Preparation and Properties of Thermo-sensitive Hydrogels of Konjac Glucomannan Grafted N-Isopropylacrylamide for Controlled Drug Delivery

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A thermo-sensitive hydrogel designed for controlled drug delivery was prepared and its properties were studied. The hydrogel was made of konjac glucomannan (KGM), copolymerized with N-isopropylacrylamide (NIPAAm) and cross-linked by N,N-methylene-bis-(acrylamide) (BIS). Several key parameters influencing the equilibrium swelling ratios of the hydrogels were studied. The swelling ratio was mainly controlled by the content of BIS, and influenced by the ratio of NIPAAm/KGM, slightly. It was possible to control the degree of swelling of the gels by changing cross-linking density of the polymer. The gels swelling ratio has possessed sensitive response to the environmental temperature. In vitro release of model drug bovine serum albumin (BSA) was studied at 20ºC and 37ºC in phosphate buffer solution (PBS) of pH 7.4. The accumulative release percentages are nearly 80% and 30% after 5 h at 20ºC and 37ºC, respectively.

INTRODUCTION

Many studies have been focused on stimuli-responsive hydrogel systems that show a phase transition in response to changes in environmental conditions such as temperature, pH, specific ions, and electric field [1,2]. Among all studied intelligent hydrogels, temperature and pH-responsive systems have drawn much attention, since temperature and pH values are important environmental factors in body and some diseases states manifest themselves by changes in temperature and/or pH of human body [3-5].

Konjac glucomannan (KGM) is a high-molecular weight water-soluble non-ionic polysaccharide and is extracted from tubers of Amorphophallus konjac plant in...
large quantities. It is a linear random copolymer consisting \( \beta \) (1, 4) linked \( D \)-mannose and \( D \)-glucose where the composition ratio of mannose to glucose is 1.6:1. This copolymer has some branching points at the C-3 position of the mannoses where an acetyl group is attached to one per 19 sugar residues. It shows such abilities as lowering of blood cholesterol and sugar level, helping with weight loss, and promoting intestinal activity, and immune function.

At the same time, this polysaccharide can be easily prepared into various derivatives because of its good biocompatibility and biodegradable activities. The deep development and exploitation of konjac glucomannan and its derivatives have been paid great attention in recent years [6]. The Food Chemicals Codex only lists the current uses of konjac flour in the United States as a gelling agent, thickener, film former, and emulsifier.

However, the studies on the applications of konjac glucomannan and its derivatives have extended much further from food and food additives to various fields such as pharmaceutical and bio-technical [7,8]. Poly \((N\)-isopropylacrylamide) hydrogel, a typical thermo-sensitive hydrogel exhibits a reversible volume phase transition at its lower critical solution temperature (LCST) 32–34ºC.

At temperatures lower than the LCST, the hydrogel swells, whereas at temperatures higher than LCST, it dehydrates to the collapsed state due to the release of all the bounded water molecules. This phase transition is reversible, and it can be modified by polymerizing the \( N \)-isopropylacrylamide monomer with more hydrophilic or more hydrophobic monomers [9-12].

In this paper, a novel hydrogel composed of konjac glucomannan (KGM) copolymerized with \( N \)-isopropylacrylamide was reported for the first time. The influence of several parameters on the equilibrium swelling ratios of the hydrogels was investigated and the in vitro release of the model drug bovine serum albumin (BSA) of the hydrogels was also studied at 20ºC and 37ºC in phosphate buffer solution (PBS) of pH 7.4.

**EXPERIMENTAL**

**Materials**

Konjac glucomannan (KGM) was purified by extracting the refined konjac powder (purity of 95%, Chengdu Root Industry Co. Ltd., Sichuan, China) in Soxhlet apparatus with benzene-ethanol (4:1, v/v), then with chloroform-ethanol (5:1, v/v). \( N \)-isopropylacrylamide (NIPAAm, Wujing Chemical Co. Ltd., Shanghai, China) was recrystallized in \( n \)-hexane before use. Bovine serum albumin (BSA; fraction V, Mw of 68 000 Da), ceric ammonium nitrate (CAN) and \( N,N \)-methylene-bis(acrylamide) (BIS) were purchased from Sigma and used without any further purification.

**Synthesis of Hydrogels**

Samples of KGM (0.162 g), some amounts of NIPAAm, and BIS were dissolved in 25 mL water for 12 h at room temperature, and then the mixture was cooled in ice water bath. Before and after the addition of the 1 wt% ceric ammonium nitrate in water solution, the mixture was bubbled with nitrogen for at least 10 min to discharge oxygen. Then, the reaction mixture was stirred for 10 min and maintained in ice water bath for 6 h and then maintained at 30ºC for 3 h. After reaction, the gels were cut into cubic shape samples, immersed in double distilled water for several times to guarantee the removal of the un-reacted monomers, homopolymers, and other small molecules. Then, the gels were dried below 60ºC to constant weight and stored for further use.

**Fourier Transform Infrared Spectroscopy Analysis**

The KGM and the hydrogels samples A1 and A5 were analyzed by FTIR spectrometer to confirm the grafting reactions. FTIR Spectra were obtained on Perkin–Elmer-2 spectrometer (KBr, pellet).

**Scanning Electron Microscopy**

For SEM analysis, the dry gels were immersed in PBS of pH 7.4 at 20ºC for 24 h. Then, the obtained hydrogels were freeze-dried. The samples were coated with gold–palladium for 70 s in an argon atmosphere before observing under the microscope (HITACHI X-650, Japan).

**Studies of Gels Swelling Properties**

The known weight samples of dry gels \((W_0)\) were immersed in the swelling medium. At certain time intervals, the gels were removed from the swelling medium, blotted each sample with filter paper to remove excess water from the their surfaces, and the weight of the swollen hydrogels \((W_1)\) was weighed. The swelling ratio (SR) was calculated as follows:
When the obtained Swellin Ratic did not change with the immersed time it was then considered as the equilibrium swelling ratio. The influence of temperature on the swelling behaviour of gels was studied by immersing the gels in buffer solutions of 20ºC and 40ºC and the changes of swelling ratio of gels with time from the beginning to the equilibrium state were also studied at these temperatures.

**Estimation of the Drug Release In Vitro**

The weighed dry gels were equilibrated in 30 mg BSA/10 mL of deionized water at 25ºC for 24 h to load BSA into the gels, and then the obtained hydrogels were weighed and freeze-dried. The drug release experiments were carried out by transferring the above lyophilized gels into 25 mL PBS of pH 7.4 and 20ºC or 37ºC, respectively. The gels were repeatedly removed and transferred into 25 mL fresh PBS at predetermined time intervals. The released drug was analyzed by the Bradford method [13] at 595 nm by a visible spectrophotometer.

**RESULTS AND DISCUSSION**

**Synthesis of Hydrogels**

The copolymerization of KGM with \(N\)-isopropylacrylamide was initiated by Ce(IV) via free-radical polymerization. Ce(IV) is a common initiator which can effectively induce saccharide units to generate free-radical sites [14-16] to react with \(N\)-isopropylacrylamide monomers to form graft copolymer. The hydrogels were obtained by incorporation of a cross-linker such as MBAAm. The mechanism of gel formation is shown in Scheme I. In order to optimize hydrogel synthesis and to find the relation between the reaction conditions and the swelling ratio of resultant hydrogels, the effects of the concentrations of \(N\)-isopropylacrylamide and MBAAm were investigated on the water uptake of gels (Tables 1 and 2). The SR was influenced more significantly by the density of BIS than by the ratios of KGM and \(N\)-isopropylacrylamide.

**FTIR Characterization of KGM and Hydrogels**

IR spectrum of KGM (Figure 1) indicates that the absorption band of carbonyl of acetyl groups is at 1737 cm\(^{-1}\), and the band at 1639 cm \(^{-1}\) is related to the intra-molecular hydrogen bonds [7,17]. When comparing the IR spectra of the hydrogels A1 and A5 with the spectrum of KGM, the intensity of some peaks are changed due to interaction or superposition of the peaks. The mechanism of gel formation is shown in Scheme I.

**Table 1. Influence of molar content of NIPA on hydrogels swelling ratio (SR).**

<table>
<thead>
<tr>
<th>Entry</th>
<th>*KGM:NIPAm molar ratio</th>
<th>SR(20ºC pH: 7.4) (mean±s.d. n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1 : 2.5</td>
<td>12.5 ± 0.2</td>
</tr>
<tr>
<td>A2</td>
<td>1 : 5</td>
<td>13.5 ± 0.1</td>
</tr>
<tr>
<td>A3</td>
<td>1 : 10</td>
<td>15.9 ± 0.3</td>
</tr>
<tr>
<td>A4</td>
<td>1 : 5</td>
<td>13.0 ± 0.3</td>
</tr>
<tr>
<td>A5</td>
<td>1 : 20</td>
<td>14.0 ± 0.2</td>
</tr>
<tr>
<td>A6</td>
<td>1 : 25</td>
<td>13.7 ± 0.3</td>
</tr>
<tr>
<td>A7</td>
<td>1 : 30</td>
<td>14.0 ± 0.2</td>
</tr>
</tbody>
</table>

KGM (0.162 g), \(H_2O\) (25 mL), molar ratio: BIS/NIPAm=1/125, CAN (5 mmol/L). (*) The molar content of KGM is calculated based on monosaccharide unit.

**Table 2. Influence of molar content of BIS on hydrogel’s swelling ratio (SR).**

<table>
<thead>
<tr>
<th>Entry</th>
<th>*NIPA: BIS molar ratio</th>
<th>SR(20ºC pH:7.4) mean±s.d. n=3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>500 : 1</td>
<td>19.1 ± 0.5</td>
</tr>
<tr>
<td>B2</td>
<td>250 : 1</td>
<td>16.1 ± 0.4</td>
</tr>
<tr>
<td>B3</td>
<td>125 : 1</td>
<td>12.4 ± 0.4</td>
</tr>
<tr>
<td>B4</td>
<td>67 : 1</td>
<td>11.3 ± 0.2</td>
</tr>
<tr>
<td>B5</td>
<td>33 : 1</td>
<td>10.9 ± 0.3</td>
</tr>
</tbody>
</table>

KGM (0.162 g), \(H_2O\) (25 mL), NIPAAm (0.4 mol/L), and CAN (5 mmol/L).
related to the groups of \( N \)-isopropylacrylamide, and KGM. It can be seen that peaks at 1651(1655) cm\(^{-1}\), 1547(1545) cm\(^{-1}\), 1458 cm\(^{-1}\), and 1385 cm\(^{-1}\) can be attributed to the characteristic peaks of amide I, amide II, methylene in (–CH\(_2\)–CH\(_2\)–), and methyl group in –CH(CH\(_3\))\(_2\), respectively. The above analysis indicated that the \( N \)-isopropylacrylamide was successfully grafted to the chains of KGM.

**Scanning Electron Microscope**

The SEM photographs of lyophilized gels B1, B2, B3 and B4 are shown in Figure 2a-2d, respectively. The pores were linked well to each other. The average pore size diameter of the lyophilized gels is more than 350 \( \mu \)m in B1, about 300 \( \mu \)m in B2 and less than 250 \( \mu \)m in B3 and B4. The pore size decreased significantly...
from B1 to B4 due to the increasing cross-linking densities of the hydrogels. Since more cross-linker has been used, then more bridges between the polymers chains have been formed.

**Gels Swelling Studies**

From Figure 3, it is clear that when the temperature was below 30°C the gel swelled well. The swelling ratio decreased slowly with temperature elevation from 20°C to 30°C and decreased sharply from 30 to 35°C. The phase transition temperature of hydrogels was about 33°C which can be obtained from Figure 3. Such temperature-dependent properties of the hydrogels come from the nature of poly (N-isopropylacrylamide) segment in the hydrogel network. When the temperature of swelling medium was lower than its LCST, the chains of poly(N-isopropylacrylamide) became hydrophilic, thus, the gel swelled well. But, when the temperature raised higher than its LCST, the chains of poly (N-isopropylacrylamide) became hydrophobic and shrunk significantly. Therefore, the gels swelling ratio decreased sharply.

The swelling kinetics of the hydrogel was studied in PBS of pH 7.4 at 20°C and 40°C, respectively. The time to achieve the equilibrium swelling was about 31 h for most samples (Figure 4). The different swelling ratios owed to the different cross-linking densities of the hydrogels.

To evaluate the re-swelling ability and the thermo-sensitive of the hydrogels, the gels were put in PBS of pH 7.4 at 20°C, and then transferred to 40°C. Such operations were repeated for several cycles. The gels were incubated at one temperature for 24 h before being transferred to another temperature. Figure 5 indicates that SR values have almost remained unchanged at 20°C or 40°C. The results have shown that the hydrogel has good re-swelling ability and maintain its response to temperature.
In Vitro Drug Release

The percentages of the drug loaded in the hydrogels of B1- B4 were 4.47, 3.54, 2.76, and 2.34 wt%, respectively. It is found that this was dependent on the cross-linking density and decreased with the increase in cross-linking density.

The drug release was investigated using BSA-loaded hydrogels in PBS of pH 7.4 and 20ºC and 37ºC, respectively. There is an obvious bursting release at the initial stage at both temperatures (Figure 6). At 37ºC, the bursting release maybe mainly due to desorption of BSA from the gels surface layer (most of the BSA was covered by the hydrophobic chains). While, at 20ºC, both the chains of poly (N-isopropylacrylamide) and KGM were hydrophilic. Thus, the BSA can diffuse easily from the network through the channels formed by pores. In the Figure 6, the obvious difference of the release percentage existed between different release temperatures. For example, the release percent is above 80% at 20ºC and about 30% at 37ºC after 5 h. After the initial bursting release, the BSA releasing became mild.

From Figure 6, the BSA release was also affected slightly by the pore size which was mainly influenced by cross-linking densities of the hydrogels, especially at 20ºC. This may be because the BSA can diffuse faster from the network through large pores than the small ones.

CONCLUSION

The konjac glucomannan grafted N-isopropylacrylamide was successfully obtained by the CAN initiated. The SR of the hydrogel can be controlled by using different BIS densities. The obtained hydrogels demonstrated excellent thermo-sensitivity. The gel can swell well at low temperature (<33ºC), and the swelling gel shrank significantly when transferred to higher temperature (>33ºC). The release of the model drug was well controlled by the temperature, but just influenced slightly by the cross-linking density of the gels. For this shortcoming, further study was needed to improve the release profile. Since, all procedures used in the preparation of the hydrogels with drugs were performed in aqueous medium, which may preserve and promote the bioactivity of protein drugs, it could be a potential polymeric carrier for site-specific bioactive protein drug delivery system.

REFERENCES

delivery, Polymer, 46, 6274-6281, 2005.