Enzymatic oxidative polymerization of pyrogallic acid for preparation of hindered phenol antioxidant

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ABSTRACT: A novel antioxidant with high molecular weight was successfully synthesized via horseradish peroxide-catalyzed oxidative polymerization of pyrogallic acid. As-synthesized poly(pyrogallic acid), a black powder completely soluble in common organic solvents, has a number-averaged molecular weight of about several thousand and consists of phenylene and oxyphenylene units. Besides, as-prepared poly(pyrogallic acid) exhibits much better thermal stability and antioxidant capacity than butylated hydroxyanisole (denoted as BHA) and butylated hydroxytoluene (denoted as BHT), two kinds of commercial antioxidants. And it was also found that the higher phenolic content the pyrogallic acid polymer possess, the better antioxidant activity the poly(pyrogallic acid) shows. More importantly, poly(pyrogallic acid) could effectively inhibit the oxidation degradation of polypropylene (denoted as PP) during plastic processing. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41591.

INTRODUCTION

As an important primary antioxidant, hindered phenols are widely used in industrial fields.[1–4] Recently, however, it is reported that hindered phenols with low molecular weight could easily departed from host materials by migration and evaporation, which decreases the efficiency of antioxidants.[5,6] Aiming to achieve long-term antioxidant efficiency in materials, antioxidants that are not easy to depart from polymer have gained much interest in recent years.[7] Of various methods for minimize the physical loss of antioxidants, increase the molecular weight of antioxidants has been widely applied in industries.[8–10] So far, a class of high molecular weight antioxidants has been produced. The most of above-mentioned hindered phenols, unfortunately, generally be synthesized by traditional chemical methods, which usually need complicated produces and harsh terms.[7] Therefore, a new strategy for synthesis of high molecular weigh hindered phenols with mild reaction conditions and facile procedures is desired.

Bearing those perspectives in mind, enzymatic polymerizations excited our interest, because of their high potential for the synthesis of polymer.[11–13] Recently, it has been reported that oxidative polymerization of phenol and its derivatives using HRP as catalyst can afforded a new class of polyphenol, which is difficult to be synthesized by conventional oxidative polymeriza-

tions.[14,15] And the structure of polyphenols is similar with hindered phenols. In addition, it is well known that hindered phenols antioxidants, known as radical scavengers, inhibit oxidation by donating a hydrogen atom to competing with the host materials in the formation of peroxy radicals.[4] Therefore, enzymatic polymerization of polyhydroxy phenol may be of particular significance for preparing antioxidant.

In this article, enzymatic oxidative polymerization of pyrogallic acid is carried out in an aqueous organic solvent using horseradish peroxidase (denoted as HRP) as catalyst. The polymer could be obtained in high yield with moderate molecular weight. Besides, antioxidant properties of poly(pyrogallic acid) were evaluated by various methods including 2,2-diphenyl-1-picryhydrazyl (denoted as DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (denoted as ABTS) and ferric reducing antioxidant potential (denoted as FRAP) assays. Particularly, the performances of poly(pyrogallic acid) as antioxidant for PP were assessed by measurements of differential scanning calorimetry and themogravimetric analysis.

EXPERIMENTAL

Materials
HRP (activity = 200 U mg^{-1}) was purchased from Shanghai Guoyuan Biotechnology Company Limited (Shanghai, China)

and used without further purification. DPPH and ABTS were obtained from TCI Shanghai (Shanghai, China). TPTZ was purchased from Energy Chemical Beijing (Beijing, China). Other reagents were purchased from various commercial suppliers and were used as received.

Measurements

The concentration of pyrogallic acid during the enzymatic polymerization was analyzed by high-performance liquid chromatograph (HPLC; Agilent 1100) equipped with a C18-reverse phase column (2.1 mm × 150 mm, 5 μm). The mixture of acetonitrile, distilled water and acetic acid with a volume ratio of 45 : 55 : 0.1 was used as the mobile phase (flow rate 0.4 mL min^{-1}); and pyrogallic acid was measured at 267 nm with an ultraviolet absorbance detector. Infrared (IR) spectra were recorded with VERTEX 70 Fourier transform infrared spectrometer (FT-IR; BRUKER Company, Germany). Nuclear magnetic resonance (^{1}H NMR) spectra were measured with an AVANE 400 MHz spectrometer (Bruker Company, Germany). The molecular weight of as-prepared pyrogallic acid polymer was estimated by gel permeation chromatography (GPC; Waters 1515 pump; Waters 2414 refractive index detector) with dimethylformamide (denoted as DMF) as the eluent (flow rate 1.0 mL min^{-1}).The calibration curves for GPC analysis were obtained using polystyrene as the standard. Thermogravimetric (TG) analysis under air atmosphere was performed with a TGA/SDTA851e instrument (Mettler-Toledo Company, Switzerland) at a heating rate of 10°C min^{-1}. The absorption spectrum of the sample was determined with a 723N-visible spectrophotometer (Chengguang instruments Company; Shanghai, China). Oxidative induction temperature of PP was performed by differential scanning calorimeter (Mettler-Toledo Company, Switzerland) in air atmosphere at the flow of 50 mL min^{-1} at heating rate of 10°C min^{-1}. Oxidative induction temperature was defined as the temperature when the heat flow started to change abruptly after melting peak.

Enzyme Oxidative Polymerization of Pyrogallic Acid

The procedure of the enzymatic polymerization in the mixture of buffer and water-miscible organic solvent was carried out as follows: 50 mL of phosphate buffer (0.2 mol L^{-1}, pH 7)-organic solvent mixed solution was placed into a flask, and then 0.63 g of pyrogallic acid was added into the flask and dissolved under mild magnetic stirring (temperature used for polymerization is 25°C). Into the resultant mixture solution was added 1mg of HRP, followed by dripping of 0.25 mL of aqueous solution of 5% hydrogen peroxide (the total dripping times is 14 and the dripping interval is 15 min) to allow the generation of black precipitate. Upon completion of dripping of H$_2$O$_2$ solution, the reaction mixture was stirred for an additional 12 h, followed by addition of 10 mL of hydrochloric acid (36–38%). Polymer precipitates were collected by vacuum filtration and washed with distilled water. As-washed polymer precipitates were finally dried at 50°C to give the target product, a black powder polymer. In addition, when we discuss the effect of buffer solution pH on the polymerization, citrate buffer and carbonate buffer were selected as reaction medium too.

Antioxidant Assay with DPPH Free Radical

The DPPH radical scavenging capacity of poly(pyrogallic acid) was evaluated according to the method of Brand–Willias.[16]

Briefly, a proper amount of DPPH free radical was dissolved in ethanol at a concentration of 120 μM to form a solution. Into 5 mL of DPPH free radical solution was added 5 mL of ethanol solutions of to-be-tested samples, and resultant mixed solutions were incubated at 37°C for 30 min. Upon completion of incubation, the absorbance (A_x) of the mixed solutions at 515 nm was measured, and the absorbance (A_0) of a blank sample containing 5 mL of ethanol and 5 mL of DPPH radical solution was also measured under the same conditions. All the measurements were conducted in triplicate, and radical scavenging activity is calculated by equals (1),

$$\text{Inhibition efficiency } (\%) = [(A_0 - A_x)/A_0] \times 100\% \qquad (1)$$

here A_0 is the absorbance of DPPH in ethanol, and A_x is the absorbance of the ethanol solution containing poly(pyrogallic acid) and DPPH. The antioxidant capacity is expressed as I_{C50}, and it is defined as the concentration (in mg L^{-1}) of polymer at which the absorbance of DPPH free radical is inhibited by 50%.[17,18]

Antioxidant Assay with ABTS Radical Cation

Traditional ABTS assays was used in this study.[19] Briefly, a proper amount of the stock solution of 7 mM ABTS was mixed with a proper amount of 2.45 mM potassium persulfate solution and incubated at room temperature in the dark for 16 h to afford concentrated ABTS radical cation solution. As-obtained concentrated ABTS radical cation solution was then diluted with ethanol until an absorbance of 1.40 ± 0.02 was obtained at 734 nm. Then 5 mL of ethanol solution of poly(pyrogallic acid) with different concentrations was separately added to 5 mL of the diluted ABTS radical cation solution. After the mixture was placed in 30°C bath for 10 min, the absorbance (A_x) was measured; and the absorbance (A_0) of the blank sample containing 5 mL of ethanol and 5 mL of ABTS radical cation solution was also measured in the same manner. All radical scavenging activity measurements were conducted in triplicate. The antioxidant capacity is expressed as I_{C50}; and the inhibition efficiency is calculated as aforementioned.

Ferric Reducing Antioxidant Potential Assay

Ferric reducing antioxidant potential was determined using the method of Iris F. F. Benzie.[20] Briefly, 100 mL of 300 mM acetate buffer (pH = 3.6), 10 mL of 10 mM TPTZ solution in 40 mM HCl and 10 mL of 20 mM FeCl$_3$ solution were mixed to afford freshly prepared FARP working reagent. About 1 mL of the ethanol solution of to-be-tested samples was allowed to react with 9 mL of the freshly prepared FRAP solution at 37°C for 30 min in a water bath generating color products, followed by the measure of absorbance at 593 nm. Then a blank containing 1 mL of ethanol and 9 mL of FRAP solution was measured for calibration. A standard curve of Fe^{2+} with various concentrations was drawn, and the reducing capacity of to-be-tested samples is expressed as the amount of mmol Fe^{2+} per gram of the samples. All measurements were conducted in triplicate.

Determination of Total Phenolics

Total phenolics were determined according to the modified method adapted by Shetty.[21] Briefly, 0.5 mL of ethanol solution of poly(pyrogallic acid) was added into test tube and mixed

with 5 mL water and 0.5 mL Folin-Ciocalteu reagent. After 5 min, 4 mL of 7.5% $NaCO_3$ was added and the reaction mixture was allowed to incubated at 25°C for 2 h, then the absorbance at 760 nm was measured. The standard curve for total phenolics was made using gallic acid standard solution under the same procedure as above. The total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of polymer.[22]

Preparation of PP/Poly(pyrogallic acid) Compounds

First, the powdery PP and poly(pyrogallic acid) are well blended. Then the resulting sample was prepared using a co-rotating twin-screw extruder (AK22, Nanjing, Ke Ya, China) at 170°C with a roll speed of 100 rpm. The addition quantity of poly(pyrogallic acid) is 0.5% for PP.

Oxidation Induction Time (Denoted as OIT)

OIT was measured using differential scanning calorimeter according to the standard method (ISO 11357-6: 2002). First, the sample was held at 25°C for 5 min under a nitrogen flow of 50 mL min^{-1}. Then the sample was heated to 210°C at a rate of 20°C min^{-1}, and held at 5 min for equilibration, still under a nitrogen flow rate of 50 mL min^{-1}. After that the gas was switched to oxygen with the flow rate of 50 mL min^{-1}. The oxidation of the sample was observed as a sharp increase in heat flow due to the exothermic nature of the oxidation reaction.

RESULTS AND DISCUSSION

Enzymatic Polymerization of Pyrogallic Acid

Table I shows the effect of buffer solution pH on the polymerization of pyrogallic acid in various buffer solutions containing

Table I. Enzymatic Polymerization of Pyrogallic Acid Catalyzed by HRP in the Mixture of 1,4-Dioxane and Various Buffer (10 : 90 vol %)

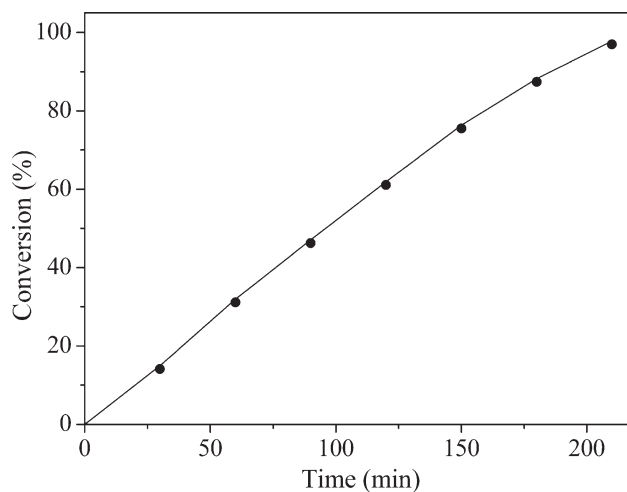| Entry	Buffer salt	pH	Yield (%)
1	Citrate	4	23
2	Citrate	5	59
3	Phosphate	6	64
4	Phosphate	7	67
5	Phosphate	8	58
6	Carbonate	9	47
7	Carbonate	10	34

Figure 1. Plot of conversions of pyrogallic acid versus reaction time in the mixture of 90% buffer and 10% 1,4-dioxane.

10% 1.4-dioxane. The polymerization in citrate buffer of pH 4 produced the polymer with lowest yield (Entry 1), and the yield rises as the pH of citrate buffer increases (Entry 2). Besides, the
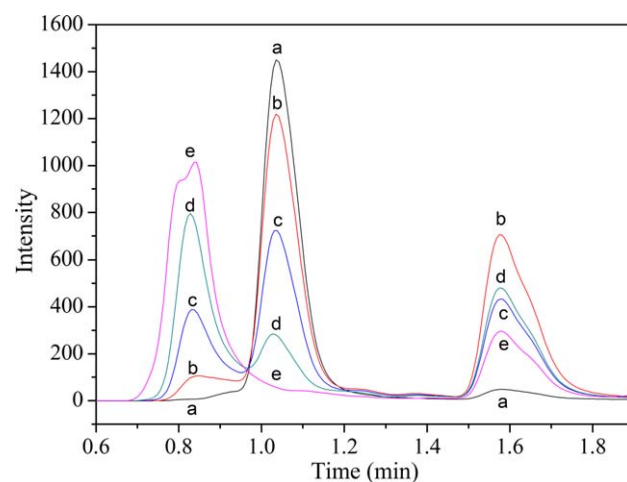

Figure 2. Variations of HPLC traces of reaction mixture during the course of the reaction (Curve a, b, c, d and e is the HPLC trace at 0, 30, 90, 150, 210 min, respectively). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
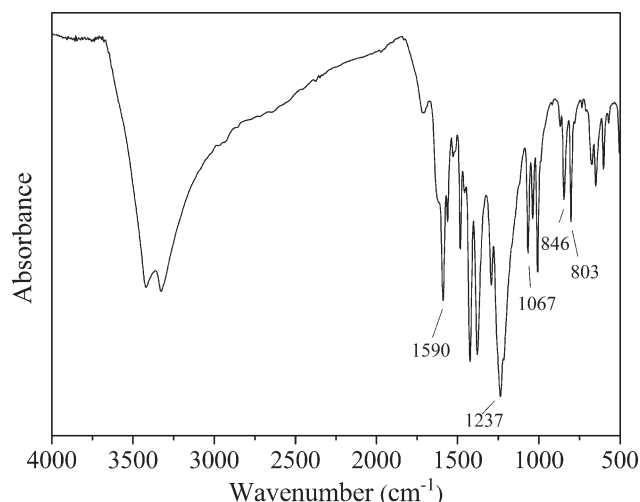
Table II. Synthesis of Poly(pyrogallic acid) in a Mixture of Phosphate Buffer and Various Organic Solvents

| Entry | Content of organic solvent (%) | Yield (%) in various organic solvent | | | | |
		DMF	DMSO	1,4-Dioxane	Ethanol	Methanol
1	5	26	60	63	8	6
2	10	38	66	70	20	19
3	20	47	63	60	33	34
4	40	59	54	55	56	53
5	50	65	42	35	35	41
6	60	44	37	12	23	30
7	80	–	–	–	–	–

Figure 3. IR spectrum of poly(pyrogallic acid) which is obtained in the mixture of 1.4-dioxane and various buffer (10 : 90 vol %).

Figure 5. ^{1}H NMR spectra of poly(pyrogallic acid) obtained in the mixture of 90% buffer and 10% 1,4-dioxane.

polymerization in carbonate buffers (pH $\geq$ 9) also produces the polymer in low yields. In the case of the reaction system using a phosphate buffer of pH 6 or 7, the polymerization produced the polymer in a relatively high yield. Therefore, it can be inferred that the pH of buffer greatly affect the yield of polymer, which is possibly because different buffers refer to different enzyme activity of HRP.[23]

Polymerization in a mixture of phosphate buffer (pH 7) and various organic solvents has been carried out (Table II). In using DMSO or 1,4-dioxane, the polymerization in the mixture of 90% buffer and 10% organic solvent give the polymer in high yield. In case of the polymerization using DMF, the reaction system containing 50% buffer produced the polymer in higher yield. In the polymerization using ethanol or methanol, the polymer has a relatively high yield in the mixture of 60% buffer and 40% organic solvent. Besides, the pyrogallic acid polymer was not obtained in the mixture of 20% buffer and 80% organic solvent. These data show that polymer yield strongly depends on the solvent composition, which is probably due to that different solvent composition refers to the formation of the monomer clusters and the catalytic activity of HRP.

To monitor the reaction process of pyrogallic acid, conversions during the enzymatic polymerization are measured by HPLC. Figure 1 shows that the polymerization of pyrogallic acid was performed efficiently and the final conversion approaches 100%. The curves of conversion monitored in other systems exhibit a similar pattern to that of one obtained in the mixture of buffer and 1,4-dioxane, implying that the reaction system scarcely affect the rate of conversion. Surprisingly, relatively low yields ($\leq$70%) were obtained in these polymerizations, although the final conversion in various systems is higher than 90%. To
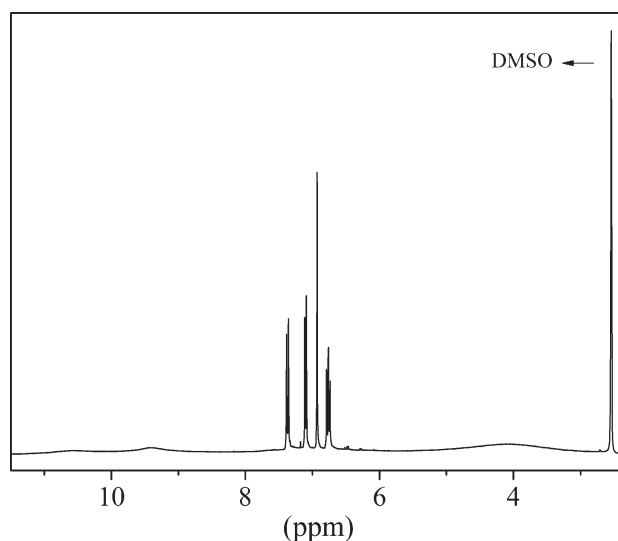
investigate the causes of this phenomenon deeply, the spectrum of HPLC of pyrogallic acid in polymerization process is shown in Figure 2. It is seen that the intensity of peaks at 1.04 min, ascribed to the presence of pyrogallic acid, tends to decrease with increasing reaction time. Besides, two new peaks, except the peak of pyrogallic acid, emerge in the curve after the addition of H_2O_2. The intensity of peaks at 0.83 min tends to increase with increasing reaction time, while the intensity of peaks at 1.58 min has no obvious regular change. The mechanism of the HRP catalysis is fairly well understood and has been the subject of many investigations. First, HRP catalyzes the one-electron oxidation of phenols by a peroxide to form the corresponding phenoxy radicals. Then the resulting phenoxy radicals react to form polymers via subsequent recombination and radical transfer steps.[24] On the basis of these reports, we suggest that the peaks at 1.58 min are ascribed to the presence of oligomers of pyrogallic acid. The reason for its intensity has no regular change is that oligomers can react with phenoxy radicals by radical transfer steps. The peaks at 0.83 min are ascribed to the presence of poly(pyrogallic acid) which is dissolved in the reaction system. The reason for its intensity increase with increasing reaction time is that more and more poly(pyrogallic acid) was formed during the reaction process. In addition, the oligomers could not be separated from the reaction medium. Thus, the yields were obtained in these polymerizations is not consistent with their final conversion.

Characterization of Poly(pyrogallic acid)

The polymers obtained in various systems are completely soluble in common organic solvents such as dimethylformamide (DMF), terahydrofuran (THF) and acetone. The chemical
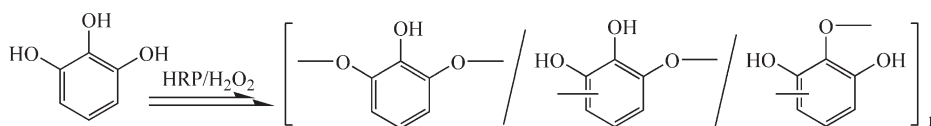

Figure 4. The structure of poly(pyrogallic acid).

Table III. Molecular Weight and Thermal Stability of Poly(pyrogallic acid)

Entry	Organic solvent	Content of organic solvent (%)	Poly(pyrogallic acid)		
			$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	T_i^a (°C)
1	DMSO	10	2.3	3.8	211
2	1,4-Dioxane	10	2.5	4.3	213
3	DMF	50	3.1	4.9	218
4	Ethanol	40	3.3	10.5	222
5	Methanol	40	3.9	13.5	226

[a] T_i means the initial temperature of weight loss is 5%.

structure of poly(pyrogallic acid) was confirmed by FTIR spectra and ^{1}H NMR. As seen from Figure 3, a broad peak centered at 3400 cm^{-1} is ascribed to the vibration of phenolic O—H bond. The peaks at 1590, 1562, 1484, 846, 803, 735, and 673 cm^{-1} are assigned to various vibration modes of C—C and C—H bonds of aromatic nuclei. Besides, a peak at 1067 cm^{-1} is ascribed to the symmetric vibration of ether bond, and the one at 1237 cm^{-1} is assigned to asymmetric stretching vibration of C—O—C and C—OH. These data indicate that as-synthesized polymer is composed of a mixture of phenylene and oxyphenylene unites (Figure 4). Obviously, the molecular chains of poly(pyrogallic acid) contain lots of hindered phenol groups. The ^{1}H NMR spectrum of poly(pyrogallic acid) is shown in Figure 5. The peaks at 6.5–7.5 ppm correspond to the protons of aromatics, and the broad peaks at 9.0–11.0 ppm are assigned to the protons of phenolic hydroxyl group. IR and ^{1}H NMR spectra of the polymers obtained in the different systems are similar to each other. In enzymatic polymerization of phenol, such a phenomenon was also observed.[23,25]

To determine the molecular weight of poly(pyrogallic acid), we acetylated it with acetic anhydride in pyridine and then detected

the acetylated product by gel permeation chromatography (GPC). It is found that the polymerization in a mixture of methanol and phosphate buffer gave a polymer soluble in DMF, whose molecular weight was relatively high (3.9×10^3), on the other hand, the molecular weight of the polymer obtained in the mixture of DMSO and phosphate buffer was low. The use of other organic solvent such as 1,4-dioxane, DMF and ethanol produced the polymer with molecular weight between 2.3×10^3 and 3.9×10^3. Corresponding GPC results are shown in Table III and indicate that molecular weight of the polymer was dependent upon the nature of organic solvents used. This may be due to the different solubility of polymer toward the reaction solvent.

Poly(pyrogallic acid) which is obtained the mixture of methanol and buffer was select as an example to explore the thermal stability of pyrogallic acid polymer. Figure 6 shows typical TG curves of BHT, BHA, pyrogallic acid, and poly(pyrogallic acid). The initial temperature of weight loss (T_i) is defined as the temperature at which the weight loss is 5% and the corresponding results, together with GPC results of pyrogallic acid polymer, are shown in Table III. It is seen that 5% weight loses of pyrogallic acid, BHT and BHA was observed at 159, 98, and 131°C, respectively, and 5% weight loses of poly(pyrogallic acid) was taken place until the temperature is above 210°C. Besides, BHT, BHA, and pyrogallic acid are completely decomposed at about 160, 190, and 230°C, respectively. Poly(pyrogallic acid), however, retains nearly 80% of weight even at an elevated temperature of 300°C, and the complete decomposition of the polymer occurs above 500°C. In addition, though T_i of polymer obtained in various systems do not change too much, thermal stability of poly(pyrogallic acid) corresponds well to its molecular weight. This indicates that the higher the molecular weight is, the lower the weight loss.[1] As well we know, an additive to be effective in stabilizing materials, it is required that the additive should be stable within the processing temperature range of the polymer.[26] Besides, processing temperature range

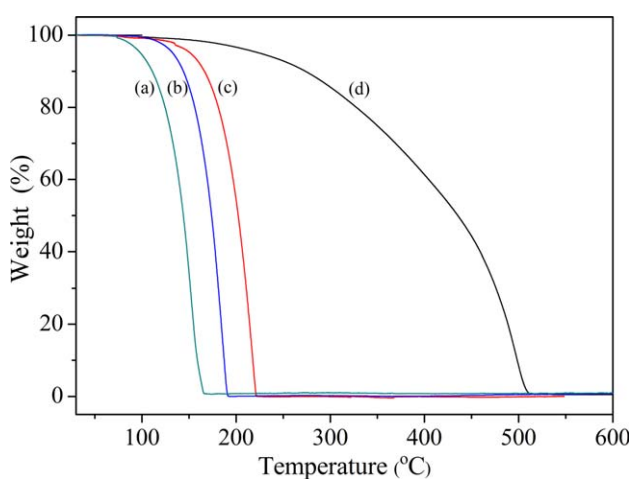**Figure 6.** TG curves of BHT (a), BHA (b), pyrogallic acid (c) and poly(pyrogallic acid) (d) obtained from the mixture of methanol and buffer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
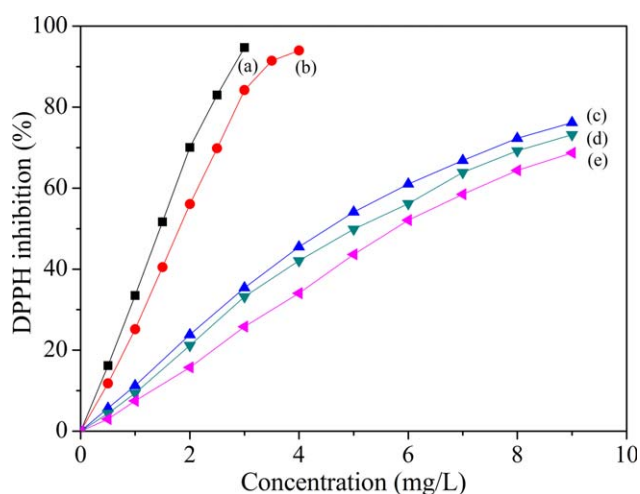

Figure 7. Scavenging capacities of poly(pyrogallic acid) obtained from 10% DMSO (a) as well as 10% 1,4-dioxane (b), 50% DMF (c), 40% ethanol (d), and 40% methanol (e) with various concentrations towards DPPH free radical. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table IV. Radical Scavenging Activity and Phenolic Content of Various Antioxidants Which are Widely Used in the Industry

Antioxidant	DPPH assay: I_{C50} (mg L^{-1})	ABTS assay: I_{C50} (mg L^{-1})	FARP value (mmolFe^{2+}/g polymer)	Phenolic content (mg GAE/g polymer)
VC	2.7	3.5	5.2	678.8
BHA	3.3	1.9	14.6	486.4
BHT	13.5	3.8	11.6	470.5

of many polymers is lower than 200°C. Therefore, we can conclude that enzymatic polymerization of pyrogallic acid can greatly improve thermal stability of resulting polymer, which could be significant to its potential applications in the antioxidant field.

Antioxidant Activity and Phenolic Content of Pyrogallic Acid Polymer

Figure 7 shows the variation of the DPPH free radical scavenging capacities of poly(pyrogallic acid) with concentration. It is seen that pyrogallic acid polymer obtained in all kinds of systems possess good inhibition ability against the DPPH free radical. Besides, the capacity of poly(pyrogallic acid) to scavenge the radical tends to significantly increase with increasing concentration. Significantly, at a concentration of 2.4 mg L^{-1}, poly(pyrogallic acid) obtained in the mixture of DMSO and phosphate buffer can inhibit DPPH free radical at a ratio of above 80%. To compare the radical scavenging capacity of poly(pyrogallic acid) to those of commercial antioxidants which are widely used in our life, radical scavenging capacity of some antioxidants were also evaluated and the corresponding results are shown in Table IV. The polymer obtained in a mixture of DMSO and phosphate buffer was found to be the most active pyrogallic acid polymer in DPPH assay; with a DPPH radical scavenging activity higher than that of VC, BHA, and BHT. The polymerization in a mixture of 1,4-dioxane and buffer gave a polymer had activity similar to that of poly(pyrogallic acid)

obtained in the mixture of DMSO and phosphate buffer, and had DPPH radical scavenging activity higher than VC, BHA, and BHT. The DPPH radical scavenging activity of poly(pyrogallic acid) obtained in other systems were lower than VC and BHA, but still higher than BHT.

Figure 8 shows the variation of scavenging ability of poly(pyrogallic acid) for the ABTS radical cation with concentration. It can be seen that poly(pyrogallic acid) possesses good inhibition ability against the ABTS radical cation, and its capacity to scavenge the ABTS radical also tends to sharply increase with rising concentration. Clearly, the results of scavenging free radical capacity evaluated by ABTS radical are closely related to the conclusions of DPPH assay. When the polymerization in 10% DMSO or 10% 1,4-dioxane, the resulting polymers were found to possess high active in the ABTS assay and they exhibit higher free radical scavenging activity than sold antioxidant such as VC, BHA, and BHT. The polymerization in other reaction systems, unfortunately, gave polymers had inferior activity in ABTS assay and show lower free radical scavenging capacity than VC, BHA, and BHT.

In the reducing power assay, the presence of reductants (antioxidant) in the fractions would result in the reduction of Fe^{3+}/ferric cyanide complex to the ferrous form by donating an electron. Increasing absorbance at 593 nm indicates an increase in reducing ability. The results of FARP assay for poly(pyrogallic
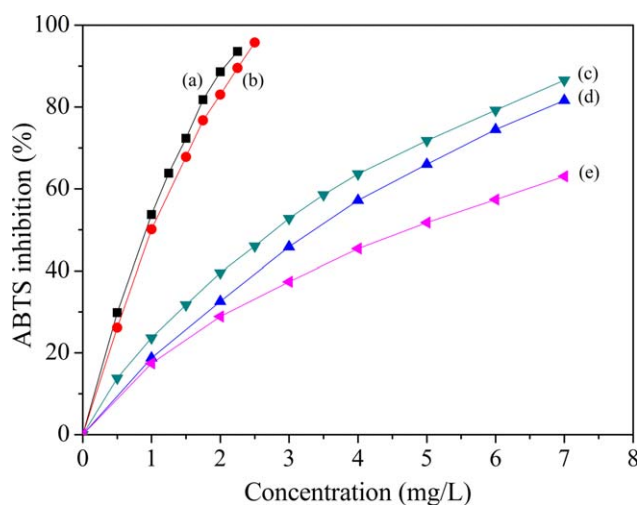

Figure 8. Scavenging capacities of poly(pyrogallic acid) obtained from 10% DMSO (a), 10% 1,4-dioxane (b), 50% DMF (c), 40% ethanol (d), and 40% methanol (e) with various concentrations towards ABTS free radical. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
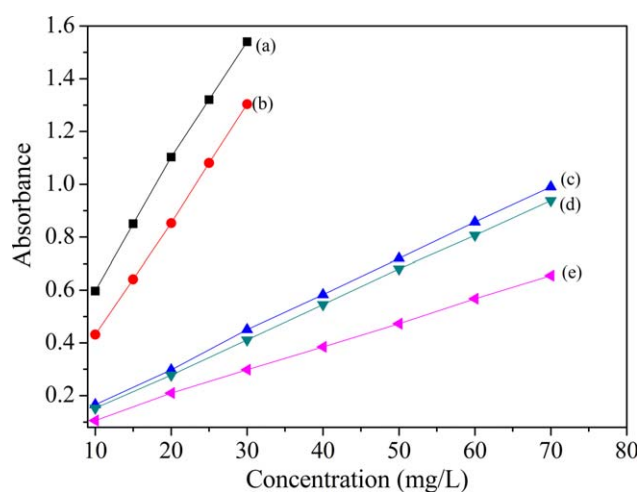

Figure 9. Ferric reducing antioxidant power of poly(pyrogallic acid) obtained from 10% DMSO (a), 10% 1,4-dioxane (b), 50% DMF (c), 40% ethanol (d), and 40% methanol (e) with various concentrations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table V. Antioxidant Activity of Poly(pyrogallic acid) Which is Obtained in Various Kinds of Reaction Systems

Reaction system	Content of organic solvent (%)	Poly(pyrogallic acid)			
		DPPH assay: I_{C50} (mg L^{-1})	ABTS assay: I_{C50} (mg L^{-1})	FARP value (mmolFe^{2+}/g polymer)	Phenolic content (mg GAE/g polymer)
DMSO and buffer	10	1.4	0.92	22.4	1013.2
1,4-Dioxane and buffer	10	1.8	1.00	19.7	965.2
DMF and buffer	50	4.5	3.19	6.1	602.6
Ethanol and buffer	40	5.0	3.26	6.0	549.7
Methanol and buffer	40	5.8	4.7	4.1	428.8

acid) were shown in Figure 9. It was found that the reducing power increases with the concentration raises. The polymer obtained in 10% DMSO or 10% 1,4-dioxane exhibit high activity in the FARP assay; it was higher than that of VC, BHA and BHT. The polymerization in 50% DMF or 40% ethanol gave polymer show lower reduction activity for Fe^{3+} than BHA and BHT, but higher activity than VC. The polymer obtained in the mixture of methanol and buffer exhibits poor activity in FARP assay, lower than that of VC, BHA, and BHT. These results indicate that poly(pyrogallic acid) obtained in 10% DMSO or 10% 1,4-dioxane may find promising applications in the antioxidant field. However, it is noteworthy that the antioxidant activity of poly(pyrogallic acid) decreased with the increasing of molecular weight. This may be explained that higher molecular weight is helpful for the ability of antioxidant at a higher temperature circumstances, but becomes a minor factor at room temperature.

Table V presents the antioxidant capacity and phenolic content of poly(pyrogallic acid). It is seen that there is a wide range of phenolic concentrations in the polymer obtained in different kinds of reaction systems. The values varied from 428.8 to 1013.2 mg GAE per gram of polymer as measured by the Folin–Ciocalteu method, which represents a variation of nearly threefold. Polymer obtained in a mixture of DMSO and phosphate buffer was found to have a very high phenolic content; it

showed higher phenolic content than VC, BHA, and BHT. Among the samples, polymer obtained in the mixture of methanol and buffer exhibited a relatively low phenolic content. The polymerization in other reaction systems gave polymer possess phenolic content lower than poly(pyrogallic acid) obtained in 10% DMSO, but higher than polymer yielded in 40% methanol. These results show that there are significant correlations between the total antioxidant capacity and phenolic content of the pyrogallic acid polymer, indicating that the higher phenolic content the poly(pyrogallic acid) possess, the better antioxidant activity the polymer shows.

Thermal Antioxidant Behavior of Poly(pyrogallic acid) for Polypropylene

Poly(pyrogallic acid) which is obtained in the mixture of dioxane and buffer was select as an example to explore thermal antioxidation behavior of pyrogallic acid polymer for PP. The addition quantity of poly(pyrogallic acid) is 0.5 wt % for PP. Figure 10 shows the TG curves of PP and poly(pyrogallic acid)-stabilized PP. It can be seen that PP loses 1% of weight at about 234°C in air, while the same amount of weight loss of poly (pyrogallic acid)-stabilized PP occurs at 268°C. Besides, 50% decomposition of the PP occurs at about 298°C, but poly(pyrogallic acid)-stabilized PP retains about 70% of weight at the
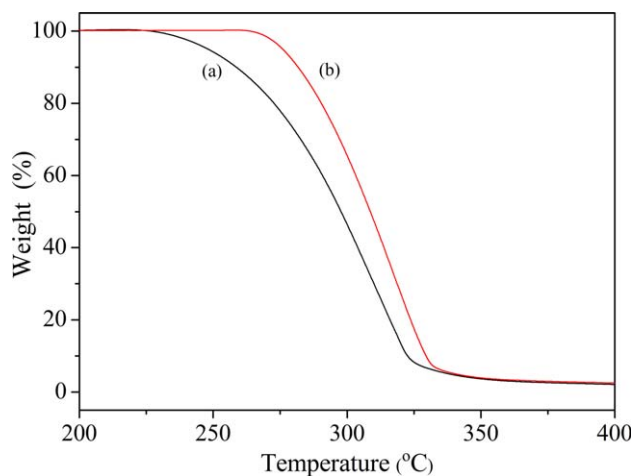

Figure 10. TG curves of PP (a) and the poly(pyrogallic acid)-stabilized PP (b) in air. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
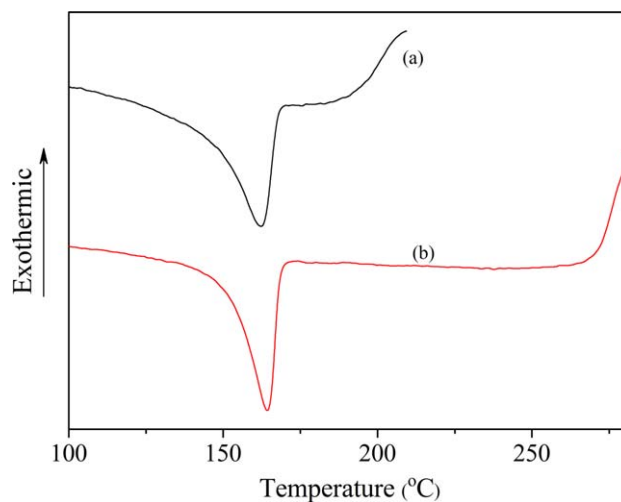

Figure 11. DSC curves of PP (a) and the poly(pyrogallic acid)-stabilized PP (b) in air. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 12. OIT curves of PP (a) and the poly(pyrogallic acid)-stabilized PP (b) in air. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
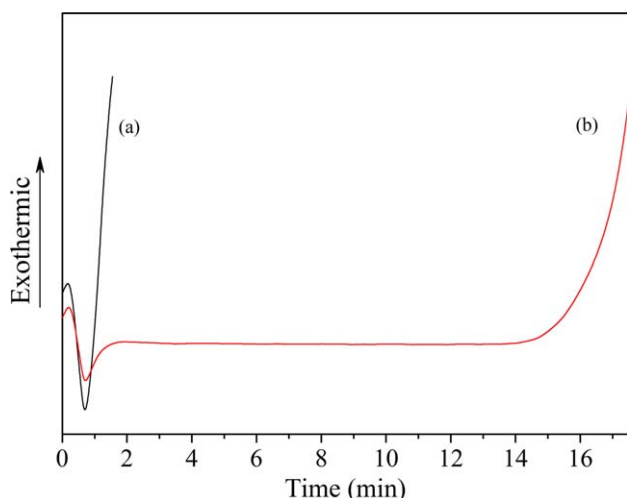
same temperature. The DSC curves of PP and poly(pyrogallic acid)-stabilized PP are shown in Figure 11. Obviously, the melting peaks of PP which appear at about 160°C do not change with the addition of poly(pyrogallic acid). The oxidative induction temperature of PP, however, greatly increases with the addition of pyrogallic acid polymer. It is because that poly (pyrogallic acid) is a kind of hindered phenol antioxidant containing —OH functional group, which supplies hydrogen atom to terminate the active free radical and generate stable free radicals to prevent the chain of PP from oxidation.[7]

Oxidation induction time (OIT) which is defined as the time it takes before the sample begins to be oxidized is another method to assess the effect of stabilizer on PP. Figure 12 shows the OIT curves of PP and poly(pyrogallic acid)-stabilized PP. Oxidation of neat PP was observed once nitrogen was switched to oxygen. OIT of poly(pyrogallic acid)-stabilized PP is about 16 min, showing longer OIT than pentaerythritol tetrakys 3-(3, 5-ditert-butyl-4-hydroxyphenyl) propionate (CHINOX 1010, OIT: 7 min) and 3, 5-ditertbutyl-4-hydroxybenzenepropanoic acid thiodi-2, 1-ethanediyl ester (CHINOX 1035, OIT: 11 min), two widely used antioxidants for PP.[1,10] In addition, the result shows that poly(pyrogallic acid) possesses more better antioxidant activity than many natural extracts such as barley husks extract, corn cobs extract and grape pomace extract.[27] It can be interpreted in the following way. The efficiency of antioxidant depends on its compatibility with host materials.[28] And high molecular weight can improve the compatibility of antioxidant.

CONCLUSIONS

To gain antioxidant with high molecular weight, efficient polymerization of pyrogallic acid has been realized with HRP as enzymatic catalyst. Findings suggest that the polymerization of pyrogallic acid can be conducted efficiently in the mixture of organic solvent and phosphate buffer (pH = 7). As-prepared poly(pyrogallic acid) is completely soluble in common organic solvents such as DMF, DMSO, THF and acetone. Besides, poly

(pyrogallic acid) exhibits much better thermal stability than pyrogallic acid as well as BHA and BHT. Particularly, poly(pyrogallic acid) possesses better antioxidant capacity compared with that of VC, BHA, and BHT, several commonly used commercial antioxidant agents. More importantly, pyrogallic acid polymer could effectively improve thermal stability of PP. Considering these results and the toxicity of polymer is far less than its monomer, we suggest that poly(pyrogallic acid) is a kind of novel and excellent antioxidant for PP.

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