Structure and Properties of Graft Copolymer of Starch and Resorcinol Synthesized Using HRP

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ABSTRACT: A modified starch tannage was synthesized by radical copolymerization of degraded starch and resorcinol (RC) using horseradish peroxidase (HRP)/H_2O_2 as an initiator. The effects of the degradation degree, system pH value, reaction temperature on the copolymerization, and tanning properties of the graft copolymer used in leather processing were investigated. The application results showed that the graft copolymer has excellent tanning properties. The shrinkage temperature (T_s) of the tanned leather reached 85.4°C, and the thickness increment ratio of the retanned leather was 23.1%. The tanning effects were significantly improved with RC modification compared with the graft copolymer of starch and vinyl monomers. The results indicated that the graft copolymer of starch and RC may be a replacement for toxic chrome and aldehyde tannage. The structure of the graft copolymer was characterized by FTIR, ^{1}H-NMR, GPC, and UV.
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Key words: degradation; enzymes; graft copolymer

INTRODUCTION

In recent years, serious environmental pollution produced by synthetic chemicals has forced people to develop more environmental friendly chemicals. Almost all synthetic chemicals are toxic and originate from petroleum and coal, which have become increasingly scarce as nonrenewable resources. Thus, the preparation of "green" chemicals using renewable and eco-friendly natural materials has received more attention.[1,2] Starch is an excellent green raw material that is renewable and does not environmentally pollute. It is considered a promising candidate for developing green chemicals and may be extensively applied in various industrial fields, such as degradable plastics, medicine, adhesives, textiles, and paper-making.[3–6] Original starch cannot be directly applied in these fields and needs chemical modification prior to application. The purpose of chemical modification is to introduce some functional groups on starch molecules to meet the application's needs. Starch modified by graft copolymerization with vinyl monomers, such as acrylic acid and acrylonitrile, using ammonium persulfate as the initiator is one of the main modification methods for the preparation of green fine chemicals.[7–10] However, the vinyl-modified starch cannot be substantially used as a substitute for toxic chrome and aldehyde tannage because the shrinkage temperature (T_s) of leather tanned with vinyl monomer modification is less than 65°C.[11,12] The results indicate that the tanning effect of vinyl-modified starch is poor.

Recently, research on enzymatic polymerization has received increasing attention. Enzymatic polymerization of phenols with peroxidases has been studied, which can produce phenolic resins without using formaldehyde. Horseradish peroxidase (HRP) is the most widely used for the enzymatic polymerization of phenol, aniline, and their derivatives. Simona studied the reactivity of HRP/H_2O_2-initiated phenolic in water and obtained a novel 4-phenylphenol *ortho* dimer.[13] Chul and Young studied the radical polymerization of aniline initiated by HRP/H_2O_2 in nonaqueous phase, and the polyaniline connected directly by benzene was generated.[14] However, the modified starch with phenols using HRP as an initiator designed to replace toxic standard tannages has been scarcely reported. In the present study, a graft copolymer was synthesized by radical copolymerization of degradation starch and resorcinol using HRP/H_2O_2 as the initiator. The effects of the reaction conditions on graft copolymerization were investigated. The structure of the graft copolymer was characterized by FTIR, ^{1}H-NMR, GPC, and UV.

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EXPERIMENTAL

Materials

Horseradish peroxidase with activity of 270 u/mg was obtained from Beijing Biosynthesis Biotechnology Co. Resorcinol (RC), hydrogen peroxide (H_2O_2, 30 wt %), high-temperature α-amylase (70–110°C; HTA), sodium bicarbonate, absolute ethanol, and methanol were supplied by the Xi'an Chemical Reagent Factory.

Starch degradation

About 30 g cornstarch and 0.02 g of α-amylase were placed into 170 g distilled water in a 500 ml three-neck round-bottom flask with a stirrer and a temperature control device. The reactant was then heated at 95°C for a suitable length of time. It was then cooled to room temperature. The product was degradation starch and used to prepare the graft copolymer of starch and RC. The schematic diagram of the degradation starch is shown in Figure 1.

Preparation of the graft copolymer of starch and RC

About 30 g of RC was added to the degradation starch solution in the flask mentioned in section 2.1. The pH value of the solution was adjusted to 5–10 with sodium bicarbonate. The graft copolymerization of starch and RC was initiated with HRP solution and H_2O_2 (30 wt %) combined as the initiator at a specific temperature. The HRP solution amount was 5 ml, and the H_2O_2 amount was 6.0 g (30 wt %). The HRP solution was added all at once, and the H_2O_2 was added in drops for 2 h. The HRP solution was prepared by dissolving 50 mg HRP in 50 ml of distilled water at 4°C. After the H_2O_2 was dropped completed, the thermal insulation reaction was performed at a specific temperature for a specific time. The temperature was then decreased to room temperature. The graft copolymer of degradation starch and RC was obtained. The chemical structure of the graft copolymer was verified by the FTIR and NMR spectra. The schematic diagram of the graft copolymerization of starch and RC is shown in Figure 2.

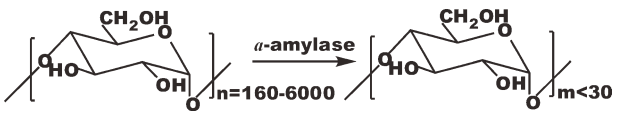

Figure 1 Schematic diagram of degradation starch with α-amylase.

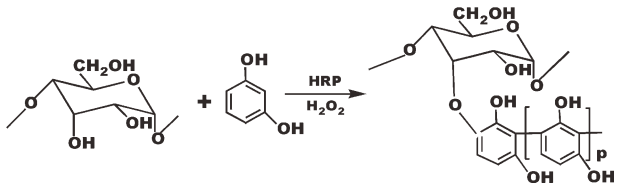

Figure 2 Schematic diagram of the graft copolymer of starch and RC.

Test and measurements

Test for FTIR

FTIR spectra were recorded on an EQUINOX-55 spectrometer (Bruker, Bremen, Germany). The sample tabletting was prepared by direct compression from 1 wt % (by weight) polymer and KBr powder and used to record the FTIR spectra.

Test for NMR

The ^{1}H-NMR spectra were obtained using an INOVA-400MHz NMR spectrometer (AVANVEIII, Palo Alto, USA) with deuterated dimethyl sulfoxide (DMSO-d_6) while the sample's solvent was internally referenced to tetramethylsilane [TMS].

Test for UV

The UV spectra of the monomer and copolymer were recorded using an ultraviolet and visible spectrometer (UV1900, Shanghai, People's Republic of China). The scanning range was from 200 to 400 nm. The sample was dissolved in distilled water into 0.01-0.05 g/L for the measurements.

Test for relative molecular mass

The relative molecular weight and polydispersity of degradated starch index (PDI) were measured using a GPC model 2414 instrument (Waters, USA). Two columns sets (Ultrahydrogel 250 and 120, 7.8 × 300 mm) were used in a series. 0.10 mol/L sodium nitrate solution was utilized as the carrying phase at a flow rate of 0.42 mL/min. The measurement was performed at 50°C, using polyethylene glycol as the standard sample.

Test for GP, GE, and CM

The graft copolymer was precipitated and washed with anhydrous ethanol, then vacuum dried at 50°C to constant weight (W_1). The product was extracted for 12 h at 60°C by soxhlet extractor with methanol as solvent and vacuum dried at 55°C to constant weight. The pure graft copolymer was obtained (W_2). All samples were measured three times, and

TABLE I
Effects of Degradation Time on Applied Properties of the Copolymer

Degradation conditions	Degradation time (min)	Degradation degree			T_s of sheepskin tanned with the copolymer[a] (°C)	
		M_n	M_w	PDI	Before tanning	After tanning
30 g starch	30	42,767	132,637	3.10	43.2	67.3
0.02 g HTA	60	16,943	46,893	2.77	42.6	73.5
170 g distilled water	90	10,490	24,131	2.30	43.4	78.6.
	120	4264	9552	2.24	44.5	85.4
95°C	150	3963	8821	2.22	45.7	85.2

[a]The copolymer was prepared with 15 g degraded starch and 30 g RC using HRP (5 mg)/H_2O_2 (6.0 g, 30 wt %) as the initiator at 30°C for 5 h in an aqueous solution.

then take the average. The graft percentage (GP), graft efficiency (GE), and the conversion of the monomer (CM) were measured by eqs. (1)–(3), respectively.

$$\mathrm{GP} = \frac{W_2 - W_0}{W_0} \times 100\% \quad (1)$$

$$\mathrm{GE} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\% \quad (2)$$

$$\mathrm{CM} = \frac{W_1 - W_0}{W_m} \times 100\% \quad (3)$$

where the W_0 and W_M are the weights of cornstarch and monomer, respectively.

Application of graft copolymer

The graft copolymer of starch and RC was used as tannage and retannage, respectively. The tanning procedure for production of a sheep garment leather is as follows.

Tanning process: Pickled sheep skin → Weighing (100% gain) → Measurement of shrinkage temperature (T_s) → Acid bating (pH = 2.5) → Neutralizing (pH = 5.5, 0.5 h) →Tanning (10 wt % graft copolymer of starch and RC for 4 h) → Feeding with formic acid (pH = 3.0, 0.5 h) → Staying overnight → Determination of T_s → Retanning → Determination of R and T_s → Dyeing → Fat liquoring → Natural drying .

Retanning process: Goat wet blue → Weighing → Washing → Soaking Back → Neutralizing (pH = 4.5) → Determination of R and T_s → Retanning (10 wt % graft copolymer of starch and RC for 4 h) →Determination of R and T_s → Dyeing → Fat liquoring → Natural drying.

The graft copolymer was used as tannage and retannage to goat skin and goat wet blue leather. The shrinkage temperature (T_s) was measured using a digital leather shrinkage temperature tester (MSW-YD4, People's Republic of China). The properties of retanned leather were evaluated by determining the thickness increment ratio (R) that was defined in eq. (3):

$$R = \frac{S_2 - S_1}{S_1} \times 100\% \quad (4)$$

whereas S_1 and S_2 are the average thicknesses of leather samples before and after retanning, respectively.

The mechanical properties of applied leather such as the tensile strength, break elongation ratio, and tear strength were determined using a

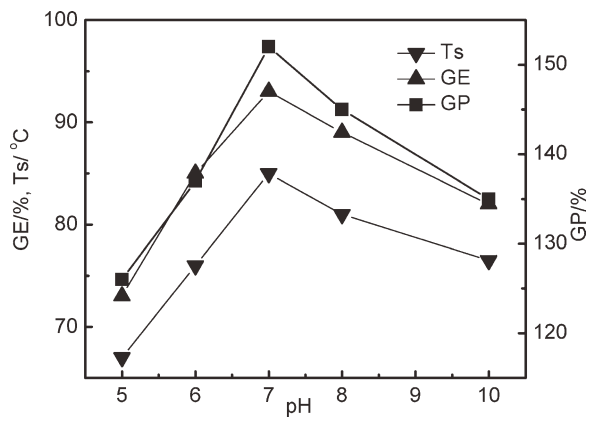

Figure 3 The effect of pH on GP, GE, and T_s.

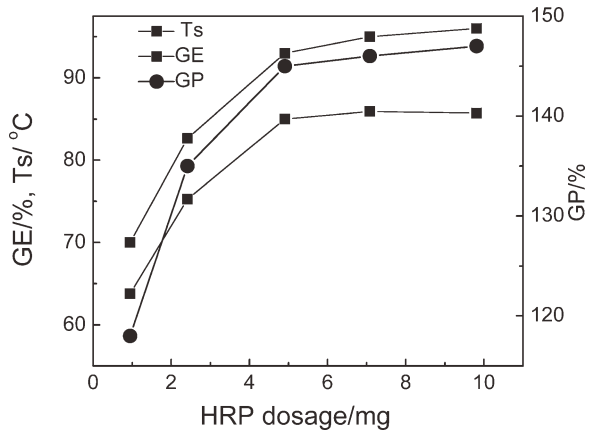

Figure 4 The effect of HRP dosage on GP, GE, and T_s.

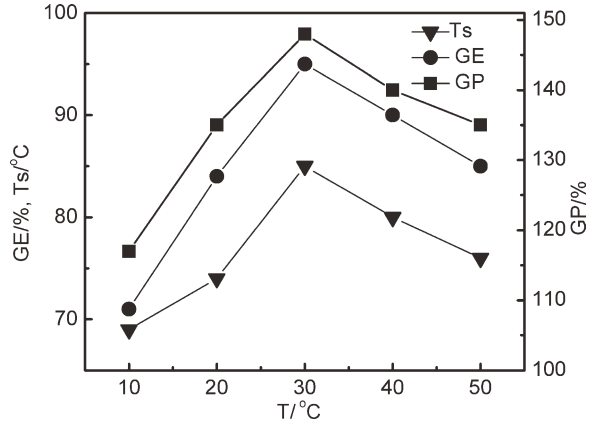

Figure 5 Effect of temperature on GP, GE, and T_s.

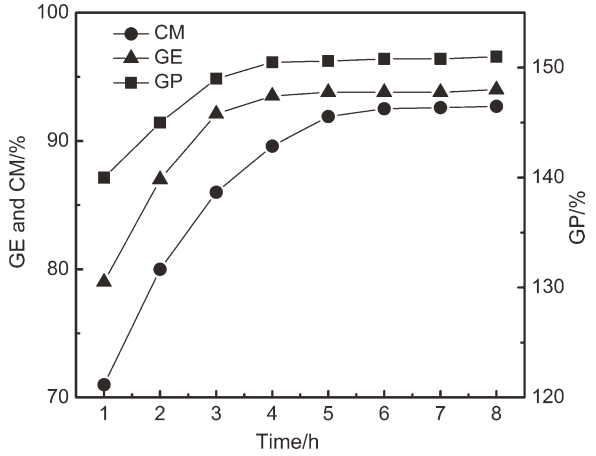

Figure 6 Effect of reaction time on copolymerization.

multifunctional electron tension-meter (TS2000-S, People's Republic of China).

RESULTS AND DISCUSSION

Effects of degradation time on molecular weight and tanning properties

The effects of degradation time on the molecular weight of starch and the application effect of the copolymer of starch and RC are shown in Table I. The M_w and M_n of degradation starch decreased rapidly with a degradation time from 30 to 120 min, then decreased with a slightly longer degradation time from 120 to 150 min. The T_s of the tanned sheepskin increased while M_w and M_n of the degradation starch decreased and reached its maximum value when the M_w and M_n of the degradation starch were 9552 and 4264, respectively. Therefore, the suitable degradation time was 120 min. The results indicate that small molecular mass degradation starch significantly improved the T_s of leather tanned with the copolymer of degradation starch and RC. The reason is that the small molecular weight of the degradation starch results in a small molecular weight of the copolymer of starch and RC, which has excellent solubility in water and good permeability in skin fibers. The results will be helpful for forming an intermolecular crosslink between skin fibers and the copolymer molecules by chemical combinations of the functional groups.

Effects of pH, HRP dosage, and polymerization temperature and time on graft copolymer

The copolymer was prepared with 15 wt % 100 g degraded starch water solution and 30 g RC using HRP (5 mg)/H_2O_2 (6.0 g, 30 wt %) as the initiator at 30°C for 5 h. The pH value of the reaction solution was changed in the range of 5.0-10.0. The effects of pH on the GE and GP of the copolymerization are shown in Figure 3. The results indicate that GE and GP first increased while the pH increased from 5.0 to 7.0 and reached maximum values of 93 and 152%, respectively, when the pH was 7.0. A further increase in pH was accompanied by a decrease in GP and GE. Therefore, the optimal system pH was 7.0. This could be explained by the effects of pH on the reaction activity of HRP. Figure 3 also shows the effect of GE and GP on T_s of the copolymer. The results indicate that the T_s of tanned sheepskin increased while GE and GP increased and reached a maximum value at the maximum value of GE and GP. The reason is that there is more hydroxyl (—OH) in the copolymer molecular chains of starch and RC when GE and GP are greater. The skin fibers

TABLE II
Application Results of Graft Copolymer of Starch and RC

Modified starch tannages	Before tanning (T_s/°C)	After tanning (T_s/°C)	Retanned leather (R) (%)	Tensile strength (N/mm^2)	Tear strength (N/mm)	Elongation at break (%)
Starch-RC tannage	43.1	85.4	16.8	8.87	36.91	48.74
Starch-RC retannage	97.6	103.7	23.1	11.82	44.2	61.95
Starch-vinyl tannage[a]	42.7	67.1	17.2	7.54	22.08	33.28
Starch-vinyl retannage[a]	97.4	98.5	27.4	10.55	36.58	56.36

[a] Modified starch was prepared by copolymerization of degradation starch with acrylic acid (AA) and acrylonitrile (AN) at 80°C for 3 h using $(NH_4)_2SO_4$ as initiator . The mass ratio of starch, AA, and AN was 20:10:5.

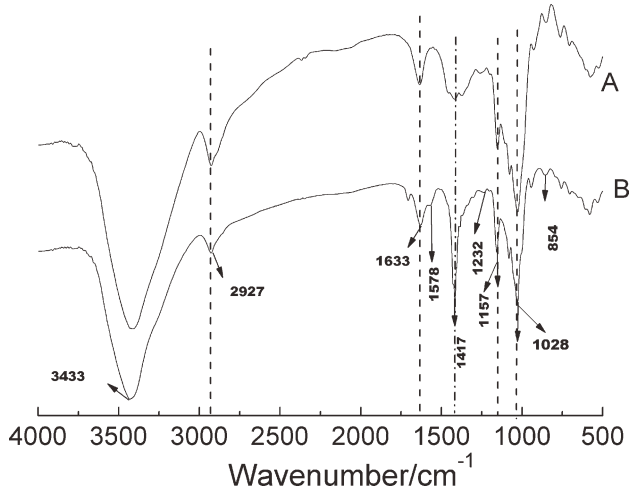

Figure 7 FTIR of the degraded starch (a) and the graft copolymer of starch and RC (b).

consist of very long molecules (collagen), which consist of amino acids that are joined together by peptide groups. There are many amino groups (—NH_2) and carboxyl groups (—COOH) on skin fibers. Furthermore, the graft copolymer has good permeability in skin fibers. Therefore, stronger chemical crosslinking can be formed between the copolymer and skin fibers.

The effects of HRP dosage on the GP, GE, and CM values were studied by setting the temperature at 30°C, pH value of 7.0, and keeping other factors constant. Figure 4 indicates that the HRP dosage has significant effects on the copolymer properties. The GP, GE, and CM values increase with the increase of HRP dosage. However, there is no obvious increment when the HRP dosage is more than 5 mg. Therefore, the optimal HRP dosage is 5 mg.

Figure 5 shows the effects of reaction temperature on GE, GM, and T_s when the other modified variables were kept constant and the copolymerization

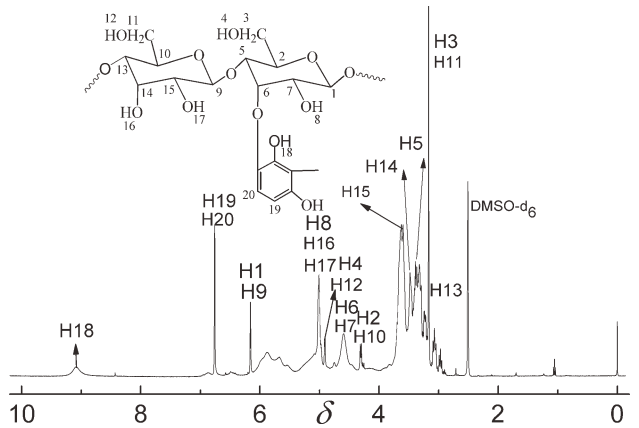

Figure 8 ^{1}H-NMR of the graft copolymer.

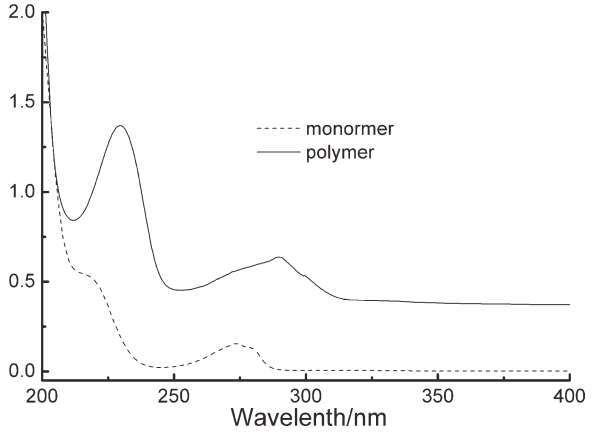

Figure 9 UV spectra of monomer and copolymer.

temperature was changed in the range of 10-50°C. The results indicate that GP, GE, and T_s increased while the reaction temperature increased, reached a maximum value when the temperature was 30°C, and then decreased with a further increase in temperature. This can be attributed to the inactivation of enzymes as the temperature became too high. Therefore, the optimal copolymer was obtained at 30°C.

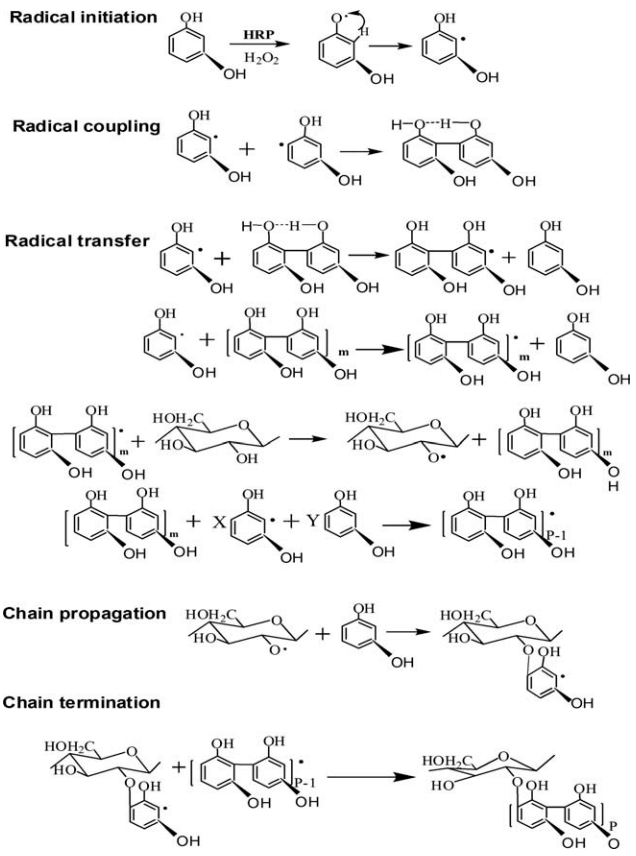

Figure 10 Schematic diagram of the grafting copolymer of starch and RC.

The reaction time was changed in the range of 1-8 h while the other reaction conditions remained unchanged. The effects of reaction time on GP, GE, and CM are shown in Figure 6. GE, GP, and CM increased rapidly when the reaction time increased from 1 to 4 h. When the reaction time was prolonged for more than 5 h, the increasing extent of GE, GP and CM improved greatly. Further prolongation of the reaction time slightly increased GE, GP, and CM. This may result from the consumption rate of RC and initiator in the reaction solution. The reaction mechanism consists of radical copolymerization, including chain initiation, chain propagation, and chain termination. The reaction rate depends on the content of RC and HRP in the reaction solution and is characteristically faster at the earlier stage and slower at the later stage. Therefore, the suitable time was 5 h.

Application results of the graft copolymer

The application results of the graft copolymer are shown in Table II. The graft copolymer (starch-RC) of starch and RC has excellent tanning properties, and the T_s of tanned leather reached 85.4°C. The average thickness increment ratio of retanned leather was 23.1%. The tanned leather had better service performance, such as softness, tightness, and fullness, and better mechanical properties than starch-vinyl copolymer tannage. Whether the materials can be chosen as a tannage depends chiefly on T_s and its tanned leather service performance. Accordingly, the modified starch can replace chrome tannage and may decrease pollution produced during the tanning process.

The structure and reaction mechanism of the graft copolymerization

The FTIR of the graft copolymer and degraded starch is shown in Figure 7. The stretching vibration absorption peak at 3418 cm^{-1} corresponds to the —O—H of starch and RC. The stretching vibration absorption peak at 2937 cm^{-1} corresponds to the $—CH_2—$ of starch. The stretching vibration absorption peak at 1028 cm^{-1} can be attributed to the —C—O— in the $—CH_2OH$ group in starch. The absorption peak at 1,156 cm^{-1} can be attributed to the C—O stretching vibration of RC. The characteristic benzene ring peaks occurred at 1615, 1501, and 1442 cm^{-1}. The absorption peak at 854 cm^{-1} showed that the copolymer contains 1,2,3,4-substituted benzene.

The ^{1}H-NMR spectrum of the graft copolymer is shown in Figure 8. The data were analyzed as in the following. ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 9.12 (H18), 6.768 (H19), 6.757 (H20), 6.16 (H1), 6.14 (H9), 5.042 (H16), 5.021 (H8), 4.997 (H17), 4.904 (H4), 4.894 (H12), 4.592 (H6), 4.59 (H7), 4.312 (H10), 4.299 (H2), 3.621 (H15), 3.476 (H14), 3.391 (H5), 3.161 (H3), 3.164 (H11), 3.065 (H13). The peak of H18 corresponds to the hydroxyl groups of RC. The characteristic peaks of H in the benzene ring were H19 and H20. ^{1}H-NMR proved that the copolymer contains hydroxyl groups and a benzene ring.

Figure 9 shows the UV spectrum for the monomer and copolymer. The spectra revealed bands at approximately 217 and 273 nm, corresponding to the absorption of the hydroxyl group and π-π* transition in the RC rings. The starch graft copolymer absorbed the most strongly in the π-π* transition, and the band shifted to the slightly longer wavelengths of 229 and 289 nm. This can be attributed to a conjugation system in the graft copolymer. The red-shifted absorption indicated that activity of phenolichydroxyl increased. Therefore, the product exhibits excellent tanning properties.

The graft copolymerization of starch with RC initiated by HRP/H_2O_2 is a radical polymerization consisting of three steps initiation, propagation, and termination. Reaction mechanism of the graft copolymerization of degraded starch and RC is depicted in Figure 10.

CONCLUSIONS

A graft copolymer of starch and RC was synthesized by graft copolymerization using HRP/H_2O_2 as the catalyst. The structure of the graft copolymer was characterized with FTIR, ^{1}H-NMR, UV, and GPC, indicating that RC was successfully grafted onto the starch. Compared with vinyl-modified starch tannage, this method exhibited better performance in enhancing the tanning properties of starch-modified tannage. The results indicated that the graft copolymer of starch and RC may replace toxic chrome and aldehyde tannage. This research introduces a new method for preparing the graft copolymer of starch and phenols via radical copolymerization in a water solution.

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