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Preparation of acetylsalicylic acid-acylated chitosan as a novel polymeric drug for drug controlled release

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ABSTRACT

The acetylsalicylic acid-acylated chitosan (ASACTS) with high degree of substitution (DS) was successfully synthesized, and characterized with FTIR, ^{1}H NMR and elemental analysis methods. The optimum synthesis conditions were obtained which gave the highest DS (about 60%) for ASACTS. Its drug release experiments were carried out in simulated gastric and intestine fluids. The results show that the drugs in the form of acetylsalicylic acid (ASA) and salicylic acid (SA) were released in a controlled manner from ASACTS only in simulated gastric fluid. The release profile can be best fitted with logistic and Weibull model. The research results reveal that ASACTS can be a potential polymeric drug for the controlled release of ASA and SA in the targeted gastric environment.

1. Introduction

Chitin derivatives are non-toxic, biodegradable and biocompatible, and therefore can be a good candidate as drug carriers or tissue engineering scaffolds in pharmaceutical, biological, medical and biochemical applications [1]. Chitosan (CTS) can be derived and produced by the deacetylation of chitin [2]. CTS has been found to possess the advantages of in-vivo biodegradability, immune antibacterial property and wound-healing activity [3]. In recent years, a large number of research works have been focused on the CTS-based drug carriers for drug delivery applications. For example, Dai et al. produced the nifedipine-loaded N-succinyl CTS/alginate hydrogel bead and studied the controlled delivery of nifedipine [4]. Zhang et al. synthesized a series of taxol-loaded CTS derivative micelles, which contained a much higher taxol concentration (2.01 mg/mL) than that in water (0.001 mg/mL) [5].

Acetylsalicylic acid (ASA), also known as aspirin, is a non-steroidal drug with antipyretic, anti-inflammatory and analgesic effects [6]. However, the oral intake of a high dose of ASA may bring about undesirable side effects including gastric ulcer, gastric bleeding, tinnitus, etc [7]. It would be better if the functions of "targeted release" and "controlled release" of ASA can be combined onto one ASA-containing drug, so that ASA would be gradually released to form a proper drug concentration in plasma, neither too high to poison nor too low to malfunction.

The combination of CTS as a drug carrier and the drug ASA would be a good candidate as a polymeric drug for the research and development of the "targeted" and "controlled" release of ASA. However, the CTS-based ASA-containing polymeric drug has seldom been reported, to the best of our knowledge. The salicylic acid (SA), which is the precursor for ASA, has been reported to be blended (entrapped or incorporated) into CTS and its derivatives in the forms of fibers, microspheres and nanoparticles, for drug delivery experiments [8–11]. Nevertheless, problems still remain in these SA-CTS blend systems. Firstly, the drug release was often difficult to be controlled, and often lack of pH sensitivity which may favor the targeted release of the drugs. For example, Boonsongrit et al. synthesized the SA-loaded CTS micro/nanoparticles and nearly all SA was released in one burst within 10 min, in both acidic and neutral solutions [8]. Such a release profile may introduce a high SA concentration in plasma in a relatively short period in both the gastric and intestine environment, which was lack of the controlled and targeted drug release features. In addition, Wang et al. produced a SA-loaded CTS/starch fiber for SA release investigation, and found that the SA release rate was fast at pH 1.0 and much slower at pH 7.4 [9]. Although a targeted SA release from the fiber can be achieved in the gastric environment at pH 1.0, the fast release profile may probably result in a high concentration of SA in the stomach in a short period and cause undesirable side effects. Secondly, SA possesses much higher stimulating effect to the stomach than ASA, and therefore it is necessary to replace SA with ASA in the polymeric drug systems.

In this paper, a novel polymeric drug was synthesized with the introduction of ASA onto CTS macromolecules, forming

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acetylsalicylic acid-acylated chitosan (ASACTS) with three main features. Firstly, instead of being blended (entrapped or incorporated) into CTS, the drug ASA was introduced onto the CTS polymer chain via the amidation reaction to form the ASACTS polymeric drug. Therefore, the controlled drug release can be realized via the gradual hydrolysis of the –NHCO– and –OCO– linkage on ASACTS, instead of a fast release of the drug in one burst in a short period. Secondly, the targeted drug release can be realized with the ASACTS polymeric drug as its drug release only occurred at pH 1 in the targeted gastric environment, and no drug release was observed in the intestine environment. Thirdly, the ASACTS polymeric drug was able to release both ASA and SA drugs, decreasing the stimulating effect of the SA to the stomach. The three main features of ASACTS would allow a gradual release of the drug only in gastric environment, forming a proper drug concentration in plasma. Therefore, the purpose of this work is to synthesize a novel polymeric drug for the targeted and controlled release of ASA and SA. The produced polymeric drug was characterized with ^{1}H NMR, FTIR and elemental analysis methods, and used for drug release experiments. It was found that the polymeric drug possessed a high ASA grafting efficiency and was able to release ASA and SA in a controlled manner in simulated gastric environment.

2. Experimental

2.1. Materials

CTS (AR) was produced by Shenzhen Brightway Biomaterials Tech Co Ltd. ASA (AR) was purchased from TCI Chemical Industry Co., Ltd. (Shanghai). SA (AR) and 1-hydroxybenzotriazole (HOBt, AR) were derived from J&K Scientific Co Ltd. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, AR) was purchased from Nanjing Debiochem Co., Ltd. N,N-dimethylformamide (DMF, AR) was derived from Tianjin Yongda Chemical Reagent Co., Ltd. All other reagents were of analytical grade, and DI water was used throughout the experiments.

2.2. Preparation of acetylsalicylic acid-acylated chitosan

With the typical method of amidation reaction between the amine and carboxylic groups to form the amide group [12,13], ASACTS was prepared with CTS as a carrier, ASA as a small-molecule drug, EDC and HOBt as the condensation dehydrants, and DMF as the solvent. Briefly, the CTS solution was prepared by dissolving 0.25 g of CTS into 15 mL of aqueous hydrochloride acid (HCl) solution (0.1 mol/L), and the solution was subsequently adjusted to pH 5 by the addition of aqueous sodium hydroxide (NaOH) solution (0.1 mol/L). The ASA solution was prepared by successively dissolving a series of predetermined amounts of ASA, EDC and HOBt (the weight ratio of ASA to EDC to HOBt was fixed to 1:1:0.8) into 20 mL of DMF, and the solution was subsequently kept in the refrigerator at −20 °C for 1 h. Then, the prepared CTS and ASA solution were mixed together, with the addition of 1 mL of pyridine. The solution mixture was allowed for the dehydration reaction for a series of predetermined reaction time at room temperature, until the sticky pinky suspension was derived. The mild reaction conditions (e.g., pH adjusted to the value of 5; dehydration at room temperature) were used in order to prevent the decomposition of ASA into SA during the reaction. The polymer in the suspension was precipitated by the addition of 200 mL of absolute ethanol. The solid was then filtrated via a Buchner funnel, washed with 100 mL of DI water twice, finally filtrated and dried in a vacuum oven at 80 °C overnight to obtain the final product. For the selection of the best reaction conditions for the synthesis of ASACTS, the weight ratio of CTS to ASA (in the range of 0.25–1.00) and the reaction time (in the range of 12–48 h) were selected as two influencing factors. The experimental design is shown in Table 1 which is based on the comprehensive experimental design method. Two factors (ratio 1 (the weight ratio of CTS to ASA) and reaction time), each with three levels (ratio 1: 0.25, 0.50, 1.00; reaction time: 12, 24 and 48 h) are designed in Table 1, with the total of 9 experiments (ASACTS samples named as F_1 to F_9). The criterion for selecting the final synthesis conditions of ASACTS is dependant on the highest value of the degree of substitution (DS).

Table 1
Synthesis conditions and the corresponding DS of ASACTS.

Sample name	Ratio 1	Reaction time (h)	N (%)	C (%)	DS (%)
F_1	1.00	12	5.36 ± 0.12	45.52 ± 0.32	41.48
F_2	0.50	12	5.22 ± 0.08	47.68 ± 0.25	49.80
F_3	0.25	12	5.32 ± 0.05	46.99 ± 0.24	45.89
F_4	1.00	24	5.24 ± 0.10	45.31 ± 0.12	43.48
F_5	0.50	24	4.86 ± 0.09	48.21 ± 0.16	59.98
F_6	0.25	24	4.92 ± 0.11	47.40 ± 0.23	56.28
F_7	1.00	48	5.07 ± 0.02	45.87 ± 0.37	48.67
F_8	0.50	48	5.08 ± 0.14	48.86 ± 0.10	57.02
F_9	0.25	48	4.94 ± 0.07	47.47 ± 0.08	55.96

Note: Ratio 1 denotes the weight ratio of CTS to ASA.

2.3. Characterization

2.3.1. Elemental analysis

The carbon (C) and nitrogen (N) contents of CTS and ASACTS samples (F_1 to F_9) were determined by elemental analysis using a CHN analyzer (Vario EL cube, Elementar, Germany), and the analyses were repeated for three times. Each sample with the weight range of 2.000–3.000 mg was wrapped in a tin vial, and dropped automatically into the combustion tube of the analyzer for the analyses of C, N and H contents.

2.3.2. Determination of degree of deacetylation (DD) of CTS

CTS is the deacetylated chitin, with a large portion of amine ($-NH_2$) groups. Therefore, the $-NHCOCH_3$ and $-NH_2$ groups are both present on the polymeric chain of CTS. As the DD of CTS is representative of the percentage portion of the $-NH_2$ groups, the relationship between DD and the molar ratio of carbon (C) to nitrogen (N) of CTS can be expressed as:

$$\frac{6 \times DD + 8 \times (1 - DD)}{1} = \frac{C\%/12}{N\%/14} \quad (1)$$

where C% and N% are the weight contents (%) of C and N elements in CTS respectively, that were derived from the elemental analysis. In addition, the constant coefficients 6 and 8 denote the number of C atoms in the two repeated units of CTS containing $-NH_2$ and $-NHCOCH_3$ groups, respectively. The DD of CTS was thus derived to be 95.53% from Eq. (1).

2.3.3. Determination of degree of substitution (DS) of ASACTS

ASACTS possesses three repetitive units, containing acetylsalicylic acid amide (ASAA), $-NH_2$ and $-NHCOCH_3$ groups, respectively, as shown in Fig. 1. As the introduction of ASA onto CTS was carried out through the amidation between the ASA and the $-NH_2$ groups of CTS, the percentage portion of the repetitive unit containing $-NHCOCH_3$ was unchanged and fixed to the value of 1 − DD (4.47% as calculated from Eq. (1)). As the DS of ASACTS was representative of the percentage portion of ASA-substituted amine groups, i.e., the portion of the repetitive unit containing ASAA, the relationship