Journal of Drug Delivery Science and Technology 39 (2017) 442–449

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Carrageenan-stabilized chitosan alginate nanoparticles loaded with ethionamide for the treatment of tuberculosis

Sharif Abdelghany*, Maha Alkhawaldeh, Hatim S. AlKhatib

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Jordan, Amman, 11942, Jordan

ARTICLE INFO

Article history:
Received 17 January 2017
Received in revised form
27 April 2017
Accepted 27 April 2017
Available online 5 May 2017

Keywords:
Chitosan-alginate nanoparticles
Carrageenan stabilized nanoparticles
Nanoparticles
Ethionamide
Controlled release

ABSTRACT

The objective of this work was to prepare ethionamide-loaded, cross linker free, chitosan alginate nanoparticles stabilized with varying amounts of carrageenan using simple inotropic gelation. The nanoparticles formulation was manipulated to optimize drug loading and release. Three different formulations containing different percentages of carrageenan (0%, 42%, and 59%) were evaluated for particles size, zeta potential, entrapment, and release. Results showed that carrageenan enhanced the stability of the nanoparticles to the formulation process and enhanced the entrapment of ethionamide in the nanoparticles (0.36 μg/mg for 0% carrageenan formulation vs 3.1 μg/mg for 59% carrageenan formulation). Moreover, nanoparticles exhibited controlled release over 96 h and the release was reduced with increasing the carrageenan content. The nanoparticles size for all formulations was found to in the range of 300 nm. These results were found concurring in size with the results obtained from TEM. Furthermore, the DSC and FTIR evaluation of the ethionamide in the freeze-dried nanoparticle preparation showed no chemical interaction between ethionamide nanocrystals and the excipients. Additionally, the ethionamide loaded nanoparticles showed significant antimycobacterial activity against H37RA mycobacterium strain using resazurin microtiter assay (REMA). Thus, it can inferred from these results that our nanoparticle formulations have a great potential in the treatment of tuberculosis.

1. Introduction

Tuberculosis (TB) is a serious communicable disease that predominately manifests in the lungs, caused by various strains of mycobacteria, primarily *Mycobacterium tuberculosis* [1]. The World Health Organization (WHO) estimates that one third of the world population is a carrier of the causative pathogen *M. tuberculosis*. TB pathogenesis is initiated by the contact and engulfment of airborne bacilli by alveolar macrophage, and followed by the multiplication and invasion of neighboring macrophages. Although epithelial type II pneumocytes and dendritic cells are involved in the prognosis of TB, alveolar macrophages are the most elucidated target to treat TB [2–4]. For standard nonresistant tuberculosis, WHO has recommended a two-phase treatment for 6 months with four antitubercular drugs. The current treatment of active TB frequently results in low patient adherence, hence may predispose into multi-drug resistant TB (MDR-TB) or extensive drug resistant TB (XDR-TB), due to multiple daily dosing [5]. In this context, ethionamide was chosen as a second generation anti TB drug that have high antibacterial activity against many bacterial strains including *Mycobacterium tuberculosis* [6]. However, high doses of this drug are associated with unfavorable side effects such as hypothyroidism in children and gynecomastia [7–9]. Providing that the dose of ethionamide can reach up to 1000 mg/day, this relegates it to a second-line anti-TB drug [10]. Therefore, the entrapment and controlled release of this drug into polymeric nanoparticles could reduce the required dose remarkably, and hence reduce the associated toxicity.

Polymeric nanoparticles have been utilized to improve the efficacy of the associated therapeutics and decrease their off-target side effect. Specifically, polysaccharide nanoparticles have been widely used in drug delivery due to their advantages over hydrophobic nanoparticles that include the simplicity of preparation, which can be accomplished in single ionic gelation/precipitation step. This also allows avoiding the use of toxic organic solvents [11–14]. In addition, the biodegradability, muco-adhesive properties, and the abundance of modifiable functional groups (hydroxyls, amines, and carboxyl) of these materials render them a preferable choice for drug delivery [15–17].

Recently, anionic polymers, including alginate and carrageenan,

* Corresponding author.
E-mail address: s.abdelghany@ju.edu.jo (S. Abdelghany).

http://dx.doi.org/10.1016/j.jddst.2017.04.034
1773-2247/

have been extensively complexed with various counter-ion materials including calcium, poly-*l*-lysine and chitosan [18,19]. Chitosan, alginate, and carrageenan are arguably the most commonly used polysaccharide polymers in drug delivery. These polymers have been utilized to efficiently entrap and release wide classes of drugs including antihistamines, anticancer, antibiotics, and others [20,21]. Antibacterial loaded polymeric nanoparticles have been extensively investigated for their potential in drug-release controlling [22]. Additionally, intracellular infections such as Tuberculosis have utilized the ability of nanoparticles to internalize mammalian cells releasing the drug in proximity of the targeted bacteria [23–26].

Ethionamide have previously studied into Polylactide-*l*-glycolide (PLGA) nanoparticles and showed an in vivo safety with no apparent toxicity on mice [27]. Moreover, ethionamide loaded PLGA nanoparticles showed improved therapeutic efficacy in a MDR-TB infected mouse model [28]. However, this drug has not yet been studied with chitosan/alginate based nanoparticles, therefore, we aimed in this study to investigate the potential of these polysaccharides matrices in obtaining controlled release of ethionamide.

The objective of the present study is to optimize the formulation of ethionamide to attain promising nanoparticles characteristics that can be further examined for their anti-mycobacterial activity. Several formulations were prepared and characterized for their physical properties, drug entrapment, and release. Particle size and Zeta potential showed favorable size and surface charge for further investigation.

2. Materials and methods

2.1. Materials

Alginate was purchased from Hayashi Pure Chemical Industries (Japan). Chitosan low molecular weight (75–85% deacetylated), and ethionamide were purchased from Sigma Aldrich, (St. Louis, MO). κ -Carrageenan sodium (molecular weight = 788.6 Da) was purchased from the British Drug Houses (BDH), England. Deionized water used in formulations is HPLC grade purified water. Dimethyl sulfoxide (DMSO) was purchased from Sigma Aldrich, USA. All other chemicals were of analytical grade. *M. tuberculosis* H37RA was purchased from American type cell culture (ATCC). Bacterial culture media Middlebrook 7H9 and 7H11, albumin dextran catalase (ADC) enrichment, oleic albumin dextran catalase (OADC), casitone, glycerol, and resazurin sodium was purchased from Sigma Aldrich, USA.

2.2. Preparation of chitosan alginate nanoparticles by ionotropic gelation

Several formulae of alginate/carrageenan chitosan were prepared using simple inotropic gelation. This method is illustrated in Fig. 1. Briefly, 9 mg of alginate was dissolved in 3 mL of methanol/water 1:1 solution and 0.1 mL of ethionamide of 25 mg/mL methanolic solution was then added to the alginate solution, readjusted at pH 5.5. Subsequently, 1.2 mL of aqueous chitosan (3 mg/mL) adjusted at pH 5.5 was added drop-wise to alginate solution with simultaneous pulse-wise ultrasonic probe sonication (Hiescher, UP200 Ht) for 2 min.

To study the impact of carrageenan in the formulation on the nanoparticles characteristics, several formulations containing different amounts of carrageenan (0 mg, 9 mg, or 18 mg) was dissolved in the alginate solution prior to the addition of chitosan solution. Moreover, a formulation containing 27 mg carrageenan without alginate was done in a similar manner. This results in a theoretical percentage of 0%, 42%, 59%, and 88.5% carrageenan in the formulation, respectively. Nanoparticles were then centrifuged and washed at 13,400 rpm (Eppendorf minispin® plus, Germany) for 30 min with deionized water. Nanoparticles were re-dispersed in equivalent volume of deionized water. Blank nanoparticles without ethionamide were prepared in a similar manner omitting the ethionamide from the 0.1 mL of the methanolic solution added. Samples were freeze dried using Vacuum freeze dryer, ENBI, Human lab instruments, Korea.

2.3. Physical characterization of nanoparticles

The particle size and zeta potential of the nanoparticles was measured using dynamic light scattering (Zetatrac particle size analyzer Microtrac, Japan). Measurements were carried out with each sizing determination done in triplicate and an average particle size expressed as the mean diameter (Z_{ave}).

2.4. Drug loading of ethionamide loaded nanoparticles

The nanoparticle supernatant and washing solutions were collected, diluted with water, and quantified for ethionamide content using modified high performance liquid chromatography (HPLC) method [29]. Briefly, the diluted supernatant of ethionamide loaded nanoparticles was injected in Venusil XBP C18, 5 μm pore, 25 cm HPLC column. The mobile phase used was isocratic, 0.02 M disodium hydrogen orthophosphate and acetonitrile (1:1) at flow rate of 1 mL/min. The analysis was performed using a Knauer HPLC system composed of Knauer Smartline® Manager 5000, Knauer Smartline® Pump 1000, a Knauer Smartline® UV Detector 2500 equipped with a degasser and a 20 μl injection loop. The data capture and analysis was performed using ClarityChrom® software. The peak area was measured and compared to a calibration curve of ethionamide at 290 nm detection wavelength. The drug loading was calculated using the following formula [30]:

$$\text{Drug loading } (\%) = \frac{\text{Total drug added } (\mu g) - \text{drug quantified in the supernatant } (\mu g)}{\text{Total weight of polymers and drug in the formulation } (mg)} \times 100$$

2.5. In vitro release study of nanoparticles

The in vitro release of ethionamide-loaded nanoparticles was investigated via in vitro dialysis cell as described before with some modifications [31]. 1 mL of nanoparticle suspension for formulations of (0%, 42%, or 59% carrageenan) was incubated at 37 °C in a donor compartment of deionized water, separated from the receptor compartment by a semipermeable cellulose membrane (molecular weight cut-off = 14,000). The receiver compartment volume was set at 5 mL and the release study was conducted over