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Research paper

Nicotine hydrogen tartrate loaded chitosan nanoparticles: Formulation, characterization and *in vitro* delivery from dry powder inhaler formulation

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ABSTRACT

This study reports the development of nanoparticles in the form of inhalable micro-aggregates of biodegradable chitosan (CS) loaded with nicotine hydrogen tartrate (NHT) for potential pulmonary delivery of nicotine from dry powder inhaler (DPI) formulations with prolonged release profile. The NHT-loaded CS particles were prepared using a water-in-oil emulsion crosslinking method. The prepared particles were characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for morphological studies; Zetasizer and Mastersizer were applied for particle size analysis. The *in vitro* aerosolization of the formulations was studied using a twin-stage-impinger (TSI) and promising aerosolization characteristics were shown. The nanoparticles were spherical with size ranges between 167 and 411 nm while micro-aggregates (3.73–4.73 μm) were formed among nanoparticles. According to differential scanning calorimetry (DSC), X-ray diffraction (XRD) analysis and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, the NHT crystallinity was lost when in the particles, indicating it was uniformly dispersed as a solid solution. On the basis of X-ray photoelectron spectroscopy (XPS) analysis, the amount of NHT loaded on the surface of CS increased proportionally with increasing drug loading in the bulk so there was no surface enhancement. The fine particle doses (FPD) of NHT ranging between 1.7 and 3.2 mg from DPI formulations were concentration dependent and increased with increased drug loading. Based on the *in vitro* release study, NHT released from CS particles with a burst release in the first 8 h and subsequent prolonged release of nicotine.

1. Introduction

The inhaled drug delivery route provides the most direct access of drug to the target area with low dose [1]. Due to the large surface area, huge blood supply and thin epithelial layer, the absorption of drugs after inhalation occurs in very short time. The inhaled drug delivery route is a convenient, non-invasive way to deliver drug both for local and systemic effects. The delivery of nicotine by smoking is an example of pulmonary drug delivery with negative health consequences due to the carcinogenic by-products of tobacco pyrolysis. Smoking related disorders remain world-wide major health concerns, causing a serious threat to human health and a tremendous economic burden. Therefore, it is of interest to determine if there is an opportunity to develop a dry powder form of inhalable nicotine formulation to reduce the direct and indirect burden associated with smoking.

Inhaled delivery of nicotine is currently available through Nicotrol® and Nicorette® Inhalers (Pfizer); however, these devices deliver nicotine into the buccal areas, not in the lower alveoli, resulting in lowering plasma maximum concentration and delayed time to reach maximum concentration for therapeutic action. In an early study, a nicotine pressurized metered-dose inhaler (pMDI) formulation in ethanol with hydrofluoroalkane, produced a microaerosol of fine droplet size that mimicked the nicotine delivered via tobacco smoke [2] and inhaled nicotine produced a median maximum peak plasma concentration, which was about 50% of the amount that was obtained by smoking a cigarette [3]. Gonda et al., delivered clean nicotine aqueous solution to the deep lungs using an AERx Essence® inhaler [4]; and produced a rapid and dose-proportional increase in plasma nicotine concentration

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within 1 min. Although nicotine was eliminated rapidly from the blood stream, prolonged craving reduction was observed without administering another dose. Recently, a breath-activated MDI nicotine formulation known as Voke® nicotine MDI (without heating system) was reported to produce mean maximum arterial nicotine concentration of 3.73 ng/mL from a 0.45 mg dose to reduce smoking urges [5]. The AERx Essence System demonstrated the ability to rapidly deliver peak arterial nicotine levels in less than a minute that are comparable to those achieved during cigarette smoking, in contrast to the pharmacokinetic profile from the Voke® MDI which peaked after 6 –10 min whereas inhalation of nicotine from the AERx produced a rapid and prolonged reduction in craving for cigarettes for at least 2 h [6]. Although the Voke® MDI offers advantage over other systems, the formulation contains co-solvents ethanol and propylene glycol that may interact with the plastic components (polyoxymethylene) of the device.

E-cigarettes, a battery powered devices that are breath activated with a micro sensor and use heat or ultrasound to produce nicotine mist from a formulation where nicotine is suspended in propylene glycol or glycerine and water. The newer generation e-cigarettes with high voltage battery power and high temperature may cause thermal breakdown of the solvents to produce toxic chemicals like carbonyls. Although the popularity of this product is increasing; however, this is not a widely popular delivery system and the safety of inhaling nicotine from it has not been studied in humans [7] and thus the scientific evidence in terms of safety is limited. The appropriate dose of nicotine which is delivered using e-cigarette also remains unclear and this product has failed to regulate smoking related problems [8] so this product has been banned in some countries [9]. The effect of long-term use of e-cigarettes is not known and without valid clinical data, the marketing efforts of these products may increase addiction to nicotine, especially in young people. Although some data demonstrate that e-cigarettes may be effective in reducing nicotine cessation, there are no such clinical data demonstrating the efficacy of these products as an efficient tool to achieve the goal [10]. In addition the liquid dosage forms of these products are less stable compared to that of solid dosage form. At present there is no DPI formulation available to deliver nicotine into the deep lungs and studies have suggested that deep lung delivery of nicotine from DPI formulation would be an effective way to eliminate the craving for cigarettes and other tobacco products [1]. DPI formulations consist of either a carrier-based interactive mixture in which micronised drug particles (<5 μm) are mixed with the large carrier particles from where drug particles are detached from the carrier surface during inhalation; or agglomerates of nanoparticles that deagglomerate by inspiratory force and disperse into deep lungs. Therefore, a novel approach of nicotine delivery into deep lungs from DPI formulations has been researched as an effective smoking cessation strategy. This offers safer delivery compared to that of smoked tobacco where heat, carcinogens and carbon monoxide produce various adverse effects.

Chitosan (CS) is considered as a suitable carrier to deliver both small-molecule drugs and macromolecules with excellent bioavailability [11]. CS based micro- and nanoparticles for pulmonary drug delivery improved the therapeutic effect of drug by modifying the drug bioavailability [12] and, in addition, drug encapsulation in micro- and nanoparticles led to reduced toxicity [13,14] and prolonged the biological half-life of the drug [15]. Sustained release delivery of CS encapsulated insulin nanoparticles and terbutaline sulfate [16] from DPI formulation have been investigated. Very recently, the potential of CS and its derivative as a matrix for enhancing the dispersibility and prolonged release of diltiazem hydrochloride from nanoparticulate DPI formulation was demonstrated by our group [17].

In this study, we have used a biodegradable and biocompatible CS polymer as carriers for NHT as DPI formulation, which can deliver nicotine into lungs with sustained release properties. The reason behind selecting NHT is that this salt form of nicotine is more stable than nicotine base. The CS has been used in this study as it has low toxicity [14] and is compatible with respiratory epithelial cells. It is anticipated that the outcome of this study will guide the development of DPI formulations of pure nicotine with sustained release profile.

2. Materials and methods

2.1. Materials

Low molecular weight (LMW) CS with MW 50–190 kDa and degree of deacetylation of 92% was purchased from Sigma-Aldrich, Australia. 50% aqueous glutaradehyde was purchased from Merck, Australia as the crosslinker for the preparation of nanoparticles. Span 80 was obtained from PCCA, Australia as the emulsifier. Nicotine hydrogen tartrate salt powder (⩾98%) was purchased from Sigma-Aldrich, Australia. All other chemicals and reagents used were analytical grade.

2.2. Preparation of nanoparticles

2.2.1. Preparation of blank CS nanoparticles

Blank CS particles were prepared by using a modified W/O emulsion-glutaraldehyde crosslinking method [17]. In order to fabricate blank CS particles, LMW CS powder was dissolved in 2% v/v acetic acid to obtain the final concentration (2%) of CS solution with pH 3.5. About 1 mL span 80 was mixed with 100 mL paraffin oil under vigorous stirring to form a fine dispersion. 3 mL of 2% CS solution was added to paraffin oil containing span 80 drop-wise, with homogenization for 5 min using a Ultraturrax (IKA®) to obtain the W/O emulsion. Then, the mixture was continued stirring at 3000 rpm (IKA® Works (Asia), Inc) and the liquid mixture was continued to mix for 3 h with the addition of 0.2 mL 50% glutaraldehyde every hour for a period of three hours. Finally, the particles were separated by centrifugation at 11,000 rpm (Avanti J-26XP, Beckman Coulter, Inc.) for 10 min, after washing in hexane once and diethyl ether three times using centrifugation (Allegra® X-15R, Beckman Coulter, Inc.) at 4000 rpm for 10 min after each wash. The blank CS particles were obtained by freeze drying at −80 °C.

2.2.2. Preparation of NHT-loaded CS nanoparticles

For preparation of NHT-loaded CS particles, 2 mL of NHT solution in milli-Q water at different concentrations (i.e., 2%, 4%, 6% w/v) was added into 2 mL of 2% CS solution (dissolved in 2% acetic acid) before adding to paraffin oil containing span 80 and subsequent crosslinking with glutaraldehyde. This gave loadings which are referred in the following studies as 1:1; 2:1 and 3:1 ratios of NHT: CS. All other conditions were kept same as above.

2.3. Characterization of particles

2.3.1. Morphological studies

The freeze dried blank CS and NHT-loaded CS particles were examined by SEM (Zeiss Sigma VP Field Emission SEM) and TEM (JEOL 2100 200 kV TEM). A small amount of freeze dried powder was sprinkled onto a silicon wafer adhered to an aluminium stub through a carbon adhesive tape. The air dried specimen stubs were coated with a conductive layer of sputtered gold (argon gas pressure of 0.5 mbar, current of 30 mA, and coating time of 75 s), followed by observing secondary electron images under a high