Colloids and Surfaces B: Biointerfaces 117 (2014) 21–28

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Formulation, characterization and cytotoxicity studies of alendronate sodium-loaded solid lipid nanoparticles

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ARTICLE INFO

Article history:
Received 15 August 2013
Received in revised form 4 January 2014
Accepted 24 January 2014
Available online 9 February 2014

Keywords:
Solid lipid nanoparticle
SLN
Alendronate sodium
Compritol
Cytotoxicity

ABSTRACT

Aim: Solid lipid nanoparticles (SLNs) are novel drug delivery system for drug targeting in various routs of administration such as parenteral, oral, ophthalmic and topical. These carriers have some advantages such as high drug payload, increased drug stability, the possibility of incorporation of lipophilic and hydrophilic drugs, and low biotoxicity. In this study, alendronate sodium was used as a hydrophilic model drug and was incorporated into SLNs.
Methods: Hot homogenization method was used for preparation of alendronate sodium-loaded SLN formulations and the encapsulation efficiency of drug in SLNs was determined by ultrafiltration method using centrifugal devices. Scanning electron microscopy (SEM) was carried out to study the morphological behaviors of prepared SLNs like sphericity. Several cytotoxicity studies including MTT, DAPI staining and DNA fragmentation assays were used for biocompatibility assays.
Results: High drug encapsulation efficiency (70–85%) was achieved by drug determination through derivatization with *o*-phthalaldehyde. The physical stability of drug-loaded SLNs in aqueous dispersions was assessed in terms of size and drug leakage during two weeks. Scanning electron microscopy images showed spherical particles in the nanometer range confirming the obtained data from size analyzer. Several cytotoxicity studies including MTT, DAPI staining and DNA fragmentation assays as well as flow cytometry analysis confirmed the low toxicity of alendronate-loaded SLNs.
Conclusion: The cost-efficient procedure, the avoidance of organic solvents application, acceptable reproducibility, ease of manufacturing under mild preparation conditions, high level of drug encapsulation, desirable physical stability and biocompatibility are the advantages of the proposed SLN formulations.

1. Introduction

Solid lipid nanoparticles (SLNs) are novel drug carrier system with submicron size particles (50–1000 nm), which consists of a solid lipid matrix at both room and body temperatures, stabilized by surfactant. SLNs are considered as substitute carriers to traditional colloidal systems, for controlled and targeted delivery. SLNs possess high biocompatibility and biodegradability which are capable of incorporating both lipophilic and hydrophilic compounds [1,2]. In addition, enhanced cell uptake of various drugs has also been described [3–5]. Their production involves a very simple emulsification/solidification process which does not require any organic solvents. This would enable successful scale up for industry. Besides, compared with nano-emulsions, which are prepared with liquid lipids, SLNs have more potential for controlled release, owing to their solid matrix [6–8].

Alendronate sodium is a hydrophillic, amphiprotic drug, which is administered orally for treatment of bone Pagets disease, postmenopausal osteoporosis, primary hyperparathyroidism, malignant hypercalcemia and metastatic bone diseases [9–12]. It increases bone formation and enhances osteoblast proliferation and maturation and leads to inhibition of osteoblast apoptosis [11].

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http://dx.doi.org/10.1016/j.colsurfb.2014.01.055
0927-7765/

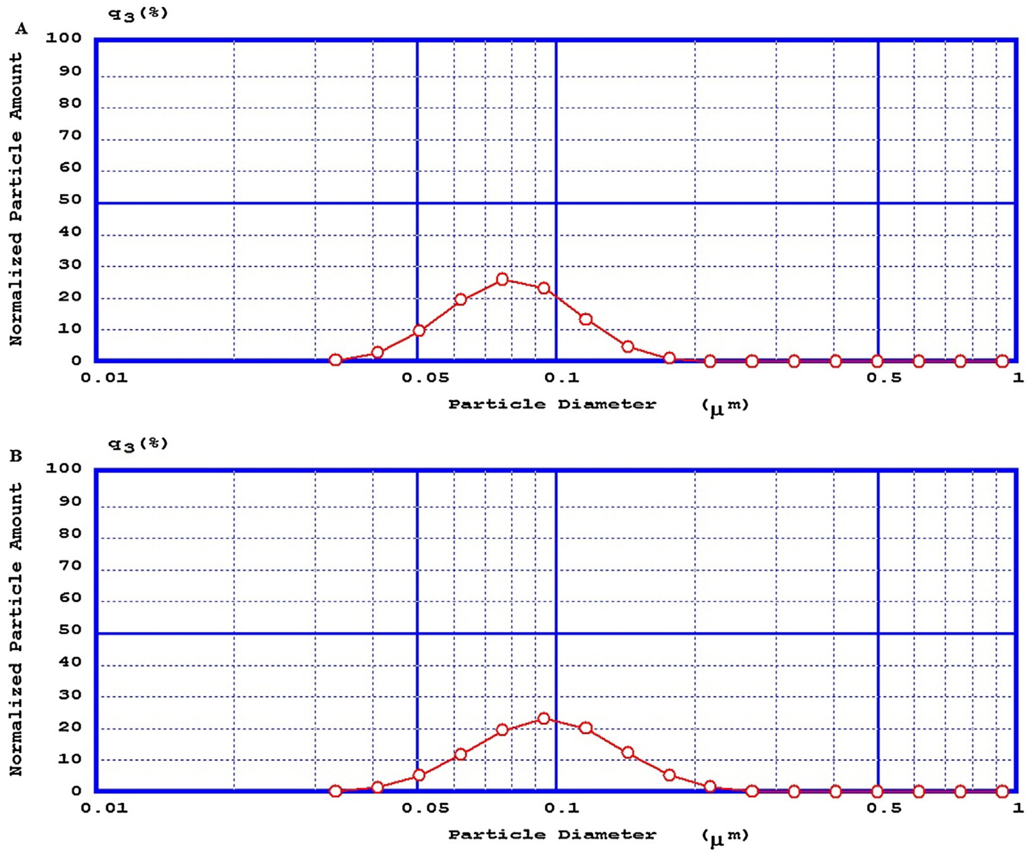

Fig. 1. The differences in formulated SLNs size between the initial (A) and 4 weeks (B) timepoint were not found to be statistically significant.

However, oral administration of alendronate sodium is associated with gastrointestinal intolerance. Gastric and esophageal lesions like erosions and ulcers have been reported due to a local irritant effect of the drug [13–15]. This medication in patients with gastrointestinal problems as well as esophageal abnormalities such as achalasia is contraindicated [15]. On the other hand, oral bioavailability of alendronate sodium is very low. Intake together with meals and beverages other than water reduces its bioavailability even more [16,17]. Poor absorption is attributed to its high hydrophilicity and complexation with divalent cations, like Ca^{2+} [16].

To overcome the poor bioavailability of alendronate sodium and the adverse gastrointestinal effects, investigation on alternative administration strategies have been performed [18]. Intravenous administration of alendronate sodium may raise the risk of nephrotoxicity. Subcutaneous and intramuscular administrations may result in local soft tissue damage and irritation at the site of injection [16,19]. Sutton and co-workers demonstrated the nasal delivery of alendronate sodium in dogs and rats as an alternative to per oral and parenteral administrations. Pulmonary delivery may help to avoid gastrointestinal tract problems such as, low bioavailability, gut irritability, unwanted metabolites, and food effects [20]. To the best of our knowledge no inhalable alendronate SLN formulations have been introduced in the literature [21]. The aim of this study was to design and optimize a novel alendronate sodium-loaded SLNs carrier system composed of a Compritol 888 ATO as lipid matrix for the pulmonary delivery of alendronate sodium. Laser light scattering and zeta potential were performed to characterize the properties of SLNs. All excipients evaluated in this study showed low toxicity on A549 cell line.

2. Materials and methods

2.1. Materials

Alendronate monosodium trihydrate was obtained by Modava Company (Iran). Poloxamer 407 and Trypsin-EDTA (0.02–0.05%) were purchased from Sigma Aldrich Co. (Poole, UK). Tween 20 was purchased from Oleon (Olegem, Belgium). RPMI1640 medium and fetal bovine serum (FBS) was supplied by Gibco, Invitrogen (Paisley, UK). Annexin V-FITC apoptosis detection kit was purchased from Oncogene Research Products (San Diego, CA, USA). Precirol® ATO 5 and Compritol 888 ATO were obtained from Gattefosse (France). A549 lung carcinoma cell line, cell culture plates and flasks were obtained from the national cell bank of Iran (Pasteur institute, Iran) and IWAKI, Japan, respectively.

2.2. Preparation of SLNs

Alendronate sodium-loaded SLN formulations were prepared by hot homogenization according to published methods [22]. Solid lipid (Precirol® ATO 5 or Compritol 888 ATO®) with 100 μl Tween 20 as a surfactant was heated at 80 °C in a boiling water bath to be melt under continuous stirring (oil phase). In a separate container, surfactant (Poloxamer 407) was dissolved in ultra-pure water and heated to the same temperature of the oil phase (aqueous phase). The drug was dissolved in 2 ml of aqueous phase and added into the oil phase under homogenization at 20,000 rpm (Heidolph, Germany) to form the initial water-in-oil emulsion. Then the hot aqueous phase was added dropwise into the oil phase under the homogenization at 20,000 rpm while maintaining the temperature