Preparation and application of dialdehyde pullulan for the construction of gelatin hydrogels

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Abstract. In this work, dialdehyde pullulans with different aldehyde contents were successfully prepared with periodate oxidation method and used as cross-linking agent for the construction of gelatin hydrogels. It was found that aldehyde contents can be varied by changing pH of the aqueous phase. The highest oxidation degree as well as the content of dialdehyde groups was found at pH 4.0. The mechanical properties of the gelatin hydrogels were significantly improved with the addition of dialdehyde pullulan. The fascinating reinforcing performance of the dialdehyde pullulan extends the potential application of hydrogels in the biomedical area.

1. Introduction

Hydrogels have been widely studied since their discovery and were rapidly developing field of research. A large number of synthetic and natural material hydrogels have been reported in the literature [1]. However, it still remains a challenge to obtain cross-linked nature polymer networks with well-defined properties, such as mechanical property.

Gelatin, a degradation of collagen in connective tissue such as animal skin, bone, is a widely used material to form hydrogels for many biomedical applications. Gelatin is a natural polymer material with good biocompatibility and biodegradability, and its structure was similar to that of living organisms. At the same time, it is a natural water-soluble biodegradable polymer material, which has the advantage that the degradation products were easily absorbed without generating inflammatory reaction. When applying the degradability of gelatin, it was often chemically modified to regulate its degradation rate to suit different needs [2]. Unfortunately, although gelatin has many advantages, gelatin-based materials have some major limitations in their application due to their low mechanical properties, poor repeatability, narrow range of structure and performance adjustments as well as low thermal stability [3]. Hence, many attempts such as physical blending, physical or chemical cross-linking and compounding methods have been made to improve the poor properties of gelatin [4].

The selection of pullulan as biomedical material is justified on the basis of the blood compatibility and biodegradation properties of the polysaccharide [5-6]. Pullulan is a linear polysaccharide which is produced by the yeast-like fungus Aureobasidium pullulans [7]. The pullulan was oxidized by sodium periodate to obtain polysaccharide containing aldehyde groups. At present, more and more attention has been paid to oxidized polysaccharides as an ideal reinforcing agent. The aldehyde group on the modified polysaccharide can react with the ε-amino group of the lysine or hydroxylysine side groups protein to form Schiff base structure, thereby forming crosslinked network. In the case of the addition...
of sodium periodate, the C2-C3 bond on the pullulan chain cleaves under oxidation and forms aldehyde groups [8]. In the work of this article, oxidized pullulan having different aldehyde contents was successfully prepared by periodate oxidation. The compressive strength of the gelatin hydrogels was significantly enhanced after oxidized pullulan cross-linking.

2. Experimental selection

2.1. Materials
Pullulan (biochemical reagent) manufacturer was Macklin Biochemical Science and Technology Co., Shanghai, China. Sodium periodate (analytical reagent) was obtained from Yongda Chemical Reagent Co., Tianjin, China. Hydroxylamine hydrochloride and other chemical agents were all analytically pure and used as received.

2.2. Sodium periodate oxidation of pullulan
To a solution of pullulan (1g) in 50 ml pure water, 0.877g (4.1mmol) sodium periodate was added. Then, adjusted pH of the aqueous phase from 2.0 to 5.0 using buffer solutions. The mixture was gently stirred in the dark for 6 h at 25 °C. The mixture was subjected to dialysis (molar mass cut-off is 1000 g·mol⁻¹) against frequently changed double-distilled water for 48 h after the oxidation procedure. Finally, the resulting polymer solutions were freeze-dried and stored at 4 °C before use. The oxidized pullulans are named as OP1, OP2, OP3 and OP4 for the solutions with a defined pH of 2, 3, 4, and 5, respectively.

2.3. Synthesis of cross-linked gelatin hydrogels
The polymer solution with 15 wt. % gelatin was dissolved in distilled water at 45 °C in a water bath under constant stirring. The oxidized pullulan solution (1.5 wt. %) was added to the gelatin solution at 45 °C under stirring condition until sticky. After ultrasonic treatment to remove the bubbles, the solution was immediately poured into the glass tube in 4 °C refrigerator condensation molding for 12 hours. The obtained hydrogels are named as Ge-OP1, Ge-OP2, Ge-OP3 and Ge-OP4.

2.4. Determination of aldehyde groups by alkali consumption
The degree of oxidation of pullulan was evaluated by measuring the aldehyde content. The oxidized pullulan was converted to hydrazine by reaction of a Schiff base with hydroxylamine hydrochloride. The specific procedure is as follows: 0.3 g of oxidized pullulan was dissolved in 25 ml of deionized water, and the pH was adjusted to 5.0 by sodium hydroxide (NaOH) solution. To the oxidized pullulan solution was added 20 ml of hydroxylamine hydrochloride at pH 5. Then the two are thoroughly stirred and mixed for 4 hours at 40 °C, then immediately titrated the solution with 0.1 mol/L NaOH to completely react with hydrochloric acid. The volume of hydrochloric acid consumed NaOH solution in the sample after the reaction is recorded as \( V_c \). The volume of NaOH solution consumed after the pure pullulan reaction under the same conditions was recorded as \( V_b \) which used as a blank control. Therefore, the aldehyde content of oxidized pullulan can be calculated by the following formula:

\[
\text{Aldehyde content (\%)} = \left( \frac{V_c - V_b}{m} \right) \times \frac{C_{NaOH}}{100}\% 
\]

(1)

where \( C_{NaOH} = 0.1 \text{ mol/L} \), \( m \) is dry weight (g) of oxidized pullulan sample, each group is tested in triplicate.
2.5. FTIR measurements
Infrared absorption spectra of pullulan, oxidized pullulan, gelatin and gelatin hydrogel samples were recorded using a Thermo Nicolet IR200 spectrometer at a scan range from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\), at a resolution of 2 cm\(^{-1}\) and 32 scans. Samples were measured as KBr pellets.

2.6. Mechanical tests
The compressive strength of hydrogel samples were prepared into cylindrical shape with diameter of 10 mm and height of 14 mm using TA XT Plus Texture Analyzer (Stable Micro System, UK), at 25 °C and 50 ± 5% RH. The tests were carried out at compression speed of 30 mm/min, until the hydrogel samples were cracked. All experiments were performed three times and the results presented here are the average values.

3. Results and Discussion

3.1. The dialdehyde content of oxidized pullulan
The pH of the reaction system affects the oxidation performance of sodium periodate. When the pH of the reaction system was high, the sodium periodate was unstable and easily changes and the oxidation effect was greatly reduced or even lost, which may cause the content of the aldehyde groups to decrease. Therefore, this experiment was carried out under acidic conditions. The aldehyde content of oxidized pullulan was controlled by changing the pH value of the solution. The results are shown in Figure 1. It can be visualized that changes in pH of the reacting solution affect the content of aldehydes in oxidized pullulan. The amount of the aldehyde groups first increases and then decreases with the increase of pH of the aqueous phase from 2.0 to 5.0, and the aldehyde content of oxidized pullulan reaches a maximum of 5.48 mmol/g at pH 4.0. When the pH of the solution was too low, although the oxidizing property of sodium periodate was improved, it is liable to undergo reduction reaction, so that the amount of sodium periodate participating in the oxidation was reduced and the aldehyde groups content was also lowered. When the pH was too high, the acidity of the reaction system was not enough and the oxidation of sodium periodate was decreased. Therefore, when the pH of the reaction system was 4, the aldehyde group content was the highest.

![Figure 1. The aldehyde content of oxidized pullulan at different pH values.](image-url)
3.2. FTIR analysis
Sodium periodate had strong oxidizing properties, which selectively oxidized the C2-C3 bond in the glucose unit of pullulan to form two aldehyde groups. Figure 2 shows the FTIR spectra of pullulan before and after oxidation. Compared with pullulan and oxidized pullulan exhibited a large amount of alcoholic hydroxyl groups and additional band at around 1730 cm\(^{-1}\) attributing to carbonyl groups in the dialdehyde pullulan, suggesting a successful oxidization reaction in pullulan macromolecular chains.

![FTIR spectra of pullulan, oxidized pullulan.](image)

3.3. Mechanical properties
The mechanical properties of gelatin hydrogels cross-linked by oxidized pullulan were examined in compression mode at room temperature (Figure 3 and 4). Figure 3 shows typical stress-strain curves of the gelatin hydrogels. Compressive strains slightly varied between 48% and 52%, and the strain values first decrease and then increase with increasing oxidation degree of dialdehyde pullulan. Figure 4 shows the influence of aldehyde content on the compressive stress of gelatin hydrogels. It is seen that the compressive stress at failure values significantly increased with the addition of oxidized pullulan. The compression stress of Ge-OP3 attains a maximum of 1.41 MPa, more than 17 times of un-crosslinked gelatin hydrogels. It is likely that the hydrogel network crosslinked with higher aldehyde content of pullulan have a higher degree of crosslinking. Hence, they require more stress to break more covalent crosslinks between aldehyde groups of oxidized pullulan and amine groups of gelatin.
Figure 3. Typical stress-strain curves of gelatin, Ge-OP1, Ge-OP2, Ge-OP3 and Ge-OP4.

Figure 4. The compression strength of different hydrogels.

4. Conclusions
In this work, dialdehyde pullulan with different aldehyde contents were successfully prepared with periodate oxidization method and used as cross-linking agent for the construction of gelatin hydrogels. The compressive strength was significantly increased with the addition of oxidized pullulan.

Acknowledgement
The financial supports from the National Natural Science Foundation Commission of China (No. 51373158, 51673177) and the foundation of Science and Technology Department of Henan Province (No. 172102410022) are gratefully acknowledged.
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