaceuticals, formulated as oral capsules, tablets and suspensions. In terms of structural composition, Compritol consists of monobehenin (~20%), dibehenin (~50%) and tribehenin (~30%). Upon the dissolution, each of these ingredients preserves an individual pattern of recrystallization [12,13]. During this process, followed by storage, the Compritol-based SLN initially crystallize in the non-stable hexagonal L_α-form and in the metastable orthorhombic perpendicular $L_{\beta'}$ or stable triclinic parallel L_β forms. The prevalence of one particular lamellar structure over the others depends on the size, shape and the physical state of the SLN formulation, considering that the rate of phase transition is known to be more sensitive for colloidal dispersion than for the bulk materials [12]. The polymorphism of Compritol originates from the various lateral packing of fatty acid chains: the L_α and $L_{\beta'}$ polymorphs are remarkable for a mixture of long-chained 1,2-diglycerides, whilst the L_β polymorph is most suitable for monoacid 1,3-di- and triglycerides [12].

In this study, Compritol SLN were loaded with rapamycin (Rap), an antifungal agent, naturally produced by *Streptomyces hygroscopicus* and collected for the first time in 1965 [14]. The activity of Rap comprises the specific inhibition of the mammalian target of rapamycin (mTOR) kinase [15]. Upon the activation by extracellular stimulation, mediated by nutrients, growth factors or stress conditions, the mTOR signaling pathway responds *via* the regulation of cellular growth, proliferation, metabolism and survival [16]. Ongoing studies indicate that the Rap-triggered inhibition of mTOR plays a pivotal role in the reduction of causes and effects of neurodegeneration [17], cardiovascular diseases [18], tumorigenesis or cancer [19], inflammatory diseases [20] and immunological response [21]. Nevertheless, given that Rap is a macrolydic immunosuppressive agent that possesses no ionizable groups in the pH range 1–10, displays poor solubility [22], high hydrolysis-potency in aqueous solution [23], and high affinity to red blood cells and other blood components [24], the oral bioavailability and the clinical applicability of Rap are extremely limited. Therefore, much effort has been made to improve the Rap delivery into human tissues. These are mainly: (i) enhancement of Rap solubility by hydrotropy strategy [22], or (ii) incorporation of drug molecules inside various nanocarriers, mostly based on phospholipid [25] or polymeric micelles [26], nanoparticles [27] and liposomes [28]. The exceptional compliance of SLN consigned to the brain-targeted delivery of Rap, is expected to attenuate the severe immunosuppressive effect of the drug in an organism, observed upon the intravenous administration, and to prolong the therapeutic, mTOR-specific activity in central nervous system after the topical administration or following the blood-brain barrier crossing.

The detailed investigation of the surface quality of Rap-SLN, stabilized with polysorbate 80 (PS80), was achieved by means of electron and atomic force microscopy (SEM, AFM). Additionally, the morphology was studied using light and small-angle neutron scattering (DLS, SANS), since only several lipid-based drug delivery systems have been investigated by SANS, until now [29,30]. Such combined analysis evidenced that Rap-SLN shape is platelet-like rather than spheroidal. The stability of the nanoformulation was evaluated by measurements of mean hydrodynamic diameter, zeta-potential and polydispersity indexes, whereas the Rap content in SLN was analyzed both quantitatively and qualitatively by means of UV spectrophotometry and Raman spectroscopy, respectively. Eventually, the effect of the Rap loading on the phase transition in lipid matrix was investigated by recording FT-IR spectra in the temperature range of 52–75 °C. Taken together, these results suggest considerable contribution of the thermodynamically-driven processes leading to the Rap release from SLN.

2. Materials and methods

2.1. Materials

Rapamycin (Rap, Sirolimus, CAS No.: 53123-88-9, purity ≥98%) was obtained from Ningbo Heyreal Import&Export Co., Ltd, China. Compritol® 888 ATO (Compritol, glyceryl behenate, 99%) was acquired from Gattefosse, Milan, Italy. Polysorbate 80 (PS80, Tween® 80, polyoxyethylene sorbitan monooleate), deuterium oxide (D_2O, heavy water, 99.98%), chloroform ($CHCl_3$, 99.5%) and dimethyl sulfoxide (DMSO, 99.9%) were from Sigma Aldrich (Poznań, Poland and Milan, Italy). No additional purification was conducted for these reactants. Ultrapure water 18 MΩ/cm from the MiliQ system (Millipore) was used throughout the work.

2.2. Preparation of rapamycin-loaded solid lipid nanoparticles

2.2.1. Cold high-pressure homogenization

The water dispersions of blank SLN and Rap-SLN at concentration of 1% w/w were prepared by using a modified cold (RT) high-pressure homogenization (CHPH) protocol, described in details elsewhere [31]. Briefly, the 10 and 20% w/w mixtures of Rap and Compritol were dissolved in 2 mL or 1.5 mL of chloroform and dosed drop-wise into 1% w/w aqueous solution of PS80,

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