Preparation of guanidine-modified starch for antimicrobial paper

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ABSTRACT

Normal paper does not have antimicrobial properties. To impart antimicrobial properties to paper for medical applications, a guanidine-modified starch was prepared via two reaction steps using guanidine hydrochloride (GH) as a modifier, and added to the paper coating formula. FT-IR demonstrated that the GH was successfully grafted onto the starch via the Schiff base reaction. After coating with the modified starch, the antimicrobial performance of the paper against E. coli and S. aureus was evaluated by the disc diffusion method. The results indicated that the paper treated with the guanidine-modified starch exhibited excellent antimicrobial properties against the E. coli and S. aureus. In addition, the dry and wet strength indexes of the coated paper increased by 25% and 100%, respectively, as compared to the control paper.

Keywords: Starch; Guanidine hydrochloride; Medical paper; Antimicrobial activity; Tensile strength

1. INTRODUCTION

Among the natural materials, cellulosic material has been considered as one of the most promising materials for medical applications, as it is renewable, biodegradable, and nontoxic. Antimicrobial and strength properties are important attributes of medical paper. To impart antimicrobial properties to cellulose paper, natural and synthetic antibacterial agents may be added as functional additives in the coating of paper. The dry and wet strength properties of paper are also important in medical applications. Strength additives can be applied in the papermaking process to improve the strength properties. The commonly used strength additives include starch, chitosan, carboxymethyl cellulose, and polyacrylamide. Starch contains repeated units of α-1-4 linked D-glucose, and has a proclivity for forming hydrogen bonds with hydroxyl groups of cellulose fibers, thus enhancing the strength properties of paper. It would be more cost effective to combine the antimicrobial and strengthening effects into a single additive for medical paper applications. However, little has been reported in the literature regarding this type of paper additive.

In present work, we developed a novel modified starch additive by grafting guanidine hydrochloride onto starch for both antimicrobial and strengthening functionalities. Dialdehyde starch (DAS) was firstly prepared by oxidizing native potato starches with sodium periodate as an oxidant. Guanidine hydrochloride was then grafted onto the DAS through the Schiff base reaction of the aldehyde group of DAS and the amino group of guanidine hydrochloride.

2. EXPERIMENTAL

2.1 Materials

Potato starch, guanidine hydrochloride (GH), and sodium periodate were purchased from Aladdin reagent Co., Ltd (China). All other chemicals were of analytical grades and used without further purification.

2.2 Preparation of dialdehyde starch (DAS)

The DAS was prepared by following the procedures as described in a previous work. 0.7 mol/L sodium periodate solution was prepared and adjusted to pH 4.0 with 2 mol/L sulfuric acid. Native potato starch was then added into the sodium periodate solution, and then stirred at 40 °C in the darkness. The molar ratio of sodium periodate to starch was 1:1. After reaction for 4 h, the suspension was filtered and the powder was washed with deionized water for three times, and freeze-dried.

2.3 Preparation of guanidine hydrochloride-grafted dialdehyde starch (DAS-GH)

2.5 g of the DAS was dispersed into 25 mL distilled water under mechanical stirring. Guanidine hydrochloride was then added into the dispersion and the pH was adjusted to 5.0 with 2 mol/L NaOH solution. The molar ratio of guanidine hydrochloride to DAS were 1:1, 1:4, 1:8, 1:12, or 1:16. After the reaction was carried out at 60 °C for 2 h, the dispersion was filtered, washed and freeze-dried.

2.4 Coating of paper

2g of the DAS-GH was dispersed into 40 mL distilled water, and heated at 90 °C for 0.5 h with continuous mechanical stirring. The obtained DAS-GH solution was cooled to room temperature; a paper strip (copy paper, 70 g/m²) was immersed into the above solution for 30 min, and then dried at 110 °C for 20 min in a hot plate dryer. The amount of DCMC-GH coated onto the paper was 40 mg/g paper.

2.5 Determination of the GH content of the grafted starch
The content of nitrogen in the DAS-GH was determined by the Kjeldahl method, and the content of nitrogen was calculated by the formula:

$$N\% = \frac{V \times C \times 14 \times 10^{-3} \times 100}{W}$$

Where $V$ is the volume of hydrochloric acid solution (HCl) used in the titration of the DAS-GH (mL), $C$ is the concentration of the standard HCl solution (mol/L), $W$ is the weight of test specimen (g).

The GH content of the grafted starch was calculated as follows:

$$GH\% = \frac{N\%}{14} \times 59$$

Table 1 lists the GH contents of the DAS-GH samples, which were prepared with various molar ratios of GH to DAS.

**2.6 Characterization of DAS-GH**

The FTIR spectra of starch, guanidine hydrochloride, and DAS-GH were recorded using a Fourier transform infrared spectroscopy (Thermo Nicolet 360) at a resolution of 4 cm$^{-1}$ in the spectral region of 500-4000 cm$^{-1}$, by making a KBr pellet with the sample.

**2.7 Strength measurement**

The dry and wet tensile strength of the DAS-GH coated paper and blank paper were measured on a strength testing machine (DCP-KZ 300, Sichuan Changjiang Paper Instrument Co., Ltd., China). The dry strength was measured according to the TAPPI standard method T494. For wet strength measurement, the samples were firstly soaked in distilled water for about 10 min, and then subjected to the tensile strength measurement. For each sample, at least ten replicates were tested and the average was reported.

**2.8 Antimicrobial activity of DAS-GH-coated paper**

A gram-negative organism (*Escherichia coli* (E. coli) ATCC 8739), and a gram-positive organism (*Staphylococcus aureus* (S. aureus) ATCC 6538) were used to examine the antimicrobial properties of the DAS-GH coated paper. The bacteria were grown in LB liquid medium (10 g.L$^{-1}$ peptone, 5 g.L$^{-1}$ yeast extract, 10 g.L$^{-1}$NaCl, and pH 7.0) for 12 hours at 37 °C, and then diluted to 105 CFU/mL with NaCl solution (0.85%, w/v). Then 0.1 mL of the diluted suspensions of E. coli or S. aureus was mixed into a LB agar medium (10 g.L$^{-1}$ peptone, 5 g.L$^{-1}$ yeast extract, 10 g.L$^{-1}$NaCl, and 15 g.L$^{-1}$ agar). A disc of 8.5 mm in diameter was punched out from DAS-GH coated paper or blank paper, and was centrally placed on the agar plates seeded with bacteria. After incubation at 37 °C for 24 hours, the inhibition zone was examined.

**3. RESULTS AND DISCUSSION**

**3.1 FTIR analyses of the DAS-GH**

The FTIR spectra of the original starch, DAS, and DAS-GH were shown in Figure 1. For the FTIR spectrum of the native starch, characteristic peaks were found at 3375 and 994 cm$^{-1}$ due to the stretching vibrations of the hydrogen bonding -OH groups and C-O stretching vibrations, respectively. Another characteristic peak at 1651 cm$^{-1}$ was attributed to the tightly bound water in the native starch. When the native starch was oxidized by periodate, the C-2 and C-3 bond of the anhydroglucose units was broken, and the aldehyde groups were formed, thus a characteristic peak was appeared at 1732 cm$^{-1}$(C=O stretch) in the spectrum of DAS. After the guanidine hydrochloride was grafted onto DAS, the major change in the spectrum for DAS-8 was the appearance of a new intensive characteristic peak at 1659 cm$^{-1}$ (N–H bending vibration), and the disappearance of the band at 1732 cm$^{-1}$(C=O stretch), due to the reaction of the aldehyde groups of DAS with the amino groups of guanidine hydrochloride. The FT-IR results indicated that guanidine hydrochloride was successfully grafted onto DAS via the Schiff base reaction.

**Table 1** The GH contents of DAS-GH prepared at different molar ratio of GH/starch

<table>
<thead>
<tr>
<th>GH/DAS (molar ratio)</th>
<th>DAS-0</th>
<th>DAS-1</th>
<th>DAS-1.4</th>
<th>DAS-4.7</th>
<th>DAS-5.8</th>
<th>DAS-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH content (%)</td>
<td>0</td>
<td>0.95</td>
<td>1.35</td>
<td>4.66</td>
<td>5.76</td>
<td>8.01</td>
</tr>
</tbody>
</table>

![Fig. 1 FTIR spectra of (a) original starch, (b) dialdehyde starch, (c) DAS-8, (d) DAS-4.7, and (e) DAS-1](image-url)
3.2 Strength property of DAS-GH-coated paper

The dry and wet strength properties of blank paper and DAS-GH-coated paper were measured and the results are shown in Figure 2. It was found that both the dry and wet strength indexes of paper were improved significantly after the coating treatment with DAS-GH. The dry strength index increased markedly from 60.2 to 75.3 Nm/g when the paper was treated with DAS-5.8, and the wet strength index also increased dramatically from 2.1 to 4.2 Nm/g. However, the dry and wet strength improvement with DAS-8 was much lower than with DAS-5.8, probably due to the excessive -NH₂ groups in the DAS-8 that may interfere with the hydrogen bonding of cellulose fibers.  

![Fig. 2 Dry and wet strength index of the paper before and after the treatment with DAS-GH](image)

3.3 Antimicrobial performance of the DAS-GH coated paper

The antimicrobial performance against *E. coli* and *S. aureus* of the paper before and after treatment with DAS-GH was evaluated using the disc diffusion method, and the results were shown in Figure 3. No inhibition zone around the blank paper was found, while obvious inhibition zones were clearly observed around the DAS-GH coated paper, demonstrating strong antimicrobial properties against *E. coli* and *S. aureus*. The inhibition zones around the treated paper discs were larger for the paper treated with the modified starch with a higher GH content, due to the strong antimicrobial activity of the GH groups.

![Fig. 3 The antimicrobial performance of the paper before and after the treatment with DAS-G,H against *E. coli* (a) and *S. aureus* (b)](image)

4. CONCLUSIONS

In this work, a multi-functional paper additive was successfully synthesized by grafting guanidine hydrochloride (GH) onto starch and applied to paper to provide antimicrobial attributes against *E. coli* and *S. aureus*, as well as strength improvement. It was found that the dry and wet strength of the paper increased 25% and 100%, respectively, after the treatment with the DAS-GH starch. The inhibition zones around the treated paper discs were larger for the paper treated with the modified starch with a higher GH content, due to the strong antimicrobial activity of the GH groups. The DAS-GH modified starch has a great potential as a multi-functional agent for medical paper.

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REFERENCES


