

Synthesis and Leather-tanning Properties of a Starch-polyacrylamide Graft Copolymer by Means of HRP

Gang Liu, Shenghua Lv, Yanfen Ma*

College of Resource and Environment, Shaanxi University of Science & Technology, Xi'an 710021, Shannxi, P.R. China

Abstract: A starch-polyacrylamide graft copolymer has been synthesized in water by means of horseradish peroxidase (HRP)/H₂O₂/acetylacetone (ACAC). It is discussed through single factor experiment that the effects of system pH, reaction temperature and amount of HRP on the residual acrylamide (AAM) content. The structure and properties of the graft copolymer are characterized by Fourier Transform Infrared spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) and Gel Permeation Chromatography (GPC). The results show that the ideal copolymer was synthesized when mass ratio of starch and AAM was 2:1, and catalyst was HRP/H₂O₂ at 30 °C for 3.5 h. The residual AAM content was about 1.0%, and the weight-average molecular weight (M_w) and number-average molecular weight (M_n) distribution of graft copolymer are wide, and compared with starch, they reduces so obviously that the water-solubility of graft copolymer increases greatly. The retanned leather has many advantages, including excellent increased rate of thickness, good dye effect and eligible mechanical properties.

Key Words: horseradish peroxidase; starch; acrylamide; graft copolymer; synthetic tannage

1 Introduction

In recent years, more and more people pay attention to enzymatic polymerization reaction, because not only it possesses the advantages including high efficiency, high selectivity and mild reaction conditions, but also it is more important that we can obtain substances with special structure which can't be synthesized by means of traditional chemical reaction. Furthermore, Klivanov^[1] found some enzyme could catalyze polymerization in organic solvent, which made Polymerization the application of enzyme catalysis get rid of aqueous solution. HRP is a kind of enzyme which has extensive application prospect in enzymatic polymerization. It is extracted from the roots of horseradish and has high catalytic activity. In the presence of hydrogen peroxide, HRP can catalyze phenols, aromatic amines and their derivatives to polymerize to obtain some polymer products with special structures without formaldehyde^[2]. It is a green preparation method.

Simona^[3] had studied the reactivity of HRP with water insoluble phenolic compounds, and obtained a novel 4-phenylphenol ortho dimer. Lalot^[4] had studied radical polymerization of acrylamide in aqueous solution using HRP/H₂O₂/ACAC ternary system as initiator and made preliminary research on kinetics. Based on the research of Lalot, Cai and Sun^[5] had studied the effect of reaction temperature and initial concentrations of HRP, ACAC, H₂O₂ and AAM upon AAM polymerization kinetics catalyzed by HRP catalyze using dilatometer, and then obtained the optimum reaction conditions. The substances with unique properties and structures could be obtained by aforementioned methods which are green synthetic methods.

In the present, the developing green leather chemicals, reducing the pollution in tanning progress and the implementing clean production are research focus in leather industry. Starch is a kind of rich, biodegradable, environmentally friendly and renewable resource, and is a important raw material made

Corresponding author. Phone: +86-(0)13891098330. E-mail: lvsh@sust.edu.cn

for green chemicals. It's one of the main research directions that modified starch is used as green chemical material now and in the future. Starch graft copolymerization is one of main methods of starch modification, and ceric ammonium nitrate or ammonium persulfate is used in traditional starch modification. The initiation ability of ceric ammonium nitrate is very high, but it has low initiating activity for styrene, methacrylic acid and vinyl acetate monomers and other disadvantages including large consumption, high cost and strict reaction conditions. Ammonium persulfate is cheap and non-toxic, and its initiation reaction is easy to control. But it needs high reaction temperature, and has slow initiation speed and low grafting ratio [6]. In this study, graft copolymerization of starch and acrylamide will be researched using HRP/H₂O₂/ACAC as catalyst, and this paper will explore the effects of system pH, reaction temperature and amount of HRP upon graft copolymerization. The structure and properties of the graft copolymer will be characterized by FTIR, NMR and GPC. Finally, the graft copolymer will be used in retanning leather.

2 Experimental

2.1 Materials

HRP with an activity of 330 u/mg was supplied by Beijing Biosynthesis Biotechnology Co., Ltd., starch was supplied by Tianjin Kermel Chemical Reagent Co., Ltd., acrylamide (AAM) was supplied by Tianjin Chemical Reagent Factory, acetylacetone (ACAC) was supplied by Guangdong Xilong Chemical Co., Ltd., hydrogen peroxide (H₂O₂, 30%, v/v) was supplied by Tianjin Chemical Reagent No. 3 Plant, potassium dihydrogen phosphate (KH₂PO₄), dipotassium hydrogen phosphate trihydrate (K₂HPO₄·3H₂O) was supplied by Tianxin Fine Chemical Development Center of Tianjin, ethanol absolute, sodium thiosulfate and potassium bromide were supplied by Xi'an Chemical Reagent Factory, potassium bromate was supplied by Beijing Yili Fine Chemicals Co., Ltd., potassium iodide was supplied by Tianjin Chemical Reagent No. 6 Plant, hydrochloric acid was supplied by Xi'an Sanpu Fine Chemicals Factory.

2.2 Preparation of graft copolymer

27.84 g of KH₂PO₄ and 10.62 g of K₂HPO₄·3H₂O dissolved in 1000 ml of deionized water to prepare phosphate buffer solution pH 7.0. The solution was calibrated by pH apparatus. Appropriate amount of HRP weighted precisely dissolved in some deionized water, then stored in a refrigerator at 4 °C until use.

The reaction was carried out in a 500-ml three-neck round-bottom flask when mass ratio of starch and AAM was 2:1, and catalyst was HRP/H₂O₂ at 30 °C for 3.5 h. Then the solution was poured into 200 ml of absolute ethanol to precipitate the product, and then the product was filtered and again washed with 100 ml of absolute ethanol. Finally, the product was refiltered and dried in vacuum oven at 80 °C for 5 h.

2.3 Application experiment

Retanning process was as follow: Goat wet blue → Weighing → Washing → Soaking Back → Neutralizing → Determination of leather thickness and shrinkage temperature (*T_s*) → Retanning → Determination of leather thickness and *T_s* → Dyeing → Fatliquoring → Natural drying

2.4 Characterization

2.4.1 FTIR Analysis

FTIR spectra were recorded on a EQUINOX-55 spectrometer (Bruker, Bremen, Germany). KBr pellets made from a 1% (by weight) polymer and KBr mixture were used to record the FTIR spectra.

2.4.2 NMR Analysis

¹H-NMR spectra and ¹³C-NMR spectra were recorded on a INOVA-400MHz spectrometer (Varian, Palo Alto, USA). Samples were prepared in deuterated dimethyl sulphoxide (DMSO-d6).

2.4.3 Determination of average molecular weight

The average molecular weight and polydispersity of the graft copolymer were characterized by GPC using a model 2414 refractive index detector (Waters, Milford, USA).

2.4.4 Determination of residual AAM content

1 g of graft copolymer weighted precisely dissolved in 100 ml distilled water, and then placed in 250-ml iodine flask. 20 ml of bromine standard solution prepared by potassium bromate and potassium bromide was pipetted to the flask, and then 10 ml of hydrochloric acid (1:1, v/v) was also added. The flask was covered with lid as soon as possible, and some distilled water was poured on the lid to prevent bromine from volatilization. The flask was placed in dark for 30 min, after that 10 ml potassium iodide solution (20%, w/w) was poured into the flask rapidly. Then the mixture in flask with 2 ml of starch indicator was titrated with sodium thiosulfate standard solution, until blue disappear. At the same time, it was recorded that the consumption volume of sodium thiosulfate standard solution. Finally, the same experiment was carried out without 1 g of graft copolymer. Equation of residual AAM content was showed in equation 1.

$$R = \frac{c(V_0 - V)M \times 0.0005}{m} \times 100\% \quad (1)$$

In equation 1, R is residual AAM content that implies the percentage of AAM monomer that was not converted into polyacrylamide (PAAM). V_0 is the consumption volume of sodium thiosulfate standard solution without graft copolymer in titration experiment (ml). V is the consumption volume of sodium thiosulfate standard solution (ml). M is molar mass of AAM (g/mol). m is the precise mass of graft copolymer (g).

2.4.5 Measurement of leather thickness

3 lines were marked as A, B and C on the wet blue from backbone area to belly area, also 3 points were marked each line, such as a_1 , a_2 and a_3 . The thickness of 9 points was measured before and after retanning. The thickness value of A was thickness average of a_1 , a_2 and a_3 , and the same to B and C. The equation for calculating the increased rate of thickness was showed in equation 2.

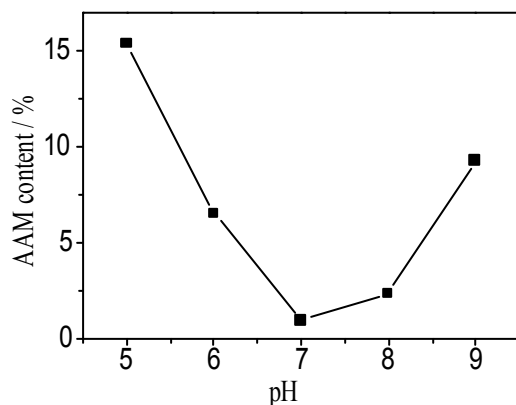
$$R = \frac{d_2 - d_1}{d_1} \times 100\% \quad (2)$$

In equation 2, R is the increased rate of thickness. d_1 is the thickness of wet blue before retanning. d_2 is the thickness of wet blue after retanning.

2.4.6 Characterization of leather properties

Leather T_g was measured by Digital Leather Shrinkage Temperature Tester MSW-YD4 before and after retanning. Mechanical properties of retanned leather were measured by Tensile Machine TS2000-S, including tensile strength, tear strength, elongation at set strength and elongation at break.

Fig. 1 The effect of the system pH upon the residual AAM content



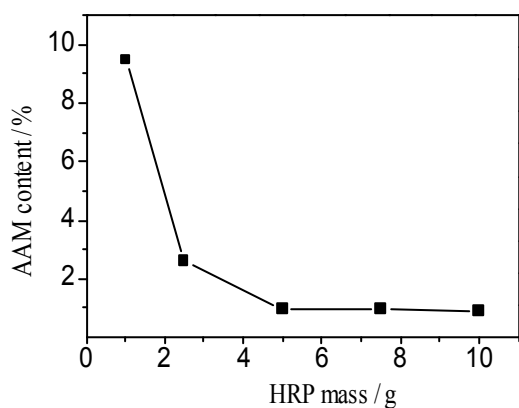


Fig. 3 The effect of the HRP mass upon the residual AAM content

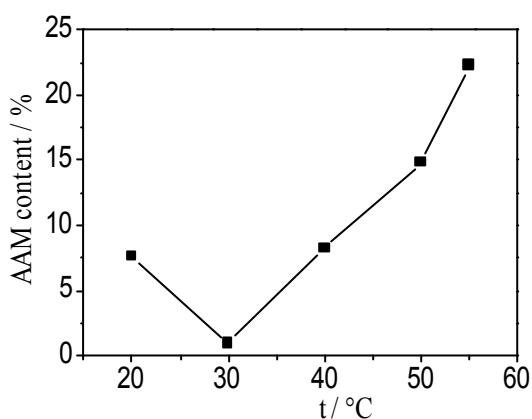


Fig. 2 The effect of the reaction temperature upon the residual AAM content

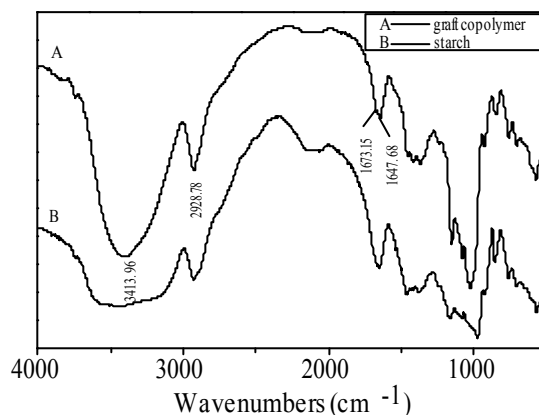


Fig. 4 The FTIR of starch and graft copolymer

3 Results and discussion

3.1 Factors of effect on the polymerization

3.1.1 System pH

Enzyme is produced by the cells of biological macromolecules, which is sensitive to pH. It can keep high activity at optimum pH. The effect of pH upon polymerization is showed in Fig. 1. In this reaction the optimum pH of HRP is 7.0, and inhibition of acid for HRP is very obvious.

3.1.2 Reaction temperature

The effect of reaction temperature upon polymerization is showed in Fig. 2. HRP lost activity easily at high and low temperature. With reaction temperature become high, residual AAM content reduces first then increases. HRP could keep high activity at 20-50 °C, and begin to lost activity higher than 50 °C. So the optimum reaction temperature is 30 °C.

3.1.3 Amount of HRP

The effect of amount of HRP upon polymerization is showed in Fig. 3. Although HRP is a very efficient biological catalysts, amount of HRP in experiments can not be too few, otherwise will not be able to initiate polymerizations. So amount of HRP in this reaction should be 5 mg.

3.2 Characterization of graft copolymer

3.2.1 FTIR Analysis of graft copolymer

The FTIR data for the graft copolymer are showed in Fig. 4. The absorption peak at 1673.15 cm^{-1} correspond to the -C=O stretching vibration of the amide I. The in plane bending vibration absorption peak corresponding to N-H is called amide II, which is appeared at about 1600 cm^{-1} in free primary amide and moved to about 1640 cm^{-1} in association primary amide. The amide II of graft copolymer may be covered by absorption peak at 1647.68 cm^{-1} of starch. Two stretching vibration absorption peaks

corresponding to N-H should appear at 3500-3050 cm^{-1} , which may cover by stretching vibration absorption peak of starch. On the other hand, comparing with wide stretching vibration absorption peak corresponding to -O-H of starch, stretching vibration absorption peak corresponding to -O-H of graft copolymer is clearly narrow, probably because amount of hydroxyl has reduced after grafting acrylamide. And stretching vibration absorption peak at 2928.78 cm^{-1} corresponding to $-\text{CH}_2-$ of graft copolymer is stronger than starch, probably because acrylamide has brought lots of methylene.

3.2.2 NMR Analysis of graft copolymer

The ^{13}C -NMR data for the graft copolymer are showed in Fig. 5. Resonance peaks from starch are distributed according to chemical shift of carbon as follow, δ 100.996 ppm (C1), δ 79.579 ppm (C4), δ 74.101 ppm (C5), δ 72.755 ppm (C3), δ 72.512 ppm (C2), δ 61.304 ppm (C6). Otherwise, resonance peak at δ 184.753 ppm is from amide, and resonance peaks at δ 56.851 ppm and δ 19.364 ppm are from graft segment. According to empirical formula [7], resonance peak corresponding to C9 should appear at δ 40 ppm, which may be covered by DMSO- d_6 resonance peaks.

The ^1H -NMR data for the graft copolymer are showed in Fig. 6. Resonance peaks from starch are distributed according to chemical shift of hydrogen as follow, δ 5.103 ppm (H1), δ 4.605 ppm (H2), δ 4.395 ppm (H3), δ 3.650 ppm (H6), δ 3.583 ppm (H6'), δ 3.416 ppm (H5), δ 3.324 ppm (H4). In addition, resonance peaks at δ 3.450 ppm (H7) and δ 1.058 ppm (H8) are from graft segment, and according to empirical formula [7], resonance peak corresponding to H9 should appear at δ 2.5 ppm, which may be covered by DMSO- d_6 resonance peaks. Resonance peak at δ 5.533 ppm and δ 5.472 ppm may from amide and hydroxyl.

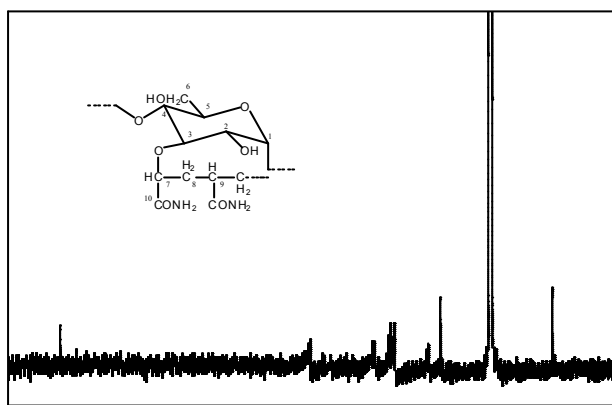


Fig. 5 The ^{13}C -NMR of graft copolymer

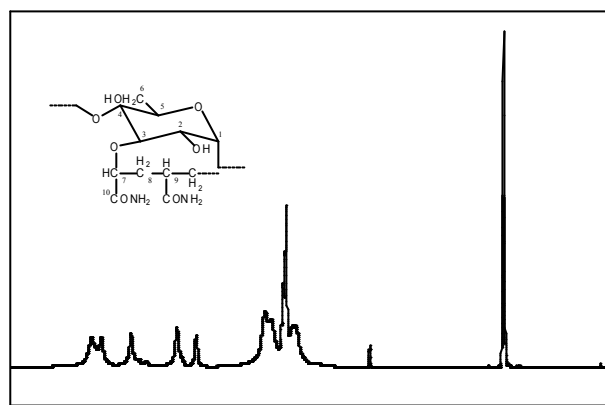


Fig. 6 The ^1H -NMR of graft copolymer

3.2.3 GPC Analysis of graft copolymer

The GPC data for the graft copolymer are showed in Table 1. Table 1 shows that the weight-average molecular weight (M_w) and number-average molecular weight (M_n) distribution of graft copolymer are wide, and comparing with starch, they reduces so obviously that the graft copolymer water-soluble increases greatly. In retanning leather, molecule with high M_w can permeate in leather where fibers are loose, and molecule with low M_w can permeate in leather where fibers are compact, thereby the graft copolymer shows good selective filling.

Tab. 1 Average molecular weights and polydispersity index of the graft copolymer

M_n	M_w	polydispersity index
30894	43869	1.42
16894	22561	1.34
9683	14016	1.45
5624	8502	1.51

3.2.4 Reaction Scheme

Reaction scheme for starch-polyacrylamide graft polymerization is showed in Fig. 7.

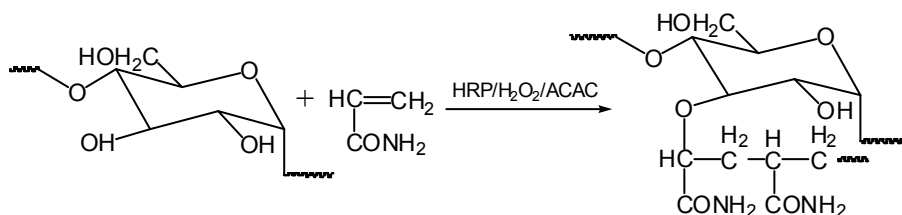


Fig. 7 The schematic diagram of graft polymerization

3.3 Retanning results analysis

Retanning leather experimentation has been carried out using the graft copolymer (marked as 1, 2, 3, 4, and 5). Table 2 shows that increase of retanned leather T_s proves the graft copolymer plays a certain role for wet blue, and that the thickness of retanned leather increases also, which is incremental along A, B, C proves the graft copolymer has good selective filling. The retanned leather had many advantages, including an excellent thickness ratio and good dye effect.

It is used as comparable leather (marked as 0) that leather retanned by starch and vinyl monomer graft copolymer initiated by ammonium persulfate. Table 3 shows leather retanned by the graft copolymer has same properties to comparable leather in elongation at set strength and elongation at break, and has better properties to comparable leather in tensile strength and tear strength, that proves the graft copolymer plays a enhanced role in retanning leather.

Tab. 2 The retanning results of synthesis tannage

Number	T_s of retanned leather ($^{\circ}\text{C}$)			Increased rate of thickness (%)		
	Before retanning	After retanning	ΔT_s	A	B	C
1	112.6	113.8	1.2	18.95	22.34	27.65
2	111.6	112.0	0.4	19.52	23.27	27.77
3	108.5	109.3	0.8	20.31	23.95	28.73
4	109.4	111.0	1.6	20.11	24.15	28.82
5	110.7	112.2	1.5	19.28	24.25	29.16

Tab. 3 The physical and mechanical properties of retanned leather

Samples	elongation at break (%)	elongation at set strength (%)	tensile strength (N/mm^2)	tear strength (N/mm)
0 transverse sample	63.44	45.91	8.70	34.61
0 longitudinal sample	60.21	43.35	9.64	38.92
1 transverse sample	63.41	40.44	11.87	45.23
1 longitudinal sample	59.57	36.41	12.81	41.42
2 transverse sample	70.03	47.56	10.20	47.67
2 longitudinal sample	67.48	43.28	11.97	43.96

4 Conclusions

A starch-polyacrylamide graft copolymer has been synthesized in water by means of horseradish peroxidase (HRP)/ H_2O_2 /acetylacetone (ACAC). It has been discussed through single factor experiment that the effects of system pH, reaction temperature and amount of HRP on the residual AAM content. The ideal copolymer was synthesized when mass ratio of starch and AAM was 2:1, and catalyst was HRP/ H_2O_2 at 30°C for 3.5 h. The structure and properties of the graft copolymer have been characterized by FTIR, NMR and GPC. FTIR and NMR results show that the designed polymerization has took place, GPC result shows that the M_w and M_n distribution of graft copolymer are wide, and comparing with starch, they reduces so obviously that the graft copolymer water-soluble increases greatly.

The graft copolymer is used in retanning leather experimentation. The results show that increase of retanned leather T_s proves the graft copolymer plays a certain role for wet blue, and that the thickness of retanned leather increases also, which is incremental along A, B, C proves the graft copolymer has good selective filling. The retanned leather has many advantages, including an excellent increased rate of thickness and good dye effect and eligible mechanical properties.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China (No.

20876091), Shannxi Natural Science Foundation of Shannxi province China (No.SJ08B06), and the Graduate Innovation Fund of Shaanxi University of Science and Technology.

References

- [1] M. P. Scolla; G. Sigal; A. M. Klibanov. *Biotechnology and Bioengineering*, 1985, 27(3): 247-252.
- [2] W. J. Hong; C. H. Zhang; G. Y. Lu. *Chemical of Life*, 2005, 25(1): 33-36.
- [3] S. Sgalla; G. Fabrizi; S. Cacchi; A. Macone; A. Bonamore; A. Boffi. *Journal of Molecular Catalysis B: Enzymatic*, 2007, 44(3): 144-148.
- [4] D. Teixeira; T. Lalot; M. Brigodiot; E. Marechal. *Macromol.* 1999, 32: 70-72.
- [5] Z. Q. Cai; J. Z. Sun; Q. Y. Zhou. *Journal of Functional Polymers*, 2004, 17(1): 81-86.
- [6] Y. S. Zhang. *Production and Application of Modified Starch Handbook*, Beijing: China Light Industry Press, 1999.
- [7] H. Zhang; Q. J. Peng; Y. M. Li. *Spectral Analysis of Modern Organic*, Beijing: Chemical Industry Press, 2005.