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Electrosprayed 4-carboxybenzenesulfonamide-chitosan microspheres for acetazolamide delivery

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ABSTRACT

4-Carboxybenzensulfonamide-chitosan (4-CBS-chitosan) microspheres were prepared by electrospraying with acetazolamide (ACZ) as a model drug. The obtained 4-CBS-chitosan microspheres with or without ACZ-loading were characterized by Fourier transform infrared spectroscopy, differential scanning colorimetry, scanning electron microscopy and particle size analyses. The crystalline form and the stability of ACZ in a basic solution was determined using X-ray single crystal analysis. 4-CBS-chitosan had 90% encapsulation efficiency for ACZ compared to 47% of encapsulation efficiency (EE) obtained from native chitosan, forming 3.1 μm diameter microspheres with a low polydispersity index (0.4). After an initial burst release (58% in 5 min), ACZ-loaded 4-CBS-chitosan gave a sustained release of ACZ (~100% over 3 h) in simulated gastric fluid (0.1 N HCl; pH 1.2), which was better than that seen for the release from ACZ-loaded chitosan (44% over 1.5 h). Thus, 4-CBS-chitosan microspheres are a possible drug carrier in acidic conditions, such as at the gastric mucosal wall.

1. Introduction

Nowadays, the idea of using mucoadhesive micro-/nanoparticles from natural biodegradable polymers represents a promising drug delivery system. Mucoadhesive polymers capable of attaching at the mucosal surfaces may prolong the residence time and improve the specific localization of target drug absorption [1].

Chitin ((1-4)-2-amino-2-deoxy-β-D-glucan) can be partly deacetylated to form chitosan, a mucoadhesive polysaccharide [2] that has increasingly been used in the pharmaceutical application and food industries, due to its non-toxic, biocompatible and enzymatic biodegradable nature plus its antimicrobial and mucoadhesive properties [3]. Chitosan microspheres are a potential carrier for the controlled release of drugs, such as antibiotics, antihypertensive agents, anticancer agents, proteins, peptide drugs and vaccines [4]. 4-Carboxybenzensulfonamide covalently linked with chitosan (4-CBS-chitosan) has been reported to reveal a stronger mucoadhesion to mucin type II and a higher swelling behavior than the corresponding unmodified chitosan [5]. Moreover, it was found to be non-toxic in vitro to the Vero, KB, MCF-7 and NCI-H187 cell lines in tissue culture, but showed potential antibacterial activity against *Esherichia coli* and *Staphlyococcus aureus*.

Therefore, it is of interest if 4-CBS-chitosan microspheres suitable for drug delivery could be easily synthesized. In this work electrospraying (ESI) was used to prepare 4-CBS-chitosan microspheres due to the advantage of ESI in that it typically provides a uniform particle size with a narrow size distribution, and it is a simple, fast one-step technique [6].

Acetazolamide (ACZ) is an inhibitor of carbonic anhydrase with a weak diuretic activity that is used for the symptomatic treatment of glaucoma, epilepsy, benign intracranial hypertension, altitude sickness and in the therapy of gastric and duodenal ulcers [7]. However, ACZ does not remain concentrated in the circulatory system but is diluted in various body fluids and is subsequently absorbed from mucosal tissues leading to systemic toxic side effects [8]. One potential strategy to reduce these side effects and increase the therapeutic treatment is the encapsulation of ACZ in a mucoadhesive polymer delivery system to keep the drug concentrated and slowly released into the target organ. However, ACZ has a low solubility in water and organic solvents, leading to a low effective bioavailability and so requiring specialized pharmaceutical formulations and specific evaluation of its bioactivity in each formulation. Moreover, it has very poor compression properties making tablet formation difficult [9]. To improve the solubility of ACZ, it can be dissolved in 0.1 N aqueous ammonia solutions, but the bioactivity of ACZ depends on the solution pH [10] and no reports about ACZ in a base condition are available. Therefore, in this work we also investigated the crystalline form and the stability of ACZ in a base condition, after

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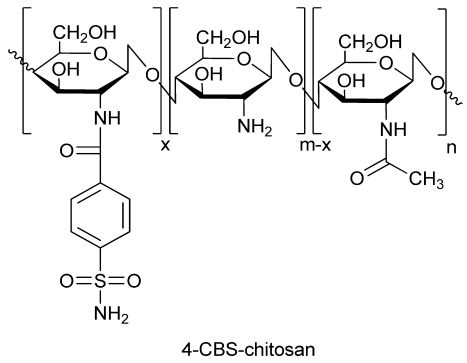

Fig. 1. Chemical structure of 4-CBS-chitosan.

being fabricated as ACZ loaded 4-CBS-chitosan microspheres, using X-ray single crystal analysis.

The aim of this work was to prepare ACZ-loaded 4-CBS-chitosan microspheres by ESI as a potential mucoadhesive carrier for the sustained release of ACZ at the gastric mucosal surface. Fourier transform infrared spectroscopy (FT-IR), differential scanning colorimetry (DSC) and scanning electron microscopy (SEM) analyses were used to characterize the microspheres.

2. Materials and methods

2.1. Materials

Chitosan with an average molecular weight (M_w) of >500 kDa was provided by Bonafide Co., Ltd., Thailand. The degree of deacetylation of chitosan was determined to be 81% by ^{1}H NMR. ACZ (99% purity), 4-CBS (99% purity), sodium tripolyphosphate (TPP) and 1-ethyl-3-(3-dimethylaminopropyl)carboiimide hydrochloride (EDAC) were purchased from Aldrich Co., USA and used without purification. Cellulose dialysis tubing (Membrane Filtration Products Inc., USA) with a molecular weight cut-off of 12–14 kDa was used to purify the modified chitosan. All other chemicals were obtained commercially as reagent grade and used as supplied.

2.2. Synthesis of 4-CBS-chitosan

4-CBS-chitosan (Fig. 1) was synthesized following the previously reported method [5]. Briefly, a mixture of 100 mL of 1% (w/v) of chitosan in 1% (v/v) acetic acid solution was prepared, and then 0.05 g 4-CBS and 0.05 g EDAC were added and refluxed for 6 h. Excess EDAC was removed by precipitation following the addition of 1 M HCl to the reaction mixture and then centrifugation. The supernatant was then neutralized with 1 N NaOH and the residual sodium acetate, NaCl, *o*-acylurea derivative and unreacted 4-CBS were eliminated by dialysis against three changes of 100 mL of ethanol. The reaction mixture was then filtered, washed with water and lyophilized at 198 mbar and −45 °C.

2.3. ACZ-loading of 4-CBS-chitosan microspheres

The particles of 4-CBS-chitosan were prepared by ESI using a high-voltage electrostatic system. 4-CBS-chitosan (1 g) was dissolved in 100 mL of l% (v/v) acetic acid and the solution placed in a syringe that was connected to a 26 gauge needle as the positive electrode of the electrostatic system. A negative electrode was placed on the grounded plate under the receiving beaker that was filled with 0.5 g ACZ dissolved in 15 mL of 0.1 N ammonia solution mixed with 25 mL of 10% (w/v) TPP. To form microsphere particles, the 4-CBS-chitosan solution was dropped into the TPP/ammonia solution at a flow rate of 2.5 mL/h, an applied voltage of 15 kV, a stirring rate of 400 rpm and an 8 cm distance between the needle tip and the negative electrode. 4-CBS-chitosan microspheres without ACZ-loading were prepared using the same method as mentioned above except omitting the ACZ. Likewise chitosan and ACZ-loaded chitosan particles were prepared as above except using chitosan in place of the 4-CBS-chitosan. The microspheres were collected by centrifugation (12,000 rpm, 25 min, RT) and washed by deionized water (DI) three times and air-died for 24 h.

2.4. Characterization of microspheres

2.4.1. Fourier transformed infrared spectroscopy (FT-IR)

FT-IR spectrophotometry (Perkin Elmer Spectrum RX-1 FT-IR system) was used to analyze the obtained chitosan, 4-CBS-chitosan, ACZ-loaded chitosan and ACZ-loaded 4-CBS-chitosan microspheres. The dried sample particles were mixed at 1% (w/w) with potassium bromide (KBr) and ground to a powder in an agate mortar and pestle. The mixture was then transferred to a hydraulic pressing machine and pressed into a disk. The sample was scanned from wavenumbers of 600–4000 cm^{-1}.

2.4.2. Differential scanning calorimetry (DSC)

Approximately 3–6 mg of each sample (chitosan, 4-CBS-chitosan, ACZ-loaded chitosan and ACZ-loaded 4-CBS-chitosan microspheres) were weighed in an aluminum pan, and then crimped-sealed for determination. DSC was performed with a NETZSCH DSC 204 instrument under a nitrogen atmosphere over a temperature range of 25–320 °C at a heating rate of 10 °C/min.

2.4.3. Scanning electron microscopy (SEM)

The anhydrous particle surface, shape and size were observed via SEM using a Philips, XL30CP model scanning electron microscope. Samples were dropped onto metal grids and coated by gold under vacuum before observation. Scanning was performed under high vacuum at an ambient temperature with a beam voltage of 10–20 kV. The photographs were taken at different magnifications.

2.4.4. Hydrated particle size measurement

The hydrated particle size, size distribution (polydispersity (PDI)) and zeta potential of the different types of particles were performed on a particle size analyzer (Zetasizer, Malvern instrument) using a He–Ne laser with 4.0 mW power at a 532 nm wavelength. Size calculation was based on dynamic light scattering (DLS) as a software protocol. The scattered light was collected at an angle of 90° through fiber optics and converted to an electrical signal by an avalanche photodiode array (APDs). The PDI was evaluated and then used as an indicator of the particle size distribution, where PDI values of less than 0.2 indicate a mono-dispersed emulsion whilst values above 0.5 show a larger particle size distribution [11]. All samples were sonicated and run in triplicate with the number of runs set to 5 and run duration set to 10 s.

2.5. Encapsulation efficiency (EE)

The dried ACZ-loaded chitosan and ACZ-loaded 4-CBS-chitosan particles (4 mg) were immersed in 10 mL ethanol at room temperature for 24 h. After stirring, the total ACZ content that had been entrapped inside and/or on the chitosan and 4-CBS-chitosan particles were released in the solution. Following centrifugation to remove the particles, the supernatant was collected and the ACZ content determined by UV–vis spectrophotometer at 266 nm. The