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Synthesis of oxidized glycerol monooleate-chitosan polymer and its hydrogel formation for sustained release of trimetazidine hydrochloride

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ABSTRACT

In this paper, a lipid material glycerol monooleate was used as the starting material to synthesize the oxidized glycerol monooleate (OGMO). OGMO was subsequently linked to chitosan (CS) via imine bonds (—C═N—) to obtain a new chitosan-based polymer (OGMO-CS), which can form hydrogels rapidly in aqueous media. Scanning electron microscopy, swelling behavior studies and degradation kinetics studies were performed to demonstrate the effect of this synthetic modification on the hydrogels formation of chitosan network and in vitro drug release. The effects of OGMO-CS type, dry hydrogels percentage, release media and drug loading on the sustained release of the model drug trimetazidine hydrochloride were evaluated. The release profiles of the hydrogels could be described by the Peppas–Sahlin mechanism, a combination of Fickian diffusion and Case-II relaxation. Based on the fact that numerous pharmaceutical lipids are available, the present study may pave the way for other lipids to be employed as modifiers of chitosan for more innovative chitosan derivatives with versatile properties and pharmaceutical applications.

1. Introduction

Hydrogels are three-dimensional cross-linked polymer network that exhibits the ability to swell in aqueous solutions to many times their original volume (Peppas et al., 2000). Their affinity to absorb water is attributed to the presence of hydrophilic groups such as —OH, —$CONH_2$, —COOH and —SO_3H within the molecular structure (Peppas and Khare, 1993). Hydrogels will swell instead of being dissolved in aqueous solutions due to the critical cross-links present in the hydrogel structure. Fully swollen hydrogels have excellent biocompatibility and low toxicity because of their similar physical properties to living tissues (Kim et al., 2008).

Chitosan (CS) is a cationic polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units (Chandy and Sharma, 1990). It has a highly stable crystalline structure that can only be dissolved in dilute acids (pH ≤ 5.0) through the protonation of the free amino groups but cannot form hydrogels spontaneously in the acidic solution. The reactive amino groups and the hydroxyl groups in CS can be either physically associated (Berger et al., 2004) or chemically cross-linked (Hennink and van Nostrum, 2012) to some functional reagents such as PEG (Kulkarni et al., 2005), Konjac Glucomannan (Yu et al., 2007), sodium hexametaphosphate (Gupta and Jabrail, 2006), starch (Tang et al., 2003), hyaluronic acid (Tan et al., 2009) or carboxylic compounds (Lu et al., 2007) to obtain many new derivatives, which possess versatile properties and have been investigated as drug delivery vehicles.

In the present study, a rarely reported lipid modifier for CS was investigated to offer the groundwork for further lipids use in modifying CS, based on the present knowledge on the diversity and safety of lipids, as well as their comprehensive applications in pharmaceutics (Akoh, 2005; Wasan, 2006). Glyceryl monooleate (GMO) was used as a novel starting material for the preparation of CS-based pharmaceutical hydrogels. GMO is a polar amphiphilic lipid that can form liquid crystalline phases depending on the water content and temperature (Engstrom, 1990). In the presence of excess water, GMO forms gels with high viscosity, known as the cubic phase. However, the water uptake of GMO is very slow, with a swelling ratio of lower than 1.5% in 12 h. Its swelling is strongly dependent on the temperature and the initial water content of GMO (Lee et al., 2003). Once contact with water, the surface of GMO produces a viscous gel, which prevents further immersion of water and

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makes GMO very difficult to be handled. All of these restrict the use of GMO and its formation of hydrogels.

Ganguly et al. used CS and GMO to prepare an in situ gel with and without a cross-linker (glutaraldehyde) (Ganguly and Dash, 2004). It is conceivable that it is hard to prepare the gel due to the gel formation of GMO alone in aqueous media and the resultant high viscosity, even with the help of a prolonged period of sonication. Besides, this system would produce reversible gel formation and imprecise control of drug release because the network formation by this interaction was purely physical (Bhattarai et al., 2010). With the addition of glutaraldehyde, the gel properties and drug release were enhanced (Ganguly and Dash, 2004). However, this toxic cross-linker may be of great concern for oral delivery (Bhattarai et al., 2010).

In this paper, we synthesized oxidized glycerol monooleate (OGMO) from GMO and subsequently used OGMO as a novel modifier to prepare new OGMO-CS polymers with different degrees of substitution and OGMO-CS based hydrogels. The effect of OGMO-CSs on the physicochemical and release profiles of the new matrix was also investigated. Trimetazidine hydrochloride (TMH), an effective anti-ischemic agent which has the marketed sustained release tablets (Marzilli, 2003), was used as the model drug.

2. Materials and methods

2.1. Materials

Chitosan (CS, MW = 1×10^4, degree of deacetylation = 85.46%) was purchased from Jinan Haidebei Marine Bioengineering Co., Ltd. (Shandong, China). Glycerol monooleate (GMO) was kindly gifted by Danisco A/S Co., Ltd (Copenhagen, Denmark). Trimetazidine hydrochloride (purity of 99.7%) was purchased from Hubei-Sihuan Pharmaceuticals Co., Ltd. Sodium periodate was provided by Guangdong Guanghua Chemical Factory Co., Ltd. (Guangdong, China). Sodium triacetoxyborohydride (STAB-H, analytical grade) was purchased from Henan Wanxiang Technology & Trade Co., Ltd. (Henan, China). Acetic acid, N,N-dimethylformamide (DMF), ethanol, sulfuric acid, hydroxylammonium chloride and other agents were analytical grade.

HepG2 cell lines were purchased from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. HepG2 cells were grown in the Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated calf serum (Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China), 100 U/ml penicillin G and 100 U/ml streptomycin (pH7.4), in a water jacketed CO_2 incubator with a humidified atmosphere of 5% CO_2 at 37 °C.

2.2. Synthesis of oxidized glyceryl monooleate (OGMO)

OGMO was synthesized by oxidizing GMO with sodium periodate (Scheme 1a), a compound with high selectivity for oxidative cleavage of vicinal glycols (Perlin, 2006). GMO (10 g) was first dissolved in absolute ethanol to obtain 50 ml of solution. 25 ml of distilled water was added. After stirring for 1 h, 0.5% sulfuric acid solution was added drop-wise to adjust the pH to 4.5. 50 ml of sodium periodate aqueous solution (18%, w/v) was added to the mixture and stirred at 45 °C for 3 h in the dark. OGMO was then collected by separating the oily upper phase from the reaction solution.

2.3. Synthesis of oxidized glycerol monooleate-modified chitosan (OGMO-CSs)

OGMO-CSs were obtained by introducing OGMO into the N-terminal of glucosamine units of chitosan through one-pot reductive amination (Scheme 1b). STAB-H, a mild and selective reducing agent, was used for the reductive amination of ketones and aldehydes (Abdel-Magid and Mehrman, 2006) in the synthesis process. 2 g of CS was suspended in 100 ml of acetic acid solution (1%, v/v) and stirred until it was completely dissolved. On the basis of the molecular weight (10,000), the number of monomers (59) and the deacetylation of CS (85.46%), 2 g of CS is equivalent to 0.01 mol of amine group. Sodium hydroxide (0.5 mol/l) was added slowly to adjust the pH (approximately 12), resulting in the precipitation of expanded CS. The oyster white suspension was then filtered. The expanded CS on the filter membrane was subsequently transferred into a flask containing 50 ml of DMF. OGMO was added to the flask and the pH of the mixture was adjusted to approximately 6.5 using acetic acid. After stirring for 6 h at ambient temperature, STAB-H was added slowly (the molar ratio of STAB-H to OGMO = 1.4:1). The reaction continued for another 24 h. The filtered cake, OGMO-CS, was collected and fully washed with absolute ethanol to completely eliminate the free OGMO. About 0.33 g (equivalent to 0.001 mol aldehyde group), 0.65 g, 0.97 g and 1.62 g of OGMO were used in the synthesis to obtain OGMO-CS1, OGMO-CS2, OGMO-CS3 and OGMO-CS4, respectively, by varying the molar ratios of amine group in CS to aldehyde group in OGMO from 10:1, 5:1, 10:3–2:1.

2.4. Characterization of OGMO, OGMO-CSs

2.4.1. Determination of the oxidized degree of OGMO

The oxidized degree (OD) of OGMO was determined by measuring the molar weight of the aldehyde groups applying the hydroxylamine hydrochloride potentiometric titration, which has been proved to be reliable without using a standard (Zhao and Heindel, 1991). After drying in a vacuum oven at 25 °C to constant weight, hydroxylamine hydrochloride (8.75 g) was dissolved in 100 ml of 70% ethanol solution and 3.0 ml of methyl orange reagent (0.05%) was then added. The solution was diluted to 500 ml with 70% ethanol solution to obtain hydroxylamine hydrochloride solution (0.25 mol/l). 0.45 g of OGMO was dissolved in 25 ml of the hydroxylamine hydrochloride solution and stirred at room temperature in the dark for 2 h. Afterwards, each sample was titrated with standardized sodium hydroxide solution until the red-to-yellow end point was achieved. Simultaneously, pH changes were recorded. The equivalent volume could be determined more precisely by the first derivative of the titration curve. The oxidation product was measured in triplicate. The OD of OGMO was calculated by:

$$\mathrm{OD}(\%) = \frac{V_{\mathrm{NaOH}} \times N_{\mathrm{NaOH}} \times 10^{-3} \times MW_{\mathrm{OGMO}}}{W_{\mathrm{sample}}} \times 100$$

where V_{NaOH} and N_{NaOH} are the equivalent volume and the concentration of the standardized sodium hydroxide solution, respectively. MW_{OGMO} is the molecular weight of OGMO. W_{sample} is the weight of the oxidation product added in the hydroxylamine hydrochloride potentiometric titration.

2.4.2. Elemental analysis

The degree of substitution (DS) of OGMO-CSs was estimated using the automatic elemental analyzer Vario EL III (Elementar Analysensysteme GmbH., Hanau, Germany).

2.4.3. Fourier transforms infrared spectroscopy (FTIR)

FTIR was performed to confirm the formation of a new modified chitosan by OGMO. The FTIR data of OGMO, CS and OGMO-CSs were collected by using Tensor-27 Infrared Spectroscopy (Bruker, Germany) over the wavenumber range of 4000–400 cm^{-1}. Each

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