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Nicotine–magnesium aluminum silicate microparticle surface modified with chitosan for mucosal delivery

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ABSTRACT

Magnesium aluminum silicate (MAS), a negatively charged clay, and nicotine (NCT), a basic drug, can interact electrostatically to form microparticles. Chitosan (CS) was used for the surface modification of the microparticles, and a lyophilization method was used to preserve the original particle morphology. The microparticles were characterized in terms of their physicochemical properties, NCT content, mucoadhesive properties, and release and permeation across porcine esophageal mucosa. The results showed that the microparticles formed via electrostatic interaction between MAS and protonated NCT had an irregular shape and that their NCT content increased with increasing NCT ratios in the microparticle preparation solution. High molecular weight CS (800 kDa) adsorbed to the microparticle surface and induced a positive surface charge. CS molecules intercalated into the MAS silicate layers and decreased the crystallinity of the microparticles, leading to an increase in the release rate and diffusion coefficient of NCT from the microparticles. Moreover, the microparticle surface modified with CS was found to have higher NCT permeation fluxes and mucoadhesive properties, which indicated the significant role of CS for NCT mucosal delivery. However, the enhancement of NCT permeation and of mucoadhesive properties depended on the molecular weight and concentration of CS. These findings suggest that NCT-MAS microparticle surface modified with CS represents a promising mucosal delivery system for NCT.

1. Introduction

Microparticles have been widely used as drug carriers and are often proposed as drug delivery systems for continuous, targeted, sustained or controlled release of active substances [1,2]. Microparticles can offer homogeneous and reproducible drug absorption, reduction of local irritation, and protection of active substances against enzymatic degradation [3]. Most microparticles have been fabricated using natural and synthetic polymers as the main components. Polymeric microparticles can have mucoadhesive properties, increasing the contact time with the mucosa and enhancing drug delivery efficiency [4,5].

Magnesium aluminum silicate (MAS) is a mixture of montmorillonite and saponite clays [6]. MAS presents a silicate-layered structure that is composed of two tetrahedral silicate sheets that sandwich an alumina or magnesia octahedral sheet [6,7]. MAS is non-toxic and non-irritating at the levels employed in pharmaceutical use [6]. Moreover, montmorillonite clays exhibit weak cytotoxicity and good adhesion to cell membranes [8,9]. The MAS silicate layers can be separated upon hydration. The silicate layers present a negative charge with a large surface area, enabling the adsorption of positively charged drugs. Recently, anionic clays have been used to adsorb drug molecules to enhance drug stability [10], reduce drug toxicity [11] and improve drug efficiency [12] because of the intercalation of drug molecules into the interlayer spaces of clays.

Nicotine (NCT) is one drug that can be adsorbed onto MAS particles, with adsorption mainly occurring via electrostatic interactions [13,14]. NCT is a diprotic base with $pK_{a1} = 3.04$ and $pK_{a2} = 7.84$, with diprotonated, monoprotonated and neutral species existing at acidic, neutral, and basic pH levels, respectively [15]. Rapid interaction between MAS and NCT under neutral and acidic pH conditions leads to the formation of NCT-MAS complex flocculates. The characteristics of NCT-MAS complexes that were prepared, dried and ground into small particles were previously investigated [14]. However, the drying and grinding process destroyed the particle morphology and changed the characteristics of the flocculate particles, which could instead be fabricated as microparticles. To better control and gain a better understanding of NCT-MAS microparticles, a lyophilization technique was used to maintain the original morphology of the NCT-MAS microparticles. Moreover, the NCT-MAS flocculates that formed at acidic and neutral pHs still had a negatively charged particle surface. Modifying the particle surface to exhibit a positive charge could enhance the mucoadhesive properties of these microparticles. The cationic polysaccharide chitosan (CS) was used for this purpose because of its mucoadhesive properties [16] and its ability to interact with and neutralize the previously

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reported negative charge of MAS [17]. Furthermore, CS has been widely used for producing mucosal drug delivery systems due to its biodegradability and biocompatibility [18,19].

This study presents the first report of NCT-MAS microparticles with and without CS surface modification. The microparticles were prepared by the electrostatic interaction between NCT and MAS at pH 4 and 7 and dried using lyophilization. Low and high molecular weight chitosan (LCS and HCS, respectively) were used for surface modification. The particle morphology, NCT entrapment efficiency, thermal behavior, crystallinity and mucoadhesive properties of the microparticles were investigated. Furthermore, NCT release and permeation across the porcine esophageal mucosa were examined to evaluate the potential application of these materials for mucosal delivery.

2. Materials and methods

2.1. Materials

MAS (Veegum® HV in the granular form) was obtained from R.T. Vanderbilt Company, Inc., USA. LCS (MW = 80 kDa) and HCS (MW = 800 kDa) with 85% deacetylation were purchased from Seafresh Chitosan (Lab) Co. Ltd., Thailand. NCT was obtained from Fluka, Switzerland. All other reagents used were of analytical grade and used as received.

2.2. Preparation of NCT-MAS microparticles

A MAS dispersion (1% w/v) was prepared by dispersing MAS powder in hot deionized water, and the pH of the MAS dispersion was adjusted to 4 or 7 using 2 M HCl. NCT solutions at 2% w/v in deionized water were also prepared at pH 4 or 7. To produce NCT-MAS microparticles, 12.5, 25, or 50 ml of 2% w/v NCT solution was added to 500 ml of 1% w/v MAS dispersion with stirring using a propeller at 300 rpm. The mixture was stirred for 1 h before adjusting the pH to 4.0 or 7.0 again using 2 M HCl or 2 M NaOH. Then, the final volume of the mixture was adjusted to 625 ml using deionized water, and the mixture was incubated for 24 h at 37 °C with shaking at 75 oscillations/min to achieve equilibrium NCT adsorption. Then, 10 ml of the mixture was collected to investigate the particle size and zeta potential of the wet microparticles. The microparticles were collected by vacuum filtration and washed twice with 25 ml of deionized water. The microparticles were subsequently dispersed again in 50 ml of deionized water, and the mixture was frozen at −20 °C and dried using lyophilization. After drying, the microparticles that passed through a 125-μm sieve were collected and kept in a desiccator until testing.

NCT-MAS microparticle surface modified with CS was prepared using the following method. Fifty milliliters of 2% w/v NCT at pH 4 were added to 500 ml of 1% w/v MAS dispersion at pH 4 with continuous stirring using a propeller at 300 rpm for 1 h. Subsequently, 12.5, 25 or 50 of 0.5% w/v HCS or LCS in 0.1 M HCl was gradually poured into the NCT-MAS dispersion with continuous stirring for 5 min before adjusting the pH to 4.0 using 2 M HCl or 2 M NaOH. Then, the final volume of the mixture was adjusted to 625 ml with deionized water and incubated for 24 h at 37 °C with shaking at 75 oscillations/min. The collection and drying processes were the same as those described above.

2.3. Particle size determination

The particle sizes of the MAS dispersions and NCT-MAS microparticles were measured using a laser diffraction particle size analyzer (Mastersizer2000 Model Hydro2000SM, Malvern Instrument Ltd., UK). The samples were dispersed in 70 ml of distilled water in a small volume sample dispersion unit and stirred at 3000 rpm for 30 s before the measurement. The volume weighted mean diameter was recorded.

2.4. Zeta potential measurement

The zeta potential of MAS and wet NCT-MAS microparticles was determined using a laser Doppler electrophoresis analyzer (Zetasizer Model ZEN 2600, Malvern Instrument Ltd., UK). The temperature of the samples was controlled at 25 °C. The samples were diluted prior to the measurement using deionized water to a count rate above 20,000 counts/s.

2.5. Scanning electron microscopy (SEM)

The particle shape and surface morphology of MAS and microparticles were observed using SEM. Samples were mounted onto stubs, coated with gold in a vacuum evaporator, and photographed using a scanning electron microscope (Jeol Model JSM-6400, Tokyo, Japan).

2.6. Differential scanning calorimetry (DSC)

DSC thermograms of samples were recorded using a differential scanning calorimeter (DSC822, Mettler Toledo, Switzerland). Each sample (2–3 mg) was accurately weighed into a 40-μl aluminum pan without an aluminum cover. The measurements were performed over 30–400 °C at a heating rate of 10 °C/min.

2.7. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of samples were recorded with an FTIR spectrophotometer (Spectrum One, Perkin Elmer, Norwalk, CT) using the KBr disc method. Each sample was pulverized, gently triturated with KBr powder at a weight ratio of 1:100 and then pressed using a hydrostatic press at a pressure of 10 t for 10 min. The disc was placed in the sample holder and scanned from 4000 to 450 cm^{-1} at a resolution of 4 cm^{-1}.

2.8. Powder X-ray diffractometry

The X-ray diffractograms of each sample were obtained on a powder X-ray diffractometer (Philips PW3710 mpd control, The Netherlands). The measurement conditions were an X-ray source of Cu radiation generated at 30 kV and 20 mA, angles from 1 to 30° 2θ and an angle step of 0.02° 2θ/s.

The thickness of the silicate MAS layer could be determined using Bragg's equation:

$$n\lambda = 2d \sin\theta \quad (1)$$

where n is 1 (the first order reflection), λ is the wavelength of the X-ray (1.54 Å), θ is the angle of the basal spacing peak of MAS, and d is the silicate layer thickness of MAS.

2.9. Determination of NCT content

Twenty milligrams of the microparticles were weighed and dispersed in 50 ml of 2 M HCl. The mixture was incubated at 37 °C in a shaking water bath for 24 h. Then, the supernatant was collected and filtered using a cellulose acetate membrane with a 0.45-μm pore size. The NCT content was analyzed using a UV–visible spectrophotometer (Shimadzu UV1201, Japan) at a wavelength of 259 nm. The NCT entrapment efficiency was computed according to the ratio of the actual drug content in the microparticles to the total drug included in the reaction.

2.10. In vitro NCT release studies

A modified Franz diffusion cell was used to characterize the release of NCT from the microparticles. The receptor compartment contained 5.3 ml of pH 6 phosphate buffer, and the temperature was controlled at 37.0 ± 0.1 °C with a continuous stirring speed of 600 rpm. A