

Synthesis of a Phenol Copolymer with Horseradish Peroxidase and the Study of Its Structure–Property Relations

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ABSTRACT: A phenol copolymer was synthesized by the free-radical copolymerization of *p*-hydroxybenzaldehyde (HBA) and *p*-phenolsulfonate with horseradish peroxidase (HRP)/H₂O₂ as an initiator. The synthesis process included aldehyde protection via acetalization, enzymatic polymerization, and aldehyde deprotection via hydrolysis of acetals. The effects of the monomer ratio and the HRP and H₂O₂ composites on the properties of poly(*p*-hydroxybenzaldehyde-*co*-*p*-phenolsulfonate) [poly(HBA-*co*-PS)] were investigated by the determination of the shrink temperature (*T*_s) of tanned leather. The poly(HBA-*co*-PS) structure was characterized by Fourier transform infrared spectroscopy, NMR, UV spectroscopy, and gel permeation chromatography. The mechanism of enzymatic copolymerization, including initiation, H transfer, radical transfer, coupling, termination, differed from that of traditional free-radical polymerization. A remarkable characteristic was that poly(HBA-*co*-PS) had a large conjugated structure in its molecules that markedly improved the activity of the aldehyde and hydroxyl groups and showed easy crosslinking in the leather fibers. The applied results showed that the *T*_s of tanned leather with poly(HBA-*co*-PS) reached 83.6°C. Poly(HBA-*co*-PS) could be used as agent for tanning leather. © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 000: 000–000, 2012

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INTRODUCTION

Traditional syntan is obtained by the polycondensation of phenols and formaldehyde. The most substantial deficits are formaldehyde pollution and difficulty in controlling its molecular weight and structure. Horseradish peroxidase (HRP) is a type of enzyme that can initiate phenol monomers with the aid of hydrogen peroxide (H₂O₂) to form polymers by free-radical polymerization without formaldehyde.^{1–4} The enzymatic polymerization mechanism is different from traditional free-radical polymerization.^{5,6} Enzymatic polymerization not only has the advantage of high efficiency, but it can also be used for easy control of the molecular weight and structure. Therefore, enzymatic polymerization by HRP/H₂O₂ initiation has attracted considerable attention in the past decade.^{7–11}

In this study, a copolymer of *p*-phenolsulfonate (PS) and *p*-hydroxybenzaldehyde (HBA) was synthesized by free-radical copolymerization with HRP/H₂O₂ initiation. The chemical structure of the copolymer was characterized by Fourier transform infrared (FTIR) spectroscopy, NMR, UV spectroscopy, and gel permeation chromatography (GPC). The effects of the composition of the monomers and HRP/H₂O₂ on the tanning prop-

erties of the copolymer were investigated by the determination of the shrink temperature (*T*_s) of leather. The copolymerization mechanism and the tanning mechanism of poly(*p*-hydroxybenzaldehyde-*co*-*p*-phenolsulfonate) [poly(HBA-*co*-PS)] are discussed in this article.

EXPERIMENTAL

Materials

HRP with an activity of 330 u/mg was supplied by Beijing Biosynthesis Biotechnology Co. (Beijing, China). HBA and PS were chemically pure. Sodium carbonate (Na₂CO₃), *p*-toluene sulfonic acid (PTSA), sodium hyposulfite (Na₂S₂O₃), H₂O₂ (30 wt %), cyclohexane, absolute ethanol, ethylene glycol, and hydrochloric acid were analytical reagents and were supplied by Xi'an Huaxing Co. (Xi'an, China).

Preparation of hydroxybenzaldehyde glycol acetal (HGA)

The HGA synthetic device is shown in Figure 1(a). HBA (0.5 mol) and ethylene glycol (0.5 mol) were placed in 100 mL of cyclohexane in a three-necked flask. The HBA and ethylene glycol mixture was then heated to 110–120°C in the presence of 1.5 g of PTSA for 3 h. The water produced from acetalization

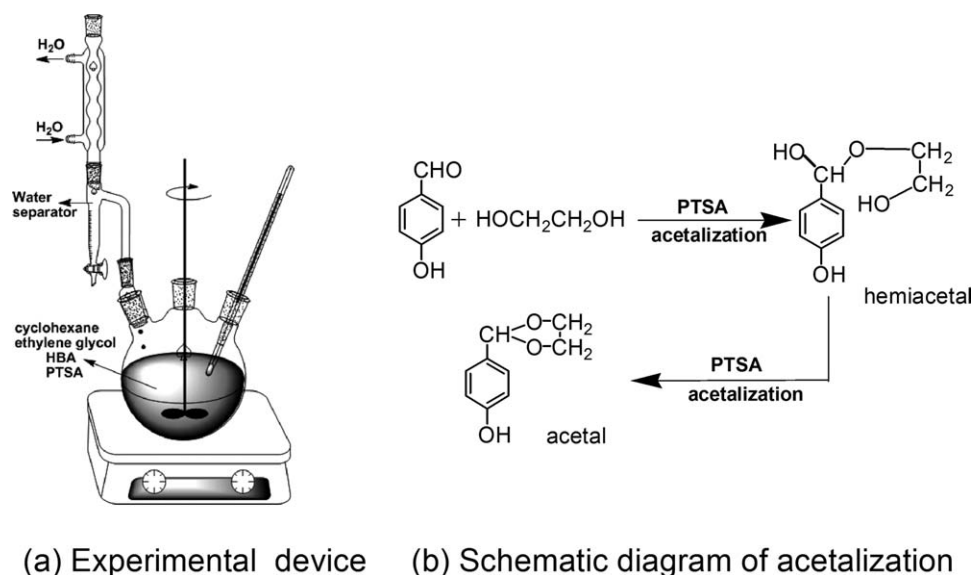


Figure 1. Schematic diagram of HGA synthesis.

was removed with a water separator. The cyclohexane solvent was then removed by distillation, and HGA was obtained. After HGA was purified by washing with absolute ethanol, the structure was determined with FTIR and NMR spectroscopy. A schematic diagram of the HGA preparation is shown in Figure 1(b). The FTIR spectra for HGA are shown in Figure 2.

FTIR from Figure 2(a) (cm^{-1}): 3470 (OH); 1690 (CHO); 1480, 1411 (C_6H_6); 1218, 1139, 1039 (C—O, C—H). FTIR from Figure 2(b) (cm^{-1}): 3450 (—OH); 1510, 1464 (C_6H_6); 1370, 1207, 1118, 1030 (—C—O—, C—H).

Preparation of Poly(HBA-co-PS)

The poly(HBA-co-PS) synthetic device is shown in Figure 3(a). An equimolar ratio of HGA and PS was placed in a suitable amount of deionized water, and the pH of the solution was adjusted to 7.5 with Na_2CO_3 powder. HRP solution (25 mL),

which was prepared by the dissolution of 10 mg of HRP in 100 mL of deionized water, was then added to the mixture. H_2O_2 (5 mL, 30 wt %) was then added dropwise to the flask for about 1 h. Copolymerization was performed at 35°C for 3 h, and the copolymer of HGA and PS was obtained. Aldehyde groups were then liberated from the acetals by the placement of 10 mL of 2 mol/L dilute hydrochloric acid into the flask at 80°C for 2 h; this yielded the final product of poly(HBA-co-PS). Poly(HBA-co-PS) was purified by repeated precipitation and washing with absolute ethanol for structural determination. A schematic diagram of the synthesis reaction is shown in Figure 3(b). The chemical structure of poly(HBA-co-PS) was characterized by FTIR spectroscopy, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$. The FTIR spectra are shown in Figure 4, and the NMR spectra are shown in Figure 5.

The FTIR spectrum from Figure 4(a) was the same as that from Figure 2(a).

FTIR from Figure 4(b) (cm^{-1}): 3460 (OH); 1687 (CH=O); 1580, 1482, 1420 (C_6H_6); 1261, 1056 (C—O, C—H). $^1\text{H-NMR}$ [400 MHz, hexadeuterated dimethyl sulfoxide ($\text{DMSO-}d_6$), δ , ppm]: 9.94–9.96 (s, H1, H3), 7.44–8.51 (s, H2, H4). $^{13}\text{C-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ , ppm): 167.17 (C11), 158.25 (C1), 150.25 (C6), 127.68 (C2), 127.56 (C3), 139.43 (C4), 138.80 (C5), 116.28 (C9), 116.24 (C7), 116.12 (C8), 114.52 (C10).

Testing and Measurement

Monomer Conversion (η) Testing. The reaction solution (10 mL) was drawn from the flask [Figure 3(a)] with a pipette every 0.5 h after the reaction occurred for 0.5 h. The solution was then immediately heated to 65°C to lose HRP efficiency, brought to room temperature, placed in a 100-mL volumetric flask, and brought to volume with deionized water. The sample (10 mL) was then pipetted from the volumetric flask to determine the amount of residual monomer with the iodometric method.¹² η was calculated with eq. (1):

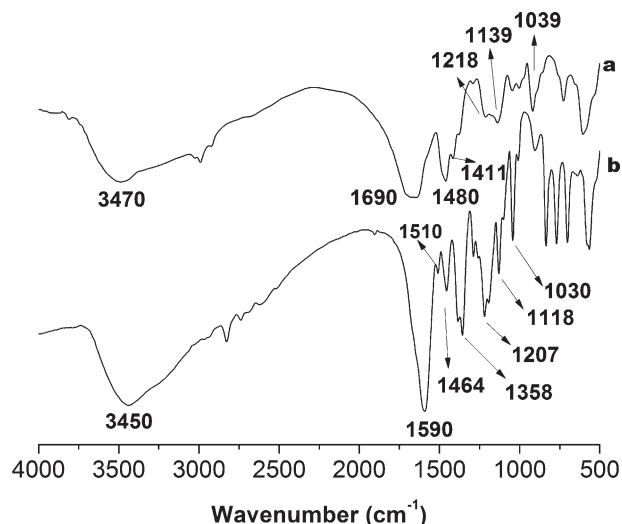


Figure 2. FTIR of (a) HBA and (b) HGA.

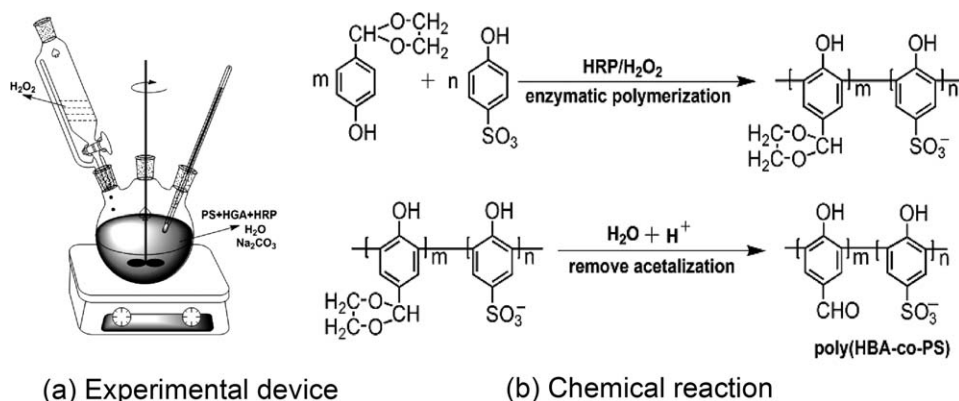


Figure 3. Schematic diagram of the poly(HBA-co-PS) synthesis.

$$\eta = \left[1 - \frac{m_0(V_0 - V)c \times 0.0025}{mn_0} \right] \times 100\% \quad (1)$$

where V_0 and V are the volumes of the titrated sample and blank consumed $\text{Na}_2\text{S}_2\text{O}_3$ standard solution (mL), respectively; c is the concentration of the $\text{Na}_2\text{S}_2\text{O}_3$ standard solution (mol/L); n_0 is the amount of monomer (HBA and PS; mol); m_0 is the total mass of the monomers (g/mol); and m is the solid content of 100 mL of the solution (g).

FTIR Testing. FTIR spectra were recorded on an EQUINOX-55 spectrometer (Bruker, Kalkar, Germany). KBr pellets made from a mixture of 0.3 mg of polymer and 5 g of KBr were used to record the FTIR spectra.

NMR Testing. ^1H -NMR and ^{13}C -NMR spectra were recorded on an INOVA 400-MHz spectrometer (Varian, Palo Alto, CA). The samples were prepared in $\text{DMSO}-d_6$.

UV Testing. The UV spectra of the monomer and copolymer were recorded with an UV1900 ultraviolet-visible (UV-vis) spectrometer (Shanghai Youke Instrument Co., Ltd., Shanghai, China). The scanning range was from 200 to 400 nm. The sample was dissolved in deionized water at 0.01–0.05 g/L for the measurements.

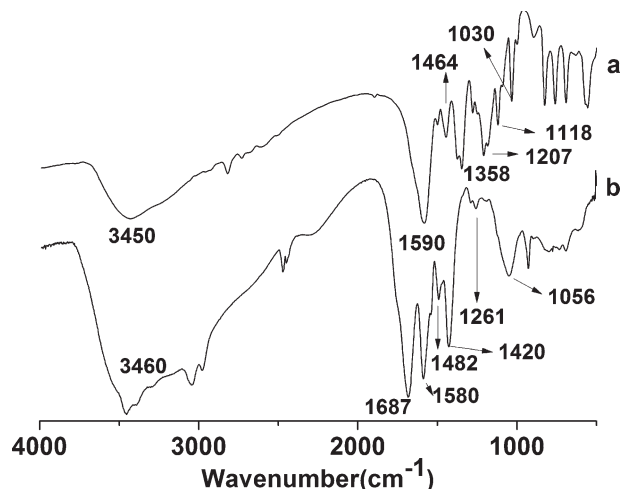
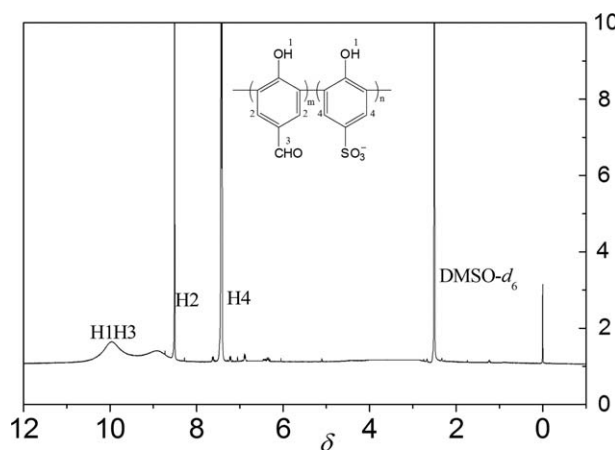
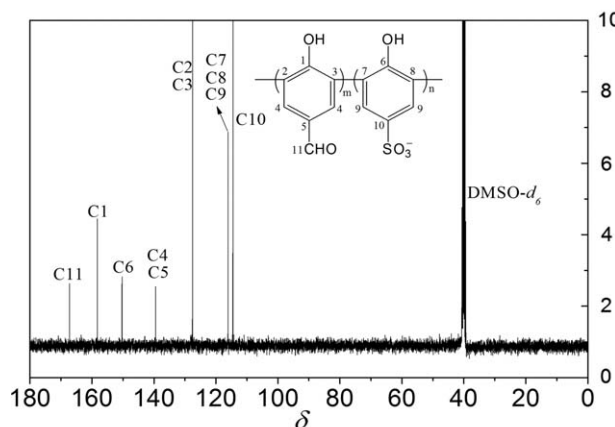


Figure 4. FTIR spectra of (a) HGA and (b) poly(HBA-co-PS).

GPC Testing. The weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity index (PDI) were characterized by GPC with a model 2414 instrument (Waters, Milford, CT). A two-column set (Ultradrogel 250 and 120, $7.8 \times 300 \text{ mm}^2$, Waters) was used in series. The mobile phase consisted of a solution of 0.10 mol/L sodium



(a) ^1H -NMR spectra of poly(HBA-co-PS)



(b) ^{13}C -NMR spectra of poly(HBA-co-PS)

Figure 5. (a) ^1H -NMR and (b) ^{13}C -NMR spectra of poly(HBA-co-PS).

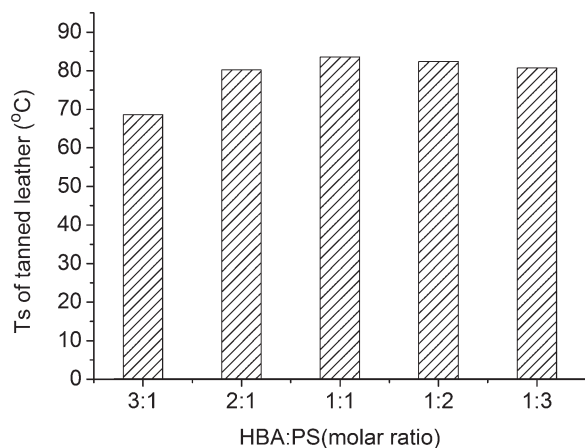


Figure 6. Effect of the monomer ratio on T_s of tanned leather. (conditions: temperature = 35, time = 3 h, HRP/H₂O₂ = 2.5 mg/5 mL).

nitrate; its flow rate was 0.42 mL/min, the test temperature was 50°C, and the standard sample was poly(ethylene glycol).

Scanning Electron Microscopy. The dispersion of leather fibers tanned with copolymer was observed by a scanning electron microscope (SEM KY1000B, Beijing, China).

Application Experiment for Poly(HBA-co-PS) Tannage

The application of the phenol copolymer was used to tan goat pickling pelt. The tanning effects were evaluated by the measurement of T_s , the mechanical properties, and the service performance of the tanned leather.

Tanning Process. The tanning process consisted of the following steps:

1. Weighing of the pickled sheep skin (100% gain).
2. T_s measurement.
3. Acid bathing (pH 2.5)
4. Neutralization (pH = 3.5, 0.5 h).
5. Tanning (10 wt % copolymer for 8 h)
6. Feeding with sodium bicarbonate (pH 5.0, 0.5 h).
7. Holding overnight.
8. Feeding with sodium bicarbonate (pH 6.0).
9. Determination of T_s .
10. Retanning.
11. Determination of T_s .
12. Dyeing.

13. Fat liquoring.
14. Natural drying.

RESULTS AND DISCUSSION

Effects of the Monomer Ratio and Molecular Weight on T_s of Tanned Leather

The other reaction conditions were the same as in the experimental design. The effects of the monomer ratio on T_s of tanned leather are shown in Figure 6. T_s of leather tanned with poly(HBA-co-PS) increased as the molar ratio of HBA and PS changed from 3 : 1 to 1 : 3. T_s reached a maximum value of 83.6°C when the molar ratio of HBA to PS was 1 : 1. The results were attributable to the activity of the aldehyde and hydroxyl groups of poly(HBA-co-PS) and its structural characteristics.

The molecular weight of the copolymer was controlled by the HRP/H₂O₂ dosage. The effects of the molecular weight of the copolymer on T_s of tanned leather are shown in Table I. The results indicate that the molecular weight of poly(HBA-co-PS) decreased with increasing HRP/H₂O₂ dosage. The best tanning effect was found at $M_w = 5938$ and $M_n = 5765$. Poly(HBA-co-PS) exhibited good solubility in water when the M_w and M_n values were less than 9561 and 9106, respectively. The results also show that the molecular weight of poly(HBA-co-PS) was easily controlled and had a narrow polydispersity. The PDI describes the uniformity of a polymer with respect to the molecular mass distribution. The minimum value of PDI is 1, which corresponds to only exactly one molecular length being present in the polymer. The PDI of poly(HBA-co-PS) close to 1 showed a narrow mass distribution. Therefore, the lower and narrow-distribution molecular weight of poly(HBA-co-PS) was easily obtained by enzymatic polymerization; this is one of the characteristics of HRP/H₂O₂ initiating polymerization. This just happened to be all the tannage needed, and it could help poly(HBA-co-PS) to permeate into the collagen fibers of the leather. This contributed to the formation of chemical crosslinking among the aldehyde and hydroxyl groups of poly(HBA-co-PS) and the carboxyl and amino groups of the collagen fibers. The results also show that the residual monomers were very low and did not pollute the environment.

Results of Poly(HBA-co-PS) Used as a Leather Tanning Agent

Table II shows the application results of the copolymer. The results show that poly(HBA-co-PS) had excellent tanning

Table I. Effects of the HRP/H₂O₂ Dosage on the Molecular Weight and T_s of Tanned Leather

| HRP/H ₂ O ₂ dosage (mg/g) | Molecular weight | | | T_s of tanned leather (°C) | | η (%) | Solubility |
|--|------------------|--------|------|---------------------------------|-------|------------|---------------------|
| | M_n | M_w | PDI | Before | After | | |
| 3.5/6.0 | 4,132 | 4,504 | 1.09 | 45.2 | 78.5 | 99.3 | Solubility in water |
| 3.0/5.5 | 4,421 | 4,731 | 1.07 | 44.4 | 80.4 | 99.2 | Solubility in water |
| 2.5/5.0 | 5,765 | 5,938 | 1.03 | 44.6 | 83.6 | 99.3 | Solubility in water |
| 2.0/4.5 | 7,568 | 7,870 | 1.04 | 45.2 | 82.4 | 99.3 | Solubility in water |
| 1.5/4.0 | 9,106 | 9,561 | 1.05 | 44.5 | 75.6 | 99.2 | Little sediment |
| 1.0/3.0 | 12,210 | 13,065 | 1.07 | 44.7 | 68.3 | 99.0 | More sediment |

Table II. Application Results of Poly(HBA-co-PS)

| | T_s (°C) | | Mechanical properties | | |
|----------------------------|----------------|---------------|-------------------------|--------------------------------------|----------------------|
| | Before tanning | After tanning | Elongation at break (%) | Tensile strength (N/m ²) | Tear strength (N/mm) |
| Leather tanning agent | | | | | |
| Poly(HBA-co-PS) | 45.3 | 83.6 | 61.57 | 13.27 | 41.62 |
| Aldehyde tanning agent | 46.6 | 84.4 | 45.23 | 13.54 | 38.65 |
| Vinyl polymer ^a | 43.5 | 73.8 | 38.32 | 6.65 | 16.23 |
| Chrome agent ^b | 44.7 | 98.2 | 65.45 | 14.87 | 43.41 |

^aThe vinyl copolymer tanning agent was prepared by the copolymerization of methyl methacrylate, acrylic acid, and acrylonitrile. The mass ratio of methyl methacrylate to acrylic acid to acrylonitrile was 20 : 10 : 2.

^bThe chrome tanning agent was supplied by Brother Enterprises Co., Ltd., Hangzhou, China.

properties, and T_s of the tanned leather reached 83.6°C. The applied leather had excellent softness, tightness, and fullness, and its mechanical properties, including tensile strength, tear strength, and elongation at break, met national standards (GB/T22889–2008, Chinese national standards). Therefore, poly(HBA-co-PS) may act as a tanning agent and could be substituted for chrome tanning agents.

Correlation of the Structure and Properties of Poly(HBA-co-PS)

Figure 7 shows the UV-vis spectrum of poly(HBA-co-PS) and its monomers. For the monomers, the UV absorption peaks at 206 and 242 and 295 nm were the K and B adsorption bands, respectively, which were caused by the conjugated system of HBA and the aromatic structure, respectively. For poly(HBA-co-PS), the same UV absorption peaks appeared at 216 and 246 and 305 nm. The results show that the absorption peaks of poly(HBA-co-PS) not only shifted slightly to longer wavelengths but were also significantly stronger than those of the monomers. The results indicate that the stronger conjugated systems were in the poly(HBA-co-PS) molecule. This may also have helped to improve the activity of the phenolic hydroxyl and aldehyde groups and form stable crosslinking networks among the collagen fibers.

The tanning mechanism of poly(HBA-co-PS) is shown in Figure 8. Although HBA and PS were introduced to the polymer chains

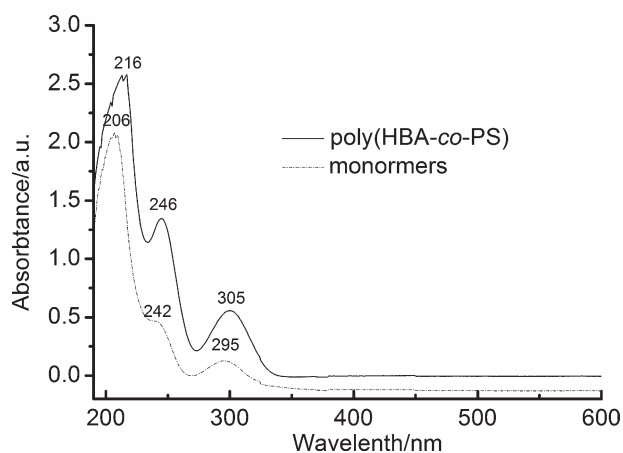


Figure 7. UV-vis spectra of poly(HBA-co-PS).

in random order, the aldehyde groups and sulfonated groups had a strong possibility of appearing alternately in the long polymer chains when the molar ratio of HBA to PS was 1 : 1; this allowed the aldehyde groups to exist stably in the copolymer chains, form strong covalent bonds and crosslinking structure, and exhibit excellent tanning effects. Similarly, two HBAs that directly linked together appeared normally, an inevitable phenomenon when the molar ratio of HBA to PS was 2 : 1. The most immediate effect may have been the two aldehyde groups that ran together and changed easily into carboxyl and hydroxyl methyl groups by an oxidation–reduction reaction, also known as *Cannizzaro reaction*; this weakened the capacity to form chemical bonds between the copolymer and collagen fibers and exhibited poor tanning effects. A schematic diagram of the oxidation–reduction reaction of the two aldehyde groups that were linked together is shown in Figure 9. The aldehyde groups that appeared least in the polymer led to poor tanning effects when the molar ratios of HBA to PS were 1 : 2 and 1 : 3. This speculation seemed to confirm the results of Figure 6. The greater number of aldehyde groups may have made the *Cannizzaro reaction* of aldehyde groups easy and decreased the tanning effects.

Polymerization Mechanism of HBA and PS with HRP/H₂O₂ Initiation

Poly(HBA-co-PS) was synthesized by the free-radical copolymerization of HBA and PS with HRP/H₂O₂ initiation. In contrast to traditional free-radical polymerization, the reaction mechanism of enzymatic polymerization may have consisted of initiation, H transfer, radical transfer, and coupling termination. A schematic diagram of the possible enzymatic copolymerization mechanism is depicted in Figure 10. This reaction mechanism resulted in the slower growth of polymer chains because the free-radical could not be transferred to monomers by the chain radical. Therefore, the molecular weight of the polymer initiated by HRP/H₂O₂ was smaller than that in conventional free-radical polymerization.

CONCLUSIONS

Poly(HBA-co-PS) was successfully synthesized by free-radical copolymerization with HRP/H₂O₂ as an initiator. The synthesis process of poly(HBA-co-PS) included aldehyde protection via acetalization, enzymatic polymerization, and aldehyde

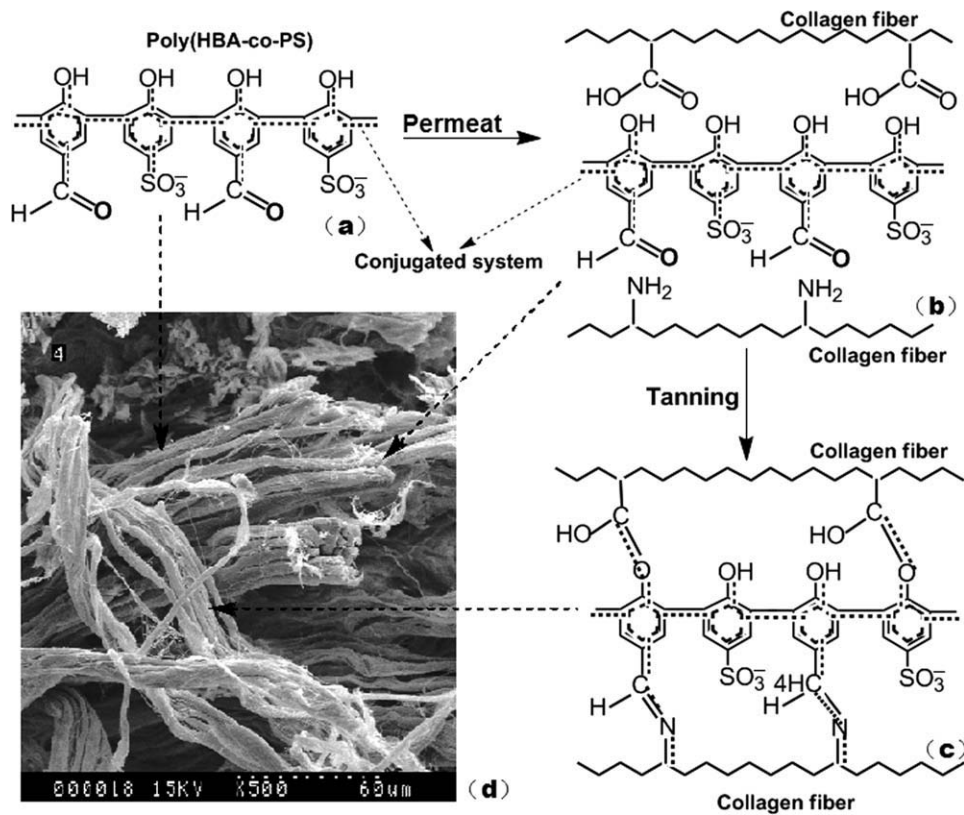


Figure 8. Tanning mechanism of poly(HBA-co-PS).

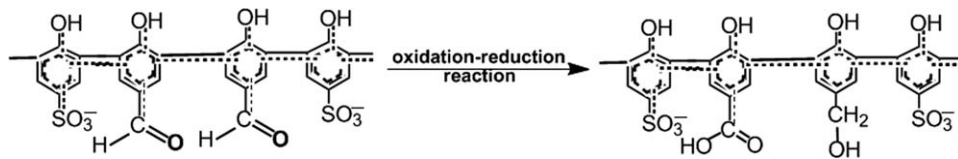


Figure 9. Schematic diagram of the oxidation-reduction reaction of aldehyde groups.

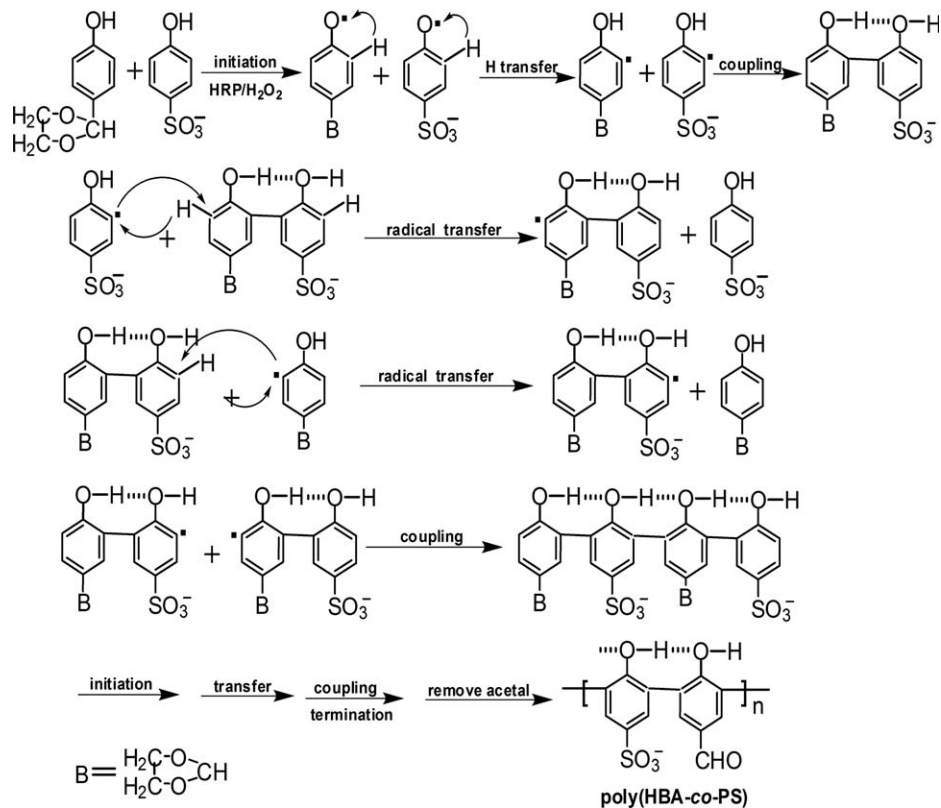


Figure 10. Schematic diagram of the enzymatic polymerization mechanism.

deprotection via the hydrolysis of acetals. A notable characteristic of the structure of the copolymer was that it contained a large conjugated system and active aldehyde and hydroxyl groups in its structure. The applied results indicated that the copolymer had excellent tanning effects and improved the T_g of leather from 45.3 to 83.6°C. The results indicate that poly (HBA-co-PS) may be used for tannage and has good prospects for applications and popularization.

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