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Preparation and properties of a novel thermo-sensitive hydrogel based on chitosan/hydroxypropyl methylcellulose/glycerol

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ABSTRACT

Chitosan-based thermosensitive hydrogels are known as injectable in situ gelling thermosensitive polymer solutions which are suitable for biomaterials. In this study, a novel thermosensitive hydrogel gelling under physiological conditions was prepared using chitosan together with hydroxypropyl methylcellulose and glycerol. Hydroxypropyl methylcellulose is to facilitate the thermogelation through large amounts of hydrophobic interactions. Glycerol in heavy concentration destroys the polymer water sheaths promoting the formation of the hydrophobic regions, and lowering the phase transition temperature. The thermosensitive hydrogels showed a physiological pH ranging from 6.8 to 6.9 and gelation time within 15 min at 37 °C. The prepared hydrogels were characterized by FT-IR, XRD, SEM, and rheological studies, mechanical studies and contact angle studies. The properties of degradability, cytotoxicity and protein release behaviors of the hydrogels were investigated. The results indicate this thermosensitive hydrogel possess good fluidity, thermosensitivity and biodegradability, as well as low-cytotoxicity and controlled release, showing the potential use in biomedical applications.

1. Introduction

Thermosensitive hydrogel, which flows freely as injectable liquid before administration and gels under physiological conditions, has attracted much attention in the field of biomedical applications [1–4]. The thermosensitive system composed by nature polysaccharide polymer, particularly, chitosan (CS). CS has been aroused general attention due to its advanced features, such as simple preparation, good biocompatibility, and biodegradability [5–7].

Chitosan, the partly acetylated (1–4)-2-amino-2-deoxy-ß-D-glucan, has been widely used in a drug delivery system and tissue engineering for its favorable biological properties [8–11]. Chitosan combined with ß -glycerophosphate disodium (ß-GP) to prepare thermosensitive hydrogel was first reported by Chenite in the year of 2000 [12]. The effective interactions responsible for the sol/gel transition mainly include chitosan interchain hydrogen bonding, chitosan-glycerol-phosphate electrostatic attractions, and chitosan-chitosan hydrophobic interactions. These interactions are the main driving force for the chitosan/ß -GP heat-induced gelation [13]. The gelation temperature is gradually decreased with increasing of the concentration of chitosan and ß-GP. Though increasing chitosan concentration is difficult because of its poor solubility and high viscosity in aqueous solution, apt to cause concentration-induced gelation. The formed hydrogels containing low concentration (always <2%) of chitosan are likely to collapse without chemical modification or crosslinking [14–17]. It is noted that hydroxypropyl methylcellulose (HPMC) a polysaccharide derivative is water-soluble, pH stable, biodegradable, biocompatible and especially, thermoreversible, being a good candidate for the thermosensitive hydrogel.

HPMC is non-ionic and linear polysaccharide obtained by modifying the hydroxyl position in cellulose, which is widely used in the formulation of foods, cosmetics and pharmaceuticals [18–21]. HPMC can undergo a thermo reversible sol-gel phase transition on heating resulting from dehydration of hydrophobically substituted regions of the polymer chains [22]. Though the gelation temperature of HPMC above 60 °C is much higher than the body temperature 37 °C, it has been recognized that some ions and molecules can depress the phase transition temperature [23,24]. And it was found that glycerol in heavy concentration can separate HPMC out of the solution at normal temperature. As a polybasic alcohol, glycerol reduces the phase transition temperature by removing water from the polymer hydration sheath and lowering the temperature at which hydrophobic interactions between polymer chains becomes

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Table 1
Compositions and characteristics of CS/HPMC/Gl solutions.

Samples No.	CHG_0	CHG_{16}	CHG_{28}	CHG_{32}
CS (wt%)	1	1	1	1
HPMC (wt%)	5	5	5	5
Glycerol (wt%)	0	16	28	32
pH value	6.82	6.85	6.90	6.90
LCST (°C)[a]	56	48	37	32
Gelation time (min)[b]	–	–	15	10

[a] LCST was determined by the Visual Tube Inversion Method.
[b] Gelation time was measured at 37 °C.

favorable [24]. Actually, glycerol depresses the phase transition temperatures of HPMC solutions to the body temperature at some ratios.

In this study, a thermosensitive hydrogel based on chitosan was prepared by introducing another water-soluble polysaccharide derivative HPMC and glycerol. The thermosensitive hydrogel is novel with high solid content of polysaccharide polymers and without chemical modification. The properties of the thermosensitive hydrogel including molecular structures, degrees of crystallinity, rheological behaviors and internal-structure morphologies were characterized using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), rheometer and scanning electron microscopy (SEM). Degradability, cytotoxicity and protein release behaviors were also investigated for the thermosensitive hydrogel.

2. Materials and methods

2.1. Materials

Chitosan (MW100KDa-300KDa, degree of deacetylation is 85%) was purchased from Chendu Konglong Chemical Reagent Factory (Sichuan, China). Hydroxypropyl methylcellulose (methoxyl: 28–30%, hydroxyl: 7.0–12%, 6 mPa s) was obtained from Aladdin industrial Corp. (Shanghai, China). All other chemicals were reagent grade and used as received.

2.2. Preparation of CS/HPMC/Gl solutions

Chitosan powders were blended with HPMC powders at first. Then, the mixture was gradually added into 0.1 M acetic acid at 50 °C and stirred at room temperature until the transparent solution obtained. 2 M sodium hydroxide was used to adjust the pH of the solutions to 6.8. Thereafter, different amounts of glycerol (Gl) were added drop-wise to the solutions under continuous stirring to prepare sample solutions (Table 1). The characteristics of sample solutions were shown in Table 1.

2.3. Lower critical solution temperature determination

The gelation temperatures of formulations were determined referring to the "Visual Tube Inversion Method" [25]. Briefly, glass vials containing 3 g sample incubated in a thermostatic water bath. The temperature was increased at a ramp rate of 1 °C and kept steady for 10 min. Afterwards, the sample surfaces were observed by tilting the vials. The steps had been repeated until the sample solutions stopped flowing on tilting. The temperatures were noted as the lower critical solution temperature (LCST) of the sample solutions (Table 1). The gelation time of the solutions were measured at 37 °C (Table 1).

2.4. Characterization

Infrared spectra (IR) were recorded on a Speclmm GX FT-IR spectrometer (GE, USA). The lyophilized samples were subjected to KBr press to obtain pellets. The measurement was in the range of 4000–500 cm^{-1} with a resolution of 4 cm^{-1}.

The structure of gels was observed using scanning electron microscope (JSM 6500LV, Japan). The thermal gelled sheets of the formulation casting on the silver papers were frozen in liquid nitrogen for 5 min and immediately, moved to vacuum freeze dryer (Lyoquest, Telstar, Spain) lyophilized at −50 °C over 24 h. The samples were fixed on adhesive carbon tapes and gold coated. They were also studied by using SEM with the accelerating voltage of 10 kV.

The XRD pattern of samples were obtained using powder XRD (Shimadzu XRD-7000 X-ray Diffractometer, Cu Kα radiation) at room temperature under a voltage of 40 kV. The sample was placed in liquid nitrogen for 4 h and ground to fine powder with a mortar and pestle before subjecting it for XRD analysis. The samples were scanned at 2θ angle range of 6°–50° at a speed of 2° min^{-1}. Then, the degree of crystallinity (Xc) of the film was calculated by:

$$Xc = (Fc/(Fc + Fa)) \times 100$$

where Xc represents the degree of crystallinity, Fc represents the areas of crystalline regions and Fa represents the areas of amorphous regions.

2.5. Rheology

Rheological measurement of the solutions was carried out by using DHR-1 rheometer (TA Instruments, USA) accompanied by a 40 mm diameter parallel geometry. The formulations were placed between parallel plats with a diameter of 40 mm, a gap of 1000 um and equilibrated at 20 °C for 4 min. The solutions were heated from 20 °C to 80 °C at a ramp rate of 2 °C/min and at a frequency of 3 Hz. Their storage modulus (G') and loss modulus (G") were measured as functions of temperature.

2.6. Mechanical analysis

The mechanical behavior of thermal hydrogels was tested by using TexturePro CT1.5 (Brookfield Engineering Labs. Inc., USA) equipped with TA5 prober. The samples were compressed by 3 mm at a loading rate of 0.5 mm/s with a pre-load of 3 g.

2.7. Contact angle

Static contact angle measurements were conducted with a sessile drop method using a Dataphysics OCA 15EC instrument (Germany) at the room temperature and 18.2 MΩ water as the probe liquid. Contact angle measurements were taken at exactly 10 s after placement of 3 μL droplets on the surface. The reported values were the averages of three consecutive measurements for each sample.

2.8. In vitro degradation

The in vitro degradation of the thermosensitive hydrogel CHG32 was performed under simulated physiological conditions. Briefly, 6 samples of 1 mL CHG32 was lyophilized and weighed (W0) and divided into 2 groups. One group was immersed in 2 mL PBS (pH 7.4), another group was in 2 mL PBS (pH 7.4, containing 1 mg/mL of lysozyme). Subsequently, all the samples incubated at 37 °C with medium refreshing daily. At designed time intervals, the samples were carefully withdrawn from the degradation medium followed