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Preparation of chitosan-ferulic acid conjugate: Structure characterization and in the application of pharmaceuticals

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ABSTRACT

A novel drug delivery system based on chitosan derivatives was prepared by introducting ferulic acid to chitosan adopting a free radical-induced grafting procedure. This paper used an ascorbic acid/hydrogen peroxide redox pair as radical initiator. The chitosan derivative was characterized by Fourier transformed infrared (FTIR), Ultraviolet-visible spectrum (UV), Differential scanning calorimetry (DSC), X-ray diffraction (XRD) and Electron microscopic scanning (SEM). What is more, preparing microcapsules with the chitosan conjugate as wall material, the drug release propertie of chitosan conjugates were compared with that of a blank chitosan, which treated in the same conditions but in the absence of ferulic acid. The study clearly demonstrates that free radical-induced grafting procedure was an effective reaction methods and chitosan-ferulic acid is a potential functionalized carrier material for drug delivery.

1. Introduction

Chitosan, a deacetylated derivative of chitin, is a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine. Chitosan is the second most abundant polysaccharide found in nature after cellulose. The sugar backbone consists of β-1,4-linked glucosamine [1], which has been known as a bioactive molecule. Chitosan has been reported that it has many bioactivity such as antitumor activity [2], immunoenhancing effects [3], wound healing effects [4], antifungal and antimicrobial properties [5], and antioxidant activity [6]. These characteristics, together with several unique properties such as biocompatible, biodegradable, non-toxic and non-antigenic properties, has widen the applications of chitosan in many fields including biomedicine, waste water treatment [7], cosmetics and food industries [8] or packaging film [9]. However, the solubility of chitosan is poor and it is only soluble in some dilute acid solutions, which greatly limits its applications. In order to improve its solubility and widen its applications, more attention has been paid to the modification of chitosan by physical and chemical modification such as acylation reactions, graft copolymerization, physical and enzymatic methods [10,11]. Among these modification methods, graft copolymerization has been applied most extensively [12].

Biofunctional chitosan derivatives, resulting from graft copolymerization of phenolic acids with chitosan are of recent interest to chitosan chemistry [13,14]. Recently, many phenolic antixodants such as gallic, caffeic and ferulic acids have been grafted onto chitosan in order to improve the water solubility and antioxidant activity of chitosan [15–19]. These biomaterials have been promoted as multifunctional packaging materials [20] and novel antioxidants [21]. What is more, phenolic acids have been proposed as a potential treatment for various disorders such as Alzheimer's disease, cancer, cardiovascular diseases, diabetes mellitus and skin disease [22,23]. This is because that grafting phenolic acids on chitosan may provide these compounds with the much needed stability and also aid their slow release. Some literature has reported that chitosan-gallic acid conjugate has anti-diabetic potential [24]. Science researchers has demonstrated synthesis of four phenolic acid-grafted chitosan derivatives with improved grafting ratio, antioxidant activity and broad spectrum antimicrobial activity against food spoilage bacteria [25] and they observed that phenolic acid grafted chitosan derivatives may find potential application in functional food development [26]. But we have not found some reports about these phenolic acid grafted chitosan derivatives applicating in the pharmaceutical field.

The aim of this study was to obtain ferulic acid grafted chitosan derivatives by an eco-friendly method and evaluate their drug release activity in vitro and in vivo. First, ferulic acid was grafted onto chitosan by a free radical mediated grafting method. This paper use ascorbic acid (Vc) and hydrogen peroxide (H_2O_2) redox pair system under nitrogen. Then, the synthesized ferulic acid grafted chitosan was characterized by UV–vis, Fourier-transform infrared (FT-IR) and thin plate chromatography analysis to confirm

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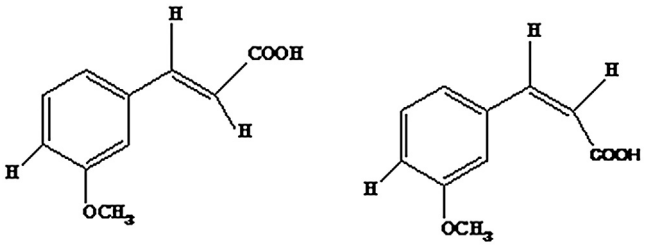

Fig. 1. Chemical structure of ferulic acid.

the conjugation. Thermal behavior and crystallographic structure of the ferulic acid grafted chitosan derivatives were determined by differential scanning calorimetry (DSC) and X-ray diffraction (XRD). Finally, the drug release activity in vitro of the ferulic acid grafted chitosan derivatives was determined. This study provides novel structural performance and in vivo drug release data for ferulic acid grafted chitosan. This study further expands the application of chitosan derivatives in pharmaceutical researchs.

2. Materials and methods

2.1. Materials

Ferulic acid (Fig. 1) was purchased from Shanghai yuanye Bio-Technology Co., Ltd, Chitosan (degree of deacetylation, 95%) and Folin-Ciocalteu reagent were obtained from Beijing lanbosite Bio-Technology Co., Ltd. Hydrogen peroxide (H_2O_2), ethanol and glacial acetic acid were provided from Kelong Chemical Reagent Factory in Chengdu. Ascorbic acid was bought from Sinopharm chemical reagent Co., Ltd. Chinese Medicine Group Chemical Reagent Co., Ltd.

2.2. Synthesis of chitosan-ferulic acid conjugates

The synthesis of chitosan-ferulic acid was performed by using Vc and H_2O_2 redox pair under inert atmosphere according to our previously reported method [18]. 10 g of chitosan was dissolved in 800 mL of 1% acetic acid solution (v/v) in a 1 L three-necked round bottom flask. Then, 20 mL of 1.0 M H_2O_2 containing 1.2 g of ascorbic acid was added dropwise to the chitosan solution. The reaction vessel was then flushed with nitrogen for 30 min with continuous stirring, followed by addition of 5 g ferulic acid, which was dissolved in 100 mL ethanol. Finally, the reaction was carried out at 30 °C under a continuous flow of oxygen free nitrogen gas for 24 h. The obtained polymer solution was dialyzed against distilled water with a cutoff membrane (MWCO 8000–14,000 Da) for 72 h to remove unreacted ferulic acid. The dialyzate was spray dried (inlet temperature 140 °C, outlet temperature 77 °C) in a pilot plant spray drier to afford individual derivatives. Blank chitosan, which act as a control, was prepared in the same conditions but in the absence of ferulic acid.

2.3. Estimation of grafted phenolic group on chitosan by folin-ciocalteu procedure

Quantification of grafted phenolic groups on chitosan was determined using Folin-Ciocalteu reagent procedure, according to the literature with some modifications [25]. Briefly, 1 mg of chitosan-ferulic acid conjugates was dissolved in distilled water (4 mL) in test tubes. Folin-Ciocalteu reagent (1 mL) was added to the sample solution, and the contents of the test tubes were mixed thoroughly. After 3 min, followed by addition of 3 mL of Na_2CO_3 (2%) solution, and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm against a control (without polymer under the same reaction conditions). The amount of total phenolic groups in each polymeric materials was expressed as ferulic acid equivalent concentrations. The standard curve of ferulic acid was made under the same reaction condition, the corresponding ferulic acid equivalent was calculated from the linear regression equation.

2.4. Characterization of chitosan-ferulic acid conjugates

To verify whether ferulic acid was grafted onto chitosan, TLC analysis was performed. Ferulic acid, plain chitosan, and chitosan-ferulic acid were developed on a silica gel plate by development with chloroform-ethyl acetate-acetic acid (5:5:1) as mobile phase [14]. The developed TLC plate was observed using an ultraviolet lamp. UV–vis absorption spectras of plain chitosan and chitosan-ferulic acid were detected with a UV-2802S spectrophotometer. Samples were dissolved in deionized water respectively, UV–vis absorption spectra were recorded in full scan mode from 200 to 400 nm. FTIR spectra were carried out using a Nicolet 6700 spectrometer as KBr pallets over a spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

To investigate the physical-chemical properties, thermal behavior and crystallographic structure of chitosan-ferulic acid were also determined. Thermal properties of chitosan and chitosan-ferulic acid were investigated using a ZRY-2P high temperature synthetic thermal analyzer (Shanghai Precision Science Co., Ltd., China) in alumina pans. Samples were heated from 30 to 800 °C at a constant rate of 10 °C/min under a nitrogen flow of 50 mL/min. XRD spectra were acquired from $2\theta = 10–80^\circ$ with a DX-2700AX X-ray diffractometer.

2.5. Microencapsulation of BSA

BSA were encapsulated following spray drying technique using ferulic acid-grafted chitosan as wall material according to our previously reported method with slight modifications [26]. The wall material was prepared following optimized protocol, starting with10 g of chitosan and 5 g of ferulic acid. The dialysate was mixed with BSA (0.15 g) in deionized water (100 mL) followed by homogenization at 15,000 rpm for 20 min. Then, the mixture was spray dried (inlet temperature 140 °C, outlet temperature 77 °C) to afford microparticles of encapsulated BSA.

2.6. In vitro release study of microencapsulation

In vitro release behavior of BSA from the encapsulated particles was studied. Briefly, 50 mg of the samples were placed inside 10 mL test tubes and were incubated in 4 mL of sodium phosphate buffer of pH 7.4 containing lysozyme (107 U/L) at 37 °C with gentle shaking (100 rpm). At specific intervals, 100 μL of the release medium was took out and replaced by pre-warmed fresh medium. The release medium was analyzed by coomassie brilliant blue method using a UV–vis spectrophotometer at 595 nm to quantify released BSA. All results were the mean of three samples.

3. Results and discussion

3.1. Synthesis of ferulic acid-chitosanConjugates

Ferulic acid-chitosan was successfully synthesized by free radical mediated reaction. By estimating the grafted phenolic group on chitosan by Folin-Ciocalteu procedure, we concluded that the mass ratio of chitosan and ferulic acid was 1:0.5 (w/w) and the reaction lasts for 24 h at 30 °C. The conjugation of the ferulic acid on the chitosan chains was performed by using a “Hydrogen peroxide-Ascorbic acid” redox initiator system. The interaction mechanism