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Research paper

Design, synthesis and *in vitro* evaluation of mucoadhesive *p*-coumarate-thiolated-chitosan as a hydrophobic drug carriers

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ABSTRACT

A hydrophobic mucoadhesive thiolated chitosan for hydrophobic drug delivery was designed and prepared by conjugating *p*-coumaric acid (pCA) to increase hydrophobic compatibility with drug via pi–pi interaction and then covalently linking homocysteine thiolactone (HT) to the pCA-chitosan to increase the mucoadhesive properties. The degree of substituted phenolics in the modified chitosan was about 7.21 ± 0.05 mg gallic acid equivalents (GAE)/g. The pCA-HT-chitosan formed from a 24 h HT conjugation reaction time showed the highest yield of grafted thiol groups (~17.6 μmol/g) and the strongest mucoadhesive property, being about 10-, 2- and 1.6-fold more than that for the unmodified chitosan at pH 1.2, 4.0 and 6.4, respectively. Piperine (PIP) as a model hydrophobic drug was encapsulated in pCA-HT-chitosan microparticles via electrospray ionization with an encapsulation efficiency of over 80%. *In vitro* release studies showed a sustained release of PIP to >75% over 12 h between pH 1.2 and 6.4.

1. Introduction

Chitosan [β-(1-4)-2-amino-2-deoxy-D-glucose] is a partially deacetylated form of chitin, a naturally occurring linear biodegradable polysaccharide that it is made up of N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine. It is an important natural polymer exhibiting several favorable chemical and biological properties such as biocompatibility, biodegradability, antimicrobial activity, and its hydrophilic and mucoadhesive nature. Accordingly, chitosan has been used in a variety of biomedical fields [1]. Although the mucoadhesion of chitosan has been widely studied, the exact basis for its mucoadhesion properties remains unclear, but is based on the formation of non-covalent bonds, such as hydrogen bonds and ionic interactions with the mucus as well as van der walls and hydrophobic interactions.

Recently, it was shown that polymers with thiol groups provide much higher adhesive properties than polymers generally considered to be mucoadhesive. Thiolated polymers (thiomers) have been developed as a category of mucoadhesive polymers with reactive thiol groups immobilized on the polymeric structure. These thiolated polymers can tightly adhere to the intestinal mucus layer through covalent bonding with mucin glycoproteins via thiol-disulfide exchange reactions. Their applications have included a trimethyl chitosan–cysteine conjugate that showed an enhanced mucoadhesion and permeation and improved oral insulin delivery [2]. Thiolated chitosan (as chitosan–glutathione) coated poly(hydroxylethyl methacrylate) nanoparticles showed enhanced mucoadhesive properties [3]. Both chitosan–thioglycolic acid coupling [4], and thiolated chitosan poly(methacrylic acid) coupling can be used to form efficient biomaterials for biomedical applications, such as mucoadhesive drug delivery [5]. The covalent attachment of homocysteine thiolactone (HT) to chitosan and N,N,N-trimethyl-chitosan (TM-chitosan) resulted in strongly improved mucoadhesive properties and a good swelling behavior [6,7].

However, the poor interaction of these thiolated polymers with hydrophobic drug molecules results in a faster drug release with low drug efficiency entrapment, which will affect their potential applications in pharmaceutical fields. Hydrophobic mucoadhesive polymers for use in hydrophobic drug delivery systems have been developed, examples being thiolated carboxymethyl chitosan-*g*-β-cyclodextrin [8] and N-(4-N,N-dimethylaminocinnamyl) chitosan chloride [9], with improved mucoadhesive properties. Such hydrophobic mucoadhesive polymers may potentially become effective hydrophobic drug delivery systems with a controlled drug release capability.

Piperine (PIP), employed in this study as a model hydrophobic drug, is the major alkaloid of black and long peppers from the family Piperaceae. It has previously been evaluated for its potential to enhance the bioavailability of propranolol and theophylline [10]

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and the oral bioavailability of curcumin via co-administration of PIP and curcumin [11]. In order to reduce gastrointestinal irritation and enhance percent of drug encapsulation efficiency and increase with prolong release rate of PIP, new drug delivery carrier was designed, prepared and *in vitro* evaluation.

The aim of this work was to design hydrophobic mucoadhesive thiolated chitosan particles that may be suitable for the delivery of hydrophobic or low water solubility drugs with high drug entrapment and prolong release of drug. This was attempted by conjugating *p*-coumaric acid (pCA) onto the amino groups of chitosan to increase hydrophobic property and compatibility part with hydrophobic drug that will increase the drug entrapment efficiency. Subsequently, the pCA-chitosan was coupled to HT to increase its mucoadhesive properties to prolong the drug carried in the gastrointestinal tract. The degree of substitution (DS) of the phenolic and thiol groups in the modified chitosan was determined by the Folin–Ciocalteu's and Ellman's methods, respectively. Periodic Acid Schiff (PAS) was used for evaluation of the mucoadhesive property of chitosan and the modified chitosans. Chitosan and the modified chitosans were synthesized as microparticles by electrospray ionization, with or without encapsulation of PIP as a model hydrophobic drug, and assayed for their encapsulation efficiency (EE), size and shape, and *in vitro* release of the encapsulated PIP in three different pH media.

2. Materials and methods

2.1. Materials

Chitosan (viscosity average molecular weight 500 kDa, degree of N-deacetylation 95%) was provided by Bonafides Co. Ltd. (Thailand). pCA, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), HT, imidazole, mucin (type II) from porcine, iodomethane, N-methyl pyrrolidone, basic fuchsin (pararosaniline), sodium metabisulphite, periodic acid and lactic acid were obtained from Aldrich Co., USA and used without further purification. 5,5-Dithio-bis(2-nitrobenzoic acid), used as Ellman's reagent for quantitatively analyzing the thiol groups, and Folin–Ciocalteu's reagent, used for quantitatively analyzing phenolics, were purchased from Aldrich Co., USA. Dialysis tubing (Mw cut-off 12–14 kDa) was obtained by Membrane Filtration Products, Inc., USA. All other chemicals were commercially available.

2.2. Synthesis of pCA-chitosan

pCA-chitosan was prepared by coupling *p*CA onto the amino groups of chitosan using EDAC following the reaction scheme presented in Fig. 1. One gram of chitosan was dissolved in 100 mL of 1% (v/v) of lactic acid and then 0.2 g of *p*CA dissolved in minimum amount of ethanol was added at a mole ratio of 1:1.5. The mixture was stirred at 60 °C for 2 h, and then precipitated with excess 1 M NaOH and re-dissolved in water. The product was dialyzed against ethanol and air-dried.

2.3. Synthesis of pCA-HT-chitosan

The pCA-HT-chitosan was synthesized based on the method by [6,7]. Briefly, 100 mL of 1% (w/v) of pCA-chitosan (Section 2.2) in 1% (v/v) lactic acid was added to an aqueous solution of imidazole (0.68 g in 2.5 mL water), followed by the dropwise addition of HT (0.5 g in 100 mL water) and EDAC at a 1:2 mol ratio of HT: EDAC. The reaction mixture was stirred at 60 °C in a nitrogen atmosphere for 12, 24 or 48 h. The reaction mixture was then adjusted to pH 7 with 1 M NaOH and precipitated with excess acetone. The pellet was re-dissolved in water and harvested by centrifugation (12,000 rpm for 2 min), dialyzed (Mw cut-off 12–14 kDa) against 1 L of water (2×) for two days and freeze-dried. The products were stored at 4 °C and were found to be stable toward air oxidation during the course of the study.

2.4. Preparation of chitosan and modified chitosan microparticles

The chitosan and modified chitosans' (pCA-chitosan and pCA-HT-chitosan) particles were prepared by electrospray ionization using a set up voltage of 23 kV, 10 cm working distance, 400 rpm stirring rate, 26 G needles and a flow rate 5 mL/h. The solution of 1% (w/v) chitosan or modified chitosan in 1% (v/v) lactic acid was electrosprayed into 50 mL of a 5% (w/v) aqueous solution of sodium tripolyphosphate (TPP) as a coagulant to form microparticles. The particles were then separated by centrifugation at 50,000*g* for 30 min, washed with water three times and then freeze dried for 24 h.

For preparation of microspheres encapsulated with PIP, as a model hydrophobic payload drug, 1%, 3% or 5% (w/w) PIP was added to 1% (w/v) chitosan or the modified chitosan in 1% (v/v) lactic acid and stirred overnight. The particles were then prepared using electrospray ionization under the same conditions as for the drug-free particles.

2.5. Characterization of chitosan and its derivatives

2.5.1. ^{1}H nuclear magnetic resonance spectroscopy (NMR)

^{1}H NMR spectra of chitosan, pCA-chitosan and pCA-HT-chitosan in 1% (v/v) trifluoroacetic acid (CF_3COOH) in D_2O were recorded on a Varian NMR spectrometer operated at 400 MHz.

2.5.2. Fourier transformed infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was used to identify the chemical structure of chitosan and the modified chitosans (pCA-chitosan and pCA-HT-chitosan). The FTIR spectra were measured with a Nicolet 6700 spectrophotometer in the region of 4000–400 cm^{-1}.

2.5.3. Thermogravimetric analysis (TGA)

The thermal degradation behavior of the chitosan, pCA-chitosan and pCA-HT-chitosan samples was studied using TGA analysis. TGA analysis was performed on a Perkin Elmer Pyris Diamond TG/DTA machine under a nitrogen flow rate of 30 mL/min.

2.5.4. Scanning electron microscope (SEM)

The morphology and surface appearance of the spheres (before and after the drug loading) were analyzed by scanning electron microscopy (SEM). The sample was mounted onto an aluminum stub using double-sided carbon adhesive tape and coated with gold–palladium. Coating was achieved at 18 mA for at least 4 min. Scanning was performed under high vacuum and ambient temperature with a beam voltage of 10–20 kV.

The zeta potential, size and size distribution (polydispersity or PDI) of the different types of particles were evaluated by dynamic light scattering (DLS) on a particle size analyzer immediately after sonication-mediated suspension of the microspheres in water. The scattered light was collected at an angle of 90° and all samples were run in triplicate with the number of runs set to five and run duration set to 10 s.

2.6. Determination of the net amount of phenol groups

The phenolic content in the modified chitosans was estimated by the Folin–Ciocalteu method reported by Medina [12]. Briefly, a sample of modified chitosan was prepared at 1 mg/mL in 1% (v/v) lactic acid solution, and then 20 μL of sample was transferred