



# Preparation and rheological properties of starch-g-poly(butyl acrylate) catalyzed by horseradish peroxidase



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## ABSTRACT

A “green” method for the preparation of starch-g-poly(butyl acrylate) (starch-g-PBA) copolymers using a biocatalyst, horseradish peroxidase (HRP), in the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and acetylacetone (Acac) was developed. The chemical structures and thermal properties of the grafted starch were analyzed by Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (<sup>1</sup>H NMR), elemental analysis, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). FT-IR, elemental analysis, and <sup>1</sup>H NMR confirmed that PBA was successfully grafted onto starch by HRP-mediated graft copolymerization. DSC and TGA results showed that the grafting of PBA chains improved the thermal stability of starch. The effects of grafting on the rheological properties of the starch pastes were also studied. Compared to control starch, the grafted starch paste had higher viscosity and showed better elastic behavior. The effects of gelatinization and addition of ethanol on the graft copolymerization were also investigated. The results indicated that gelatinization and addition of ethanol improved the grafting percentage (GP, 5.66%) and grafting efficiency (GE, 19.39%).

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## 1. Introduction

Starch, a natural polysaccharide, is one of the most widely used natural polymers in the field of medicine and in the manufacturing of paper, food, textiles, adhesives, biofuel, and many others. Its abundance, low toxicity, renewability, and biodegradability are the main features of starch, which make it highly attractive for industrial applications [1,2]. However, it is well known that native starch has many disadvantages in terms of its properties, which make it unsuitable for industrial applications and limit the scope of its uses. Therefore, in order to improve the potential value of starch and widen its utility, native starch was modified using physical, chemical, and other methods [3,4]. Recently, chemical modification of starch has attracted tremendous attention, due to its high reaction efficiency. Chemical modification of starch (which includes oxidation, hydrolysis, esterification, etherification, graft copolymerization, etc.) is a powerful means to improve the properties of starch [5].

The purpose of graft copolymerization is to attach side chains with some specific properties to the main polymeric backbone [6]. In the past decades, starch was graft copolymerized with some vinyl monomers using different chemical initiators. Depending on the purpose of graft copolymerization, the vinyl monomers with the desirable functionalities were used. The vinyl monomers used for starch modification mainly include acrylamide [7], acrylic acid [8], methyl acrylate [9], vinyl acetate [10], styrene [11], and butyl acrylate (BA) [12].

Poly(butyl acrylate) (PBA) is a high molecular weight polymer that has good elongation at break and excellent hydrophobicity, due to its long alkyl ester chains [1,12]. Previous reports suggest that BA was used in the chemically initiated grafting reaction with starch to improve the properties of starch and expand the scope of its applications [13–17]. However, it should be noted that chemical modification also has some disadvantages such as difficulty in terms of controlling the reaction and the complexity of graft copolymerization process [18]. The remaining chemical initiators in the grafted products could cause some negative effects on the environment. Shortcomings associated with the above mentioned methods call for research of new green chemistry methods to tackle the above problems. In this context, enzyme such as horseradish peroxidase (HRP), due to its eco-friendly nature and mild reaction

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process, has great potential to be used as a biotechnological catalyst. The potential use of HRP to initiate free radical polymerization of vinyl monomers was first investigated by Derango et al. [19]. The HRP-mediated reaction mechanism of acrylamide polymerization was investigated by Lalot et al. [20,21]. The free polymerization of methyl methacrylate catalyzed by HRP was reported by Bhanu et al. [22]. Moreover, HRP-catalyzed acrylamide polymerizations in aqueous media with and without surfactants and polymerization in concentrated emulsions using sorbitan monooleate as the emulsifier were also studied [23]. Shogren et al. were the first to study that the synthesis of starch-acrylamide graft copolymers using an initiator system comprising of HRP/H<sub>2</sub>O<sub>2</sub>/2,4-pentanedione [24]. In addition to this, Lv et al. synthesized starch-g-p(DMDAAC) using HRP initiator and correlated its structure to sludge dewaterability. The results showed that the structure that was formed by grafting of DMDAAC oligomers onto the starch backbone in the form of branched chains had stronger hydrophobic regions and higher cationic degree [25].

It is well-known that the hydrophilicity of native starch is very high and the viscoelastic properties of starch paste are not satisfactory, which greatly limit its applications in textile sizing. In order to overcome these shortcomings, a kind of flexible macromolecule like PBA, which has excellent hydrophobicity, was grafted onto starch molecules using chemical or physical initiators [15]. However, there are no reports on the preparation of starch-g-PBA by green methods, especially the graft copolymerization of starch and BA catalyzed by HRP/H<sub>2</sub>O<sub>2</sub>/Acac. Hence, the present study focuses on the synthesis of starch-g-PBA using HRP/H<sub>2</sub>O<sub>2</sub>/Acac. Furthermore, the effects of gelatinization and ethanol on the graft copolymerization and also the effects of graft modification on the rheological properties of starch paste have been investigated. The illustration of the grafting reaction of BA onto the starch catalyzed by HRP is shown in Fig. 1.

## 2. Materials and methods

### 2.1. Materials

HRP with an activity of 380 U/mg was provided by Aladdin Reagent Ltd. (Shanghai, China). Starch ( $\leq 13\%$  water content) and hydrogen peroxide [30% (w/v)] were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). All other chemicals used in the experiment were also obtained from Sinopharm Chemical Reagent Co. and were of analytical reagent grades.

### 2.2. Synthesis of graft copolymers

Starch (5.0 g, dry weight) mixed with 80 mL water was gelatinized by stirring for 10 min at 100 °C in a three-necked, round-bottomed flask and then cooled to room temperature. Then, 10 mL potassium phosphate buffer (0.1 M, pH 7.0), 5.0 g BA, and specific amounts of Acac and HRP were successively added to the three-necked flask. The mixture was degassed by purging with nitrogen for 30 min under stirring. Next, 100  $\mu$ L of H<sub>2</sub>O<sub>2</sub> was added drop-wise within 30 min. Finally, the reaction mixture was stirred for 5 h at 40 °C.

After the reaction, the mixture was poured into absolute ethanol to remove unreacted BA and other reagents and the crude product was obtained. The crude product was weighed after drying to constant weights under vacuum at 50 °C. The crude product also contained some amount of homopolymer. The homopolymer was separated by extraction with acetone for 12 h to obtain pure grafted starch. Control starch was obtained using the same reaction conditions except that it was carried out without HRP/Acac/H<sub>2</sub>O<sub>2</sub>.

The grafted starch was weighed accurately under absolutely dry conditions and hydrolyzed with 100 mL hydrochloric acid (1.0 mol/L) in a water bath at 98 °C. After certain time intervals, several drops of the reaction mixture were withdrawn using a dropping pipette and added into a solution of I<sub>2</sub>-KI until the solution did not turn blue. Then, the mixture was neutralized with a solution of sodium hydroxide (1.0 mol/L) and washed until the washing did not form a white precipitate when tested with a solution of AgNO<sub>3</sub>. The solid product, PBA grafted onto starch molecule, obtained after centrifugation and washing was dried to constant weight under vacuum at 50 °C.

### 2.3. Characterization

The grafting parameters, grafting percentage (GP) and grafting efficiency (GE), of grafted starch were evaluated by the weighing method, which reflected the amount of PBA grafted onto the starch. And all the samples were dried to constant weights under vacuum at 50 °C. The GP and GE were calculated using following equations:

$$GP(\%) = \frac{M_1}{M_2} \times 100 \quad (1)$$

$$GE(\%) = \frac{M_1}{M_1 + M_3} \times 100 \quad (2)$$

where:  $M_1$  was the weight of grafted chains (g),  $M_2$  was the weight of grafted starch (g), and  $M_3$  was the weight of homopolymer (g).

The FT-IR analysis was performed on a Nicolet IS10 FT-IR spectrometer (Thermo Fisher Scientific, USA). The spectra were recorded in the absorption mode in the range of 4000–500 cm<sup>-1</sup> with a 4 cm<sup>-1</sup> resolution, using KBr pellets made from a mixture of polymer (1 wt%) and KBr. <sup>1</sup>H NMR analysis was conducted to confirm the grafting of starch with monomers and the formation of grafted starch. Each of the purified sample (20 mg) was dissolved in 450–500  $\mu$ L of DMSO-*d*<sub>6</sub>. The <sup>1</sup>H NMR spectra of these samples were recorded on a Bruker Avance III spectrometer (Bruker, Germany), which operated at a frequency of 400 MHz and using tetramethylsilane (TMS) as an internal standard. An elemental analyzer (Vario ZL III, Elementar, Germany) was used for the analysis of C, H, and O elements in grafted starch and the control samples. Differential scanning calorimetry (DSC) was conducted on TA-Q200 (Waters, Shanghai) with N<sub>2</sub> flow rate of 20 mL/min. The temperature was varied from –80 to 250 °C at a heating rate of 10 °C/min. Thermogravimetric analysis (TGA) was carried using a TGA-Q500 thermal analysis system (TGA Instruments, USA) in the temperature range of 30–600 °C at a heating rate of 20 °C/min.

### 2.4. Rheological measurements

Steady shear and dynamic viscoelastic properties of the control and grafted starch were measured using a rheometer (Physica MCR 301, Anton Paar GmbH, Austria), equipped with a cone and plate system (1° cone angle, 5 cm diameter and 0.05 mm gap). The experiments were carried out in the log mode. For steady shear measurements, the fresh paste sample was placed in the rheometer and equilibrated at 25 °C. The sample was sheared continuously to obtain shear rate versus shear stress data. The test was programmed to increase the shear rate from 0 to 300 s<sup>-1</sup> within 3 min (upward flow curve) and decrease immediately from 300 to 0 s<sup>-1</sup> in the next 3 min (downward flow curve).

The dynamic viscoelastic measurement was performed with frequency sweeps in the range of 1–10 r/s with constant deformation (0.5% strain) within the linear viscoelastic region. The frequency sweep tests were also performed at 25 °C. The storage modulus ( $G'$ ), loss modulus ( $G''$ ), and complex viscosity as a function of angular frequency were obtained. The ungelatinized starch was used

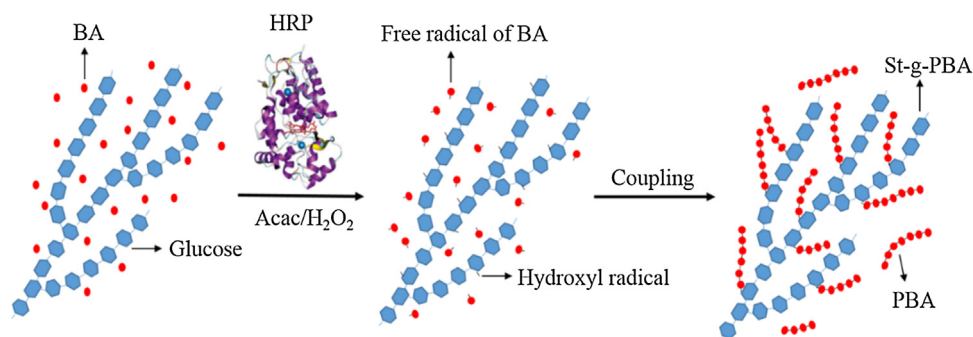


Fig. 1. Schematic illustration of the grafting reaction of BA onto the starch catalyzed by HRP.

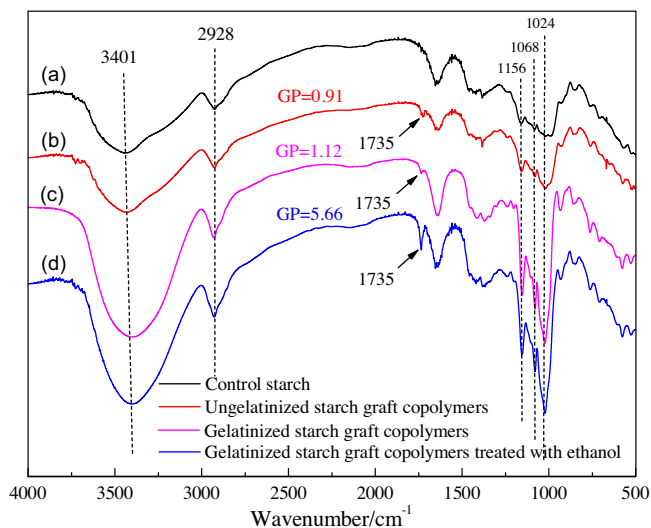


Fig. 2. FT-IR spectra of control starch and grafted copolymers prepared with different treatments.

for preparing starch-g-PBA copolymers, which was gelatinized and used in the measurement of rheological properties.

### 3. Results and discussion

#### 3.1. FT-IR analysis

To demonstrate the grafting reaction and study the effects of gelatinization and ethanol on the graft copolymerization, the FT-IR spectra of starch and grafted starch prepared under four different conditions are illustrated in Fig. 2. The FT-IR spectrum of starch is shown in Fig. 2A. A broad peak appeared at approximately  $3401\text{ cm}^{-1}$ , due to the hydroxyl groups of starch and water. The bands at  $2928$  and  $1640\text{ cm}^{-1}$  could be attributed to the C–H stretching vibrations and bending vibrations of the associated water molecules, respectively. The bands at  $1156$  and  $1068\text{ cm}^{-1}$  were to the result of the C–O stretching vibrations of the glucose ring. The sharp peak at  $1024\text{ cm}^{-1}$  was attributed to the C–O stretching frequency of the C–O–C bond in starch, which suggested an  $\alpha$ -1,6 linkage [8,26].

The FT-IR spectrum of the ungelatinized starch graft copolymers (Fig. 2B) showed all the characteristic absorption peaks of starch. Additionally, the peak at  $1735\text{ cm}^{-1}$  was due to  $\text{COO}^-$  stretching vibration in PBA [27], which indicated that PBA had been successfully grafted onto the starch molecule. Additionally, in order to improve the graft copolymerization, the grafted copolymers were prepared using gelatinized starch and BA. The FT-IR spectrum of the gelatinized starch graft copolymers (Fig. 2C) showed a weak

absorption peak of  $\text{COO}^-$  at  $1735\text{ cm}^{-1}$ , which indicated that a small amount of BA had been grafted onto the starch molecules. Gelatinization process destroyed the starch granules and the hydrogen bonds between the starch molecules, which had a positive effect on the graft copolymerization. Due to increased number of starch molecular chains getting exposed in the grafting reaction as a result of gelatinization increased the chances of contact between the hydroxyl radicals and the monomer. Therefore, the GP of gelatinized starch (GP = 1.12%) was slightly higher than the GP of ungelatinized starch.

However, due to poor solubility of BA in water, it was required that the reaction be carried out in a heterogeneous system. In order to improve the solubility of BA in water, ethanol was introduced into the graft copolymerization system of gelatinized starch. The FT-IR spectrum of the gelatinized starch graft copolymers produced by including ethanol in the reaction process is shown in Fig. 2D. By contrast, the characteristic absorption peak of  $\text{COO}^-$  at  $1735\text{ cm}^{-1}$  was obviously stronger than the  $1735\text{ cm}^{-1}$  peaks in Fig. 1B and C, which was also consistent with the increased peaks GP (GP = 5.66%). Based on above analysis, gelatinization and addition of ethanol in the homogeneous system could boost the grafting reaction. This was found to promote the catalysis of HRP in the course of graft copolymerization reaction. Therefore, the FT-IR results not only suggested that PBA had been successfully grafted onto the starch molecules, but also demonstrated the advantages of gelatinization and addition of ethanol on graft polymerization.

#### 3.2. $^1\text{H}$ NMR analysis

In order to further prove that PBA had been successfully grafted onto the chains of starch molecules,  $^1\text{H}$  NMR spectra of control starch, grafted starch, and PBA were recorded. The structures and chemical shifts of starch, grafted starch, and PBA are shown in Fig. 3. As shown in Fig. 3A, the  $^1\text{H}$  NMR spectrum of starch showed peaks at 5.39–5.44 ppm, due to protons at C1 and C4. The peak appearing at 5.10 ppm was attributed to the C5 proton. The peak at 4.88 ppm was attributed to hydroxyl proton linked to C6. Peaks at 4.58 and 3.38 ppm were due to the protons and  $\text{OH}$  protons linked to C2 and C3, respectively. The  $^1\text{H}$  NMR spectrum of starch was consistent with previous studies [10,28].

Compared to the  $^1\text{H}$  NMR spectrum of starch, some new peaks could be seen in  $^1\text{H}$  NMR spectrum of grafted starch (Fig. 3B). New peaks appearing in the range of 0.88–1.52 ppm could be attributed to alkyl protons of the grafted side chains [1,17]. Peaks at 2.09 and 3.33 ppm were due to the C9 and C11 protons, respectively. Furthermore, a significant difference could be seen in case of the peak at 5.43 ppm, which was due to the C7 proton in the first unit of the grafted chains. This presence of this new peak suggested the successful linking of PBA with the starch molecules. Moreover, the change in the chemical shift of the C7 proton as a result of graft

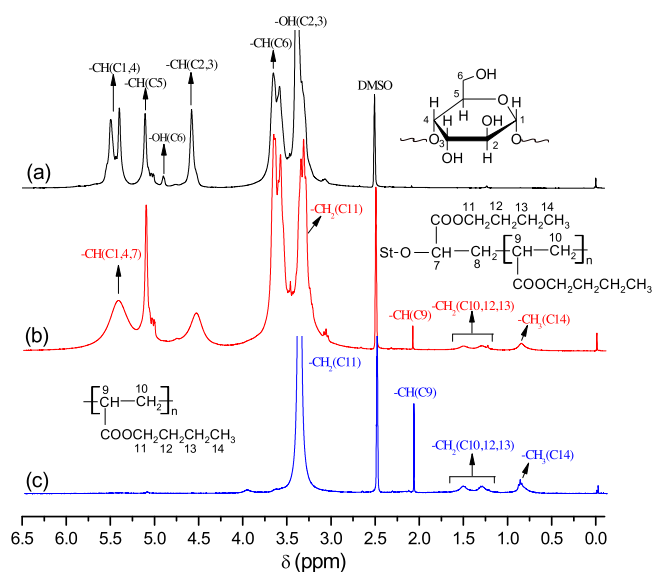


Fig. 3. <sup>1</sup>H NMR spectra of control starch (a), St-g-PBA copolymers (b) and PBA (c).

Table 1

Elements content of control starch and grafted starch.

Samples (Theoretical value)	C (%)	H (%)	O (%)
Control starch	38.62 (44.44)	7.53 (6.17)	53.58 (49.39)
Grafted starch (GP = 5.66% GE = 19.39%)	39.58 (45.93)	7.61 (6.40)	52.81 (47.67)

copolymerization was also evident. Obviously, the peak at 4.91 ppm disappeared in Fig. 3B. The disappearance of this peak was a powerful indication that the hydroxyl group at the C6 position was the active site of coupling with the free radicals of C7, with the formation of a new chemical bond (-CH<sub>2</sub>-O-CH-). In addition, all the split peaks in the <sup>1</sup>H NMR spectrum of PBA (Fig. 3C) could also be seen in Fig. 3B. Therefore, based on <sup>1</sup>H NMR analysis, the graft copolymerization of starch with PBA could be further confirmed.

### 3.3. Elemental analysis

To study the effects of grafting reaction on the elemental compositions of the products, the elemental analysis of the products was carried out. Results of the elemental analysis are presented in Table 1. The C and H contents of grafted starch were found to be 39.58% and 7.61%, which was higher than that of control starch (38.62% and 7.53%), respectively. However, compared to control starch, the O content of grafted copolymers decreased marginally. It should be noted that the measured value of C content was less than the theoretical value, whereas the measured values of H and O contents were higher than the theoretical values. This could be attributed to the residual water in the products. In addition to this, the changes in the trends of measured values were consistent with the changes in the trends of theoretical values. Therefore, results of elemental analysis further proved that the graft copolymerization of starch with PBA occurred and that changes in the compositions of the elements of starch were due to the grafting reaction.

### 3.4. DSC analysis

The DSC curves of control starch and grafted starch are presented in Fig. 4. As shown in Fig. 4A, the strong and broad endothermic peak of control starch in the range of 75–190 °C, with the peak temperature at 122.87 °C, could be attributed to the crystalline melting of starch. This was the result of hydrogen bonds

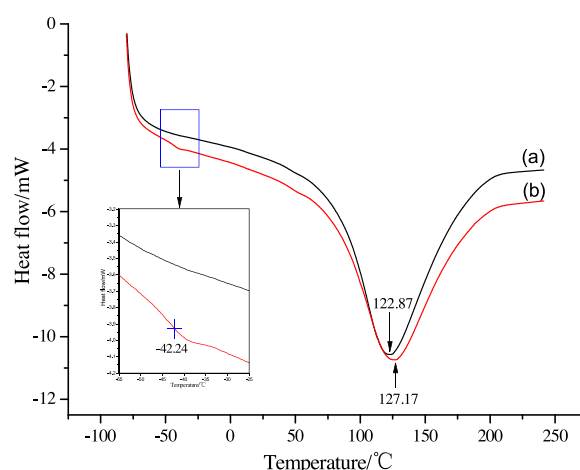


Fig. 4. DSC curves of control starch (a) and grafted starch (b).

formed between starch molecules and residual water. The high melting temperature reflected that starch had high cohesion energy and high soft temperature, which was in accordance with previous studies [16,26]. The DSC curve of grafted starch is shown in Fig. 4B. A weak endothermic peak was observed at -42.24 °C. According to some literature references [4,29], the glass transition temperature of PBA is -55 °C. Hence, it could be concluded that the endothermic peak at -42.24 °C was the result of grafting of side chains of PBA. Furthermore, the melting temperature of grafted starch (127.17 °C) was higher than control starch, which illustrated that PBA could increase the melting temperature of starch and improve its thermal stability. Therefore, DSC indicated that PBA had been grafted onto the starch molecules and this changed the melting temperature of starch.

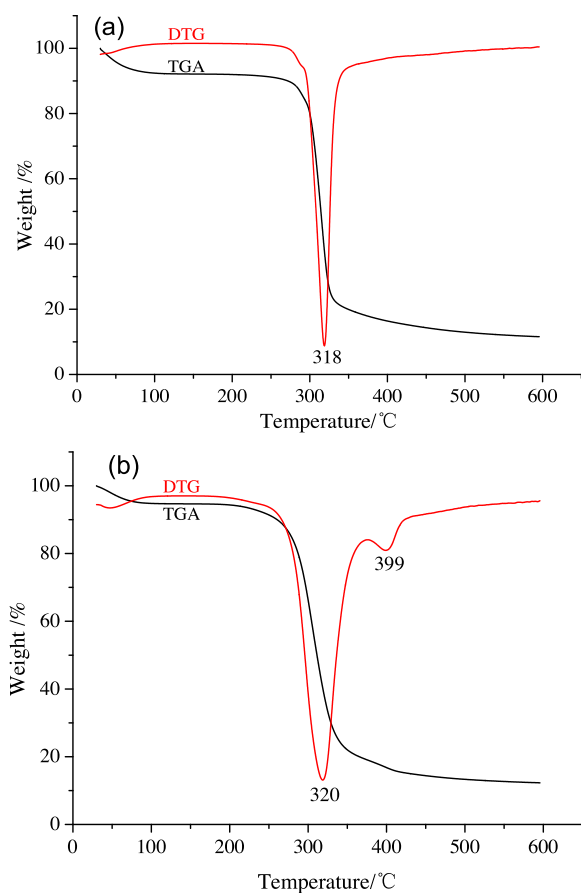
### 3.5. Thermogravimetric analysis

TGA-DTG was a viable and suitable method for studying the effects of PBA grafting on the thermal properties of grafted starch. The TGA and DTG curves of control starch and grafted starch are presented in Fig. 5 and the thermal decomposition data of control starch and grafted starch are presented in Table 2. The TGA curve of control starch (Fig. 5A) showed a characteristic three-stage decomposition pattern. The first stage of decomposition that occurred from 30 to 98 °C was due to the evaporation of moisture from the starch. The main decomposition of grafted starch occurred in the second stage between 254 and 354 °C, with 73.1% weight loss that was attributed to the degradation of starch molecules [17]. The DTG curve of control starch showed the maximum decomposition temperature to be 318 °C. The third stage from 354 to 600 °C occurred due to the formation and evaporation of some volatile compounds [30,31]. The char residue was 11.6% at 600 °C. In contrast to this, as shown in Fig. 5B, the grafted starch showed a four-stage decomposition pattern. The first and second stages were separately attributed to the evaporation of the moisture and the decomposition of starch, as in the case of the control. However, the moisture content of grafted starch was less than the control, which was attributed to the increase in slight hydrophobicity of the grafted starch, in form of the grafted chains of PBA. The third decomposition stage that occurred between 375 °C and 429 °C in case of grafted starch, corresponding to 4.7% weight loss, was caused by the decomposition of PBA grafts [32]. This further illustrated that PBA had been grafted onto the starch molecules. Furthermore, it should be noted that the maximum decomposition temperature of starch was slightly increased up to 320 °C and the char residue was 12.30% at 600 °C, which suggested that the grafted starch had higher thermal stabil-



**Table 2**  
Thermal decomposition data of control starch and grafted starch.

Sample	Degradation stage	Temperature range (°C)	T <sub>max</sub>	Weight loss (%)
Control starch	1	30–98	39	7.7
	2	254–354	318	73.1
	3	354–600	–	7.6
Grafted starch	1	30–94	47	5.0
	2	255–375	320	75.3
	3	375–429	399	4.7
	4	429–600	–	2.7

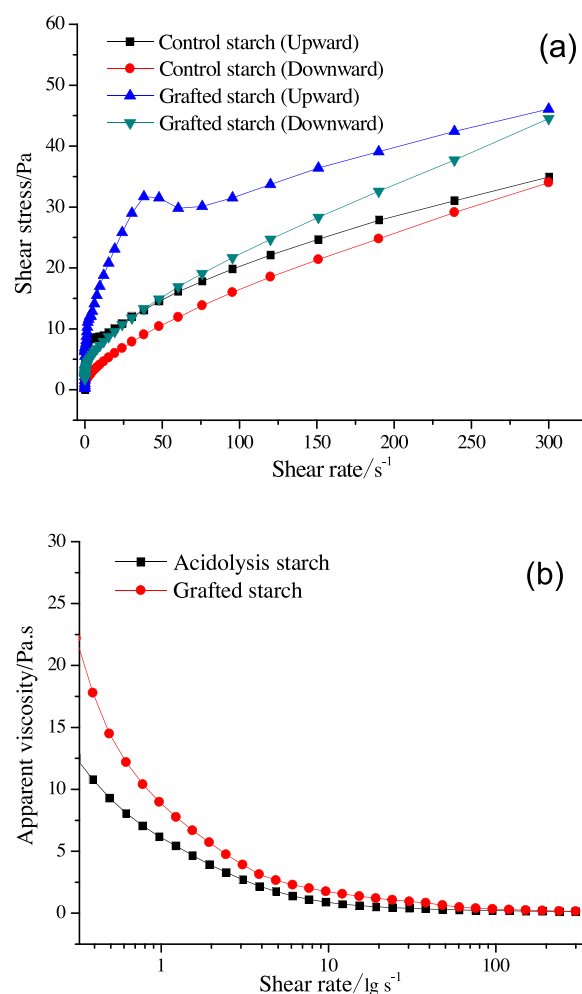


**Fig. 5.** TGA and DTG thermograms of control starch (a) and grafted starch (b).

ity than the control [29]. Therefore, the introduction of hydrophobic PBA chains could improve the thermal properties of starch to some extent, which was consistent with the DSC results.

### 3.6. Steady shear properties

The steady shear spectra of control starch and grafted starch pastes are presented in Fig. 6. For the range of shear rate used in this study, all the pastes showed mainly the shear-thinning characteristics with yield stress behavior. As shown in Fig. 6A, compared with the control, the grafted starch paste required higher shear stress under the same shear sweep. Moreover, the hysteresis loop area of the grafted starch paste between the upward and downward flow curves was obviously larger than the control. This suggested that the grafted starch had longer time for recovery to its original structure after shearing [33]. Moreover, the apparent viscosity of the grafted starch (Fig. 6B) also decreased with increase of shear rate, but was higher than the control at low shear rate. These results indicated that the introduction of grafted side chains increased the degree of branching on the starch molecules and intensified the



**Fig. 6.** Shear stress (a) and apparent viscosity (b) change versus shear rate of the samples.

intermolecular entanglement of the grafted starch, which increased the apparent viscosity and shearing sensitivity of starch paste.

### 3.7. Dynamic viscoelastic properties

The dynamic spectra (frequency-dependence of  $G'$  and  $G''$ ) of the control and grafted starch pastes are presented in Fig. 7. As shown in Fig. 7A, the storage moduli ( $G'$ ) of the samples were higher than the loss moduli ( $G''$ ) and both moduli showed only slight variations with angular frequency, which revealed weak gel-like behavior in all paste samples [34]. The  $G'$  and  $G''$  values of grafted starch paste were higher than the control, suggesting that the grafted starch paste showed more a viscous and elastic behavior than the control. Based on the values of  $G'$  and  $G''$ , the dynamic mechanical loss tangent ( $\tan \delta$ ) values are shown in Fig. 7B. The  $\tan \delta$  values of the grafted starch paste were obviously lower than the con-

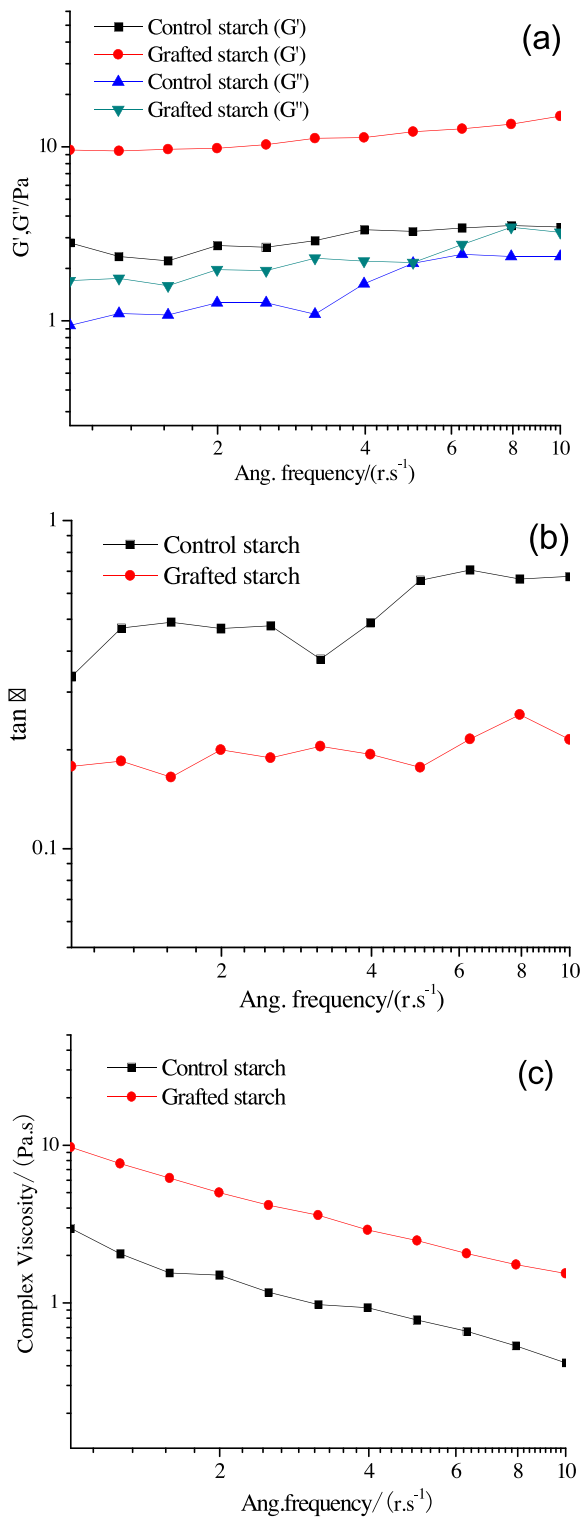


Fig. 7. Dynamic modulus (a),  $\tan \delta$  (b) and complex viscosity (c) curves of the samples.

control, which illustrated that the elastic behavior of grafted starch paste dominated over its viscous behavior [35]. Although all the samples showed gel behavior ( $G' > G''$ ), they also showed a slight frequency dependence, within the range of frequency used in the experiment. As shown in Fig. 7C, the complex viscosity of the samples decreased with increase of angular frequency, which suggested that the introduction of graft side chains did not change the shear-thinning behavior of starch paste. In addition, the complex viscosity

of the grafted starch paste was higher than that of the control. This result was attributed to the copolymerization of grafted side chains with starch backbones. This led to complicated molecular geometries in the paste. Therefore, it could be concluded that the viscoelastic properties of the starch paste markedly increased after the grafting reaction.

#### 4. Conclusions

In this study, a graft copolymer of starch with BA was synthesized using HRP/Acac/H<sub>2</sub>O<sub>2</sub> system. The grafting of PBA onto the molecules of starch, using HRP catalyst, was confirmed by FT-IR, <sup>1</sup>H NMR, and elemental analysis. Moreover, the FT-IR results demonstrated that gelatinization and ethanol favored graft copolymerization of starch with BA. The results of DSC and TGA illustrated that the introduction of PBA chains could improve the thermal stability of starch. Meanwhile, the viscosity of starch paste increased, and the grafted starch paste showed more elasticity after graft modification. This environmentally friendly process provides an attractive alternative for the preparation of modified starch for industrial applications.

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#### References

- [1] Z. Shi, N. Reddy, L. Shen, X.L. Hou, Y.Q. Yang, Effect of monomers and homopolymer contents on the dry and wet tensile properties of starch films grafted with various methacrylates, *J. Agric. Food Chem.* 62 (2014) 4668–4676.
- [2] C. Lan, L. Yu, P. Chen, L. Chen, W. Zou, G. Simon, X.Q. Zhang, Design, preparation and characterization of self-reinforced starch films through chemical modification, *Macromol. Mater. Eng.* 295 (2010) 1025–1030.
- [3] Q.X. Guo, Y.Q. Wang, Y. Fan, X.W. Liu, S.Y. Ren, Y.Z. Wen, B.J. Shen, Synthesis and characterization of multi-active site grafting starch copolymer initiated by KMnO<sub>4</sub> and HIO<sub>4</sub>/H<sub>2</sub>SO<sub>4</sub> systems, *Carbohydr. Polym.* 117 (2015) 247–254.
- [4] P. Liu, Z.X. Su, Surface-initiated atom transfer radical polymerization (SI-ATRP) of n-butyl acrylate from starch granules, *Carbohydr. Polym.* 62 (2005) 159–163.
- [5] M.W. Meshram, V.V. Patil, S.T. Mhaske, B.N. Thorat, Graft copolymers of starch and its application in textiles, *Carbohydr. Polym.* 75 (2009) 71–78.
- [6] K.M. Mostafa, Graft polymerization of methyl acrylic acid on starch and hydrolyzed starches, *Polym. Degrad. Stabil.* 50 (1995) 189–194.
- [7] J.L. Willett, V.L. Finkenstadt, Preparation of starch-graft polyacrylamide copolymers by reactive extrusion, *Polym. Eng. Sci.* 43 (2003) 1666–1674.
- [8] Z. Zhang, P.R. Chen, X.F. Du, Z.H. Xue, S.S. Chen, B.J. Yang, Effects of amylose content on property and microstructure of starch-graft-sodium acrylate copolymers, *Carbohydr. Polym.* 102 (2014) 453–459.
- [9] S. Silong, W.M.Z.W. Yunus, M. Rahman, M.Z.A. Rahman, M. Ahmad, M.J. Haron, Kinetics and mechanism of free radical grafting of methyl acrylate onto sago starch, *J. Appl. Polym. Sci.* 77 (2000) 784–791.
- [10] V. Vargha, P. Truter, Biodegradable polymers by reactive blending trans-esterification of thermoplastic starch with poly(vinyl acetate) and poly(vinyl acetate-co-butyl acrylate), *Eur. Polym. J.* 41 (2005) 715–726.
- [11] C.G. Cho, K. Lee, Preparation of starch-g-poly(styrene) copolymer by emulsion polymerization, *Carbohydr. Polym.* 48 (2002) 125–130.
- [12] Z.F. Zhu, R.X. Zhuo, Controlled release of carboxylic-containing herbicides by starch-g-poly(butyl acrylate), *J. Appl. Polym. Sci.* 81 (2001) 1535–1543.
- [13] P. Liu, Z.X. Su, Surface-initiated atom transfer radical polymerization (SI-ATRP) of n-butyl acrylate from starch granules, *Carbohydr. Polym.* 62 (2005) 159–163.
- [14] V.M. Patel, D.N. Parikh, J.V. Patel, P.D. Pandya, Synthesis and characterization of butyl acrylate graft sodium salt of partially carboxymethylated starch, *J. Appl. Polym. Sci.* 102 (2006) 3334–3340.
- [15] H.J. Chu, H.L. Wei, J. Zhu, Ultrasound enhanced radical graft polymerization of starch and butyl acrylate, *Chem. Eng. Prog.* 90 (2015) 1–5.

- [16] C. Liu, Y. Shao, D.M. Jia, Chemically modified starch reinforced natural rubber composites, *Polym* 49 (2008) 2176–2181.
- [17] J. Mou, X.R. Li, H.H. Wang, G.Q. Fei, Q. Liu, Preparation, characterization, and water resistance of cationic acetylated starch-g-poly(styrene-butyl acrylate) surfactant-free emulsion, *STARCH-STARKE* 64 (2012) 826–834.
- [18] S.H. Lv, X.L. Yan, R.J. Gao, Preparation and properties of copolymer of methacrylic acid and acrylamide onto degraded chitosan initiated by HRP/H<sub>2</sub>O<sub>2</sub>/ACAC, *Appl. Mechan. Mater.* 80 (2011) 396–399.
- [19] A.R. Derango, L.C. Chiang, R. Dowbenko, J.G. Lasch, Enzyme-mediated polymerization of acrylic monomers, *Biotechnol. Tech.* 6 (1992) 523–526.
- [20] T. Lalot, M. Brigodiot, E. Marechal, A kinetic approach to acrylamide radical polymerization by horseradish peroxidase-mediated initiation, *Polym. Int.* 48 (1999) 288–292.
- [21] A. Durand, T. Lalot, M. Brigodiot, E. Marechal, Enzyme-mediated initiation of acrylamide polymerization: reaction mechanism, *Polym* 41 (2000) 8183–8192.
- [22] K. Bhanu, R.A. Gross, Horseradish peroxidase mediated free radical polymerization of methyl methacrylate, *Biomacromolecules* 1 (2000) 501–505.
- [23] K. Bhanu, R.A. Gross, HRP-mediated polymerizations of acrylamide and sodium acrylate, *Green. Chem.* 4 (2002) 174–178.
- [24] R.L. Shogren, J.L. Willett, B. Atanu, HRP-mediated synthesis of starch-polyacrylamide graft copolymers, *Carbohydr. Polym.* 75 (2009) 189–191.
- [25] S.H. Lv, T. Sun, Q.F. Zhou, J.J. Liu, H.D. Ding, Synthesis of starch-g-p(DMDAAC) using HRP initiation and the correlation of its structure and sludge dewaterability, *Carbohydr. Polym.* 103 (2014) 285–293.
- [26] M. Miao, R. Li, C. Huang, B. Jiang, T. Zhang, Impact of  $\beta$ -amylase degradation on properties of sugary maize soluble starch particles, *Food. Chem.* 177 (2015) 1–7.
- [27] M.C. Li, J.K. Lee, U.R. Cho, Synthesis characterization, and enzymatic degradation of starch-grafted poly(methyl methacrylate) copolymer films, *J. Appl. Polym. Sci.* 125 (2012) 405–414.
- [28] Y. Yang, Z.B. Tang, Z. Xiong, J. Zhu, Preparation and characterization of thermoplastic starches and their blends with poly(lactic acid), *Int. J. Biol. Macromol.* 77 (2015) 273–279.
- [29] Y. Li, L. Liu, X.F. Shen, Y.E. Fang, Preparation of chitosan/poly(butyl acrylate) hybrid materials by radiation-induced graft copolymerization based on phthaloylchitosan, *Radiat. Phys. Chem.* 74 (2005) 297–301.
- [30] M. Elomaa, T. Asplund, P. Soininen, et al., Determination of the degree of substitution of acetylated starch by hydrolysis, <sup>1</sup>H NMR and TGA/IR, *Carbohydr. Polym.* 57 (2004) 261–267.
- [31] V.D. Athawale, V. Lele, Syntheses and characterization of graft copolymers of maize starch and methacrylonitrile, *Carbohydr. Polym.* 41 (2000) 407–416.
- [32] X.C. Ge, Y. Xu, Y.Z. Meng, R.K.Y. Li, Thermal and mechanical properties of biodegradable composites of poly(propylene carbonate) and starch-poly(methyl acrylate) graft copolymer, *Compos. Sci. Technol.* 65 (2005) 2219–2225.
- [33] Y. Viturawong, P. Achayuthakan, M. Suphantharika, Gelatinization and rheological properties of rice starch/xanthan mixtures: effects of molecular weight of xanthan and different salts, *Food Chem.* 111 (2008) 106–114.
- [34] M. Gurruchaga, I. Echeverria, I. Gonni, Synthesis and rheological characterization of graft copolymers of butyl and hydroxyethyl methacrylates on starches, *J. Appl. Polym. Sci.* 108 (2008) 4029–4037.
- [35] M. Mensitieri, L. Ambrosio, L. Nicolais, D. Bellini, M.O. Regan, Viscoelastic properties modulation of a novel autocrosslinked hyaluronic acid polymer, *J. Mater. Sci. Mater. Med.* 7 (1996) 695–698.