

Synthesis of Poly(4-aminophenol) by Horseradish Peroxidase and the Evaluation of Its Adsorptivity for Silver Ions

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ABSTRACT: In this study, poly(4-aminophenol) was successfully prepared by an enzymatic reaction in water, with its molecular weights ranging from 12,000 to 52,000. The polymerization of 4-aminophenol produced high yields over a wide pH ranging from 4 to 10. In addition, poly(4-aminophenol) was also used for silver-ion adsorption because it was composed of amino and hydroxyl groups. We found that the adsorption capacity of poly(4-aminophenol) was relevant to the time, pH value, temperature, and concentration of silver ions, and the greatest adsorption capacity of poly(4-aminophenol) for silver ions was 220 mg/g. Moreover, a series of tests showed that the adsorption of silver ions was mainly achieved by a reduction reaction, and the silver nanoparticles were formed with a face-centered cubic structure, and their average particle size was about 10 nm. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 2014, 131, 40367.

KEYWORDS: poly(4-aminophenol); enzymatic; silver ion; adsorption

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INTRODUCTION

In recent years, the enzymatic polymerization of phenol and its derivatives has attracted much attention for its mild reaction conditions, and it is highly significant to provide novel polymeric materials, which are difficult to obtain by conventional methods.^{1–4} Although the reaction can be conducted in a variety of systems, there are still many problems. For example, the use of organic solvents will cause environmental problems, reduce the activity of enzyme catalysts, and prolong the reaction time.^{5–8} The addition of templates in the buffer system makes it difficult to obtain the pure product.^{9–14} Ionic liquids have also been used, and they have a good effect on the polymerization of phenol, but they cost too much.¹⁵ Recently, our group has found that surfactants are able to form an aqueous micelle system; this increases the conversion of phenol and gives rise to polymerized phenol,^{16,17} but much water is still needed to wash the surfactant away. As a consequence, we need to find new methods to solve the previous problems. In this study, poly(4-aminophenol) synthesized by an enzymatic reaction was obtained in water without any other addition; this has never been reported until now.

With the development of industry, large amounts of effluent containing heavy-metal ions have been generated, so it seems very essential to develop effective adsorbents to manage the environment. In recent years, polyphenols extracted from plants

such as persimmon and *Aegle marmelos* leaf have been studied for the adsorption of heavy-metal ions^{18–22} because polyphenols are mainly composed of benzene rings and many hydroxyl groups with the capacity of complexation.^{23–26} However, some deficiencies still exist, such as complex extracts, dubious structure, limited species, and the influence of seasons and regions, so it would be very attractive if we could find a polymer that is similar to the polyphenol from plants.

In this study, poly(4-aminophenol) was obtained by enzymatic polymerization in water. Poly(4-aminophenol) contained both hydroxyl and amino groups and could be used as an adsorbent in a further reaction to adsorb and restore silver ions, and silver nanoparticles (SNPs) were finally formed. The novelty in this study is that enzymatic polymerization and the adsorption of silver ion were combined together, and this will attract more attention in the future.

EXPERIMENTAL

Materials

Horseradish peroxidase (HRP; Reinheit Zahl (RZ) = 2.5, activity = 200 U/mg) was purchased from Shanghai Guoyuan Biotechnology Co. and used without further purification. Other chemicals were provided by various commercial suppliers and were used as received.

Table I. Enzymatic Polymerization of 4-Aminophenol at Different Temperatures

Entry ^a	Temperature (°C)	Yield (%)	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$
1	25	90.1	2.8	3.2
2	30	90.4	3.5	3.7
3	40	87.7	3.3	3.4
4	50	67.3	3.1	3.2
5	60	36.6	2.8	3.0

M_n , number-average molecular weight.

^aThe reaction was completed in distilled water, and there were 14 H₂O₂ additions.

Synthesis of Poly(4-aminophenol)

HRP was used as the catalyst for the enzymatic polymerization of 4-aminophenol, and hydrogen peroxide was used as the oxidizing agent. 4-Aminophenol (0.55 g, 5.0 mmol) and HRP (1.0 mg) were dissolved in H₂O (50 mL), whereas 5% hydrogen peroxide (0.25 mL) was added in the mixed solution every 5 min up to a total of 14 times of addition. The mixture was then magnetically stirred at room temperature for 100 min. At last, the brown powdery precipitate was obtained as the crude product. The crude product was filtered, fully washed with water, and dried in a vacuum oven until its weight was kept unchanged. The as-dried product was finally used for analysis and adsorption.

Silver-Ion Adsorption by Poly(4-aminophenol)

Poly(4-aminophenol) (25 mg) was added to a metal solution (10 mL), and the solution was stirred at 25°C for 24 h. After adsorption, the solution was centrifuged and filtered to measure the concentration of silver. To determine the saturated adsorption capacity for silver ions (q), the concentration of silver ions was changed from 100 to 1000 ppm. For the pH value dependence study, 100-ppm silver-ion solutions were adjusted with 0.5 and 5×10^{-3} mol/L HNO₃. The concentration of silver ions was determined with a Z-2000 atomic absorption spectrophotometer. The data were used to calculate the percentage adsorption ($A\%$) and q by eqs. (1) and (2), respectively:

$$A\% = \frac{(C_0 - C_e)}{C_0} \times 100\% \quad (1)$$

where C_0 and C_e were the initial and equilibrium concentrations of silver ions (mg/L), respectively.

$$q = \frac{V \times (C_0 - C_e)}{W} \quad (2)$$

where W is the weight of polymer added (mg) and V is the volume of the metal solution (mL).

Acetylation of Poly(4-aminophenol)

Hydroxyl and amino groups are contained in the molecular structure of poly(4-aminophenol). The chromatographic column would be affected by these groups, and the determination of the molecular weight could be interfered with, so these groups had to be protected before the analysis of the molecular weight.

Poly(4-aminophenol) (0.1 g) was added in 5.0 mL of pyridine containing 5% acetic anhydride. The mixed solution was magnetically stirred at 95°C for 2 h, and then, 50 mL of H₂O was added to make the product subside. Finally, the product was collected by filtration and fully washed with water.

Characterization of Poly(4-aminophenol) and the SNPs

The IR spectrum was recorded with an Avatar 360 Fourier transform infrared (FTIR) spectroscope (Nicolet Co.). Thermogravimetric analysis (TGA) was conducted under an air atmosphere with a TGA/SDTA 851e facility (Mettler-Toledo Co., Switzerland) at a heating rate of 10°C/min. After acetylation, the molecular weight of the as-synthesized polymer was measured with gel permeation chromatography coupled with static laser light scattering (DAWN EOS and OPTILAB rEX, Wyatt Technology Corp.; flow rate of fluent tetrahydrofuran = 1.0 mL/min). The concentration of silver ion was determined with a Z-2000 atomic absorption spectrophotometer (Hitachi Co., Japan). X-ray diffraction (XRD) patterns were measured with a Philips X'Pert Pro X-ray diffractometer (Philips Co., The Netherlands; 2θ range = 20–90°). Transmission electron microscopy (TEM) was performed to observe the particle size and shape with TEM-2010 (Japan). X-ray photoelectron spectroscopy (XPS) was measured with an AXISULTRA spectrometer (Kratos Co., United Kingdom; monochromatized Al K α radiation). Nuclear magnetic resonance (NMR) spectra were obtained from a Bruker Avance 400M system.

RESULTS AND DISCUSSION

Influence of the Different Reaction Conditions on the Enzymatic Polymerization of 4-Aminophenol

At first, we set out to investigate the effect of the temperature on the polymerization of 4-aminophenol in water. The temperature ranged from 25 to 60°C. It is apparent from Table I that the temperature played an important role in this reaction. From 25 to 60°C, the yield decreased. This was probably due to the reduction in the activity of enzymes with increasing temperature. When the temperature was 25 and 30°C, the yield was above 90%. When the temperature was 60°C, the yield was only 36%. The high temperature was not conducive to the yield or the molecular weight, and that was because the activity of the enzyme was deactivated when the temperature was high. From Table I, we drew the conclusion that both temperatures, 25 and

Table II. Enzymatic Polymerization of 4-Aminophenol with the Addition of Amounts of Hydrogen Peroxide

Entry ^a	H ₂ O ₂ (times)	Yield (%)	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$
1	0	0	—	—
2	7	86.0	2.2	2.5
3	14	90.1	2.8	3.2
4	21	94.2	3.0	3.4
5	28	92.8	3.4	3.8
6	35	93.9	3.9	4.3

M_n , number-average molecular weight.

^aThe reaction was completed in the distilled water without buffer salt, and the reaction temperature was 25°C.

Table III. Enzymatic Polymerization of 4-Aminophenol in Different Kinds of Buffer Solution

Entry ^a	pH	Buffer salt	Yield (%)	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$
1	4	Citrate-phosphate	92.0	1.7	1.8
2	5	Citrate-phosphate	91.2	2.0	2.3
3	6	Citrate-phosphate	100	2.3	2.4
4	7	Citrate-phosphate	100	2.4	2.6
5	8	Citrate-phosphate	41.4	3.0	3.4
6	6	Phosphate	99.9	1.0	1.2
7	7	Phosphate	90.2	2.3	2.4
8	8	Phosphate	48.1	3.9	4.3
9	9	Carbonate	40.1	4.6	4.9
10	10	Carbonate	20.6	4.5	5.2

M_n , number-average molecular weight.

^a The reaction was performed at 25°C, and the time of H₂O₂ addition was 14.

30°C, were favorable for the reaction. In the following synthetic reactions, we chose 25°C as the reaction temperature.

Next, the effect of the addition amount of hydrogen peroxide on the polymerization in our study was examined, and the results are summarized in Table II. The polymerization could not proceed without hydrogen peroxide (entry 1, Table II). This was because hydrogen peroxide acted as an oxidizing agent to catalyze 4-aminophenol to form the corresponding radical of 4-aminophenol, and the free radicals were not produced without hydrogen peroxide in the polymerization, and a similar result was also reported by our group.¹⁶ We observed that the molecular weight of the polymer increased with the amount of hydrogen peroxide, but the yield did not increase too much, and that was relevant to the chain propagation mechanism of radical coupling in enzymatic polymerization. In the actions of H₂O₂ and HRP, phenoxy/phenylic/anilino radicals were formed, and then, the polymer chains grew by a radical-radical coupling reaction.²⁷

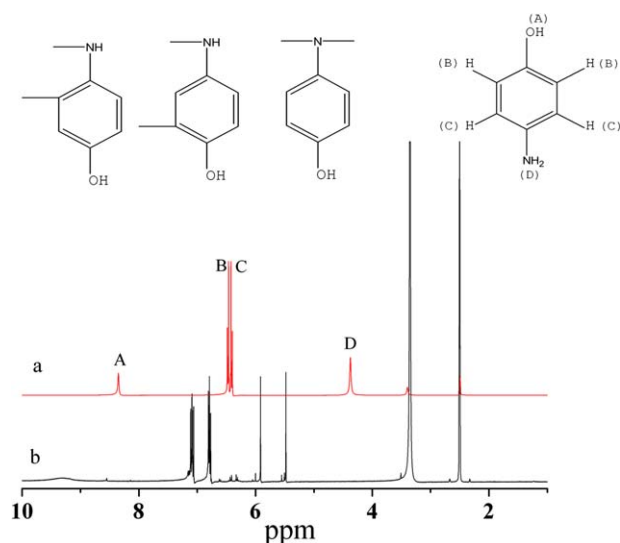


Figure 1. ¹H-NMR spectra of (a) 4-aminophenol and (b) poly(4-aminophenol) in DMSO-*d*₆. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The influence of the buffer solution on the enzymatic polymerization of 4-aminophenol was also examined, and the results are presented in Table III. The experiments were conducted in three different kinds of buffer solution. When the pH value ranged from 4 to 7, it produced a high yield in both the citrate-phosphate and phosphate buffer solutions, especially when the pH values were 6 and 7 in the citrate-phosphate buffer solution (entries 3 and 4 in Table III). We achieved such a high yield without the addition of organic solvents and other annexing agents, and this has never been reported. The molecular weight of the polymer increased when the pH values increased, and the yield decreased. When the pH value was 10, the polymerization produced a polymer with a low yield of only about 20%, but the weight-average molecular weight (M_w) was up to 52,000 (entry 10 in Table III). We conjectured that the alkaline conditions made some of the oligomer dissolve, so the yield was decreased, but the molecular weight increased. On the whole, the pH value had an important influence on both the yield and molecular weight, and we could choose different pH values to gain polymers with different molecular weights in future reactions.

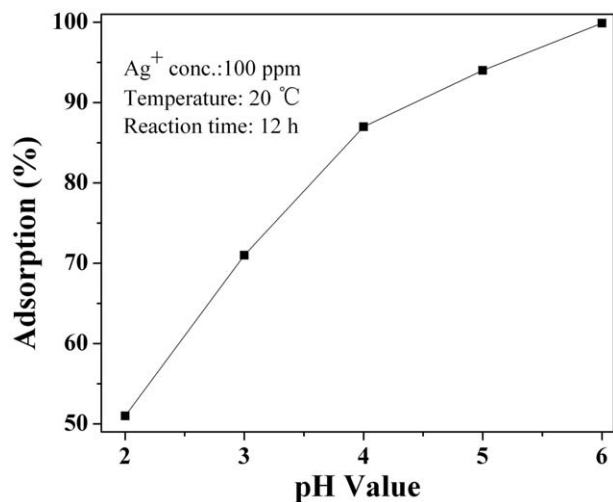


Figure 2. pH value dependence of the silver-ion adsorption by poly(4-aminophenol).

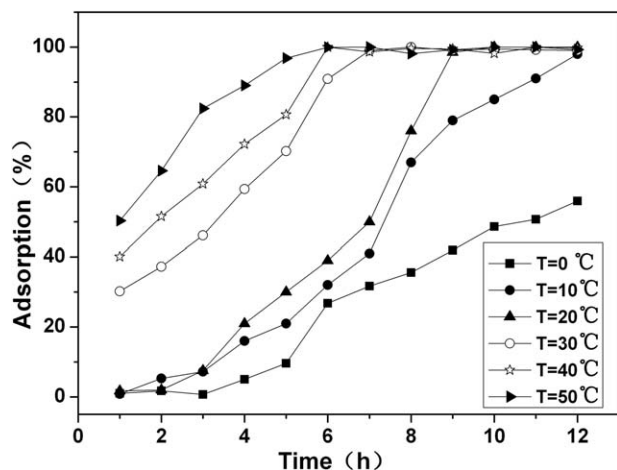


Figure 3. Time course curve of the silver-ion adsorption by poly(4-aminophenol) at different temperatures (T_s).

To investigate the structure of the polymer, the $^1\text{H-NMR}$ spectrum of poly(4-aminophenol) (Figure 1, curve b) was measured. For comparison, the $^1\text{H-NMR}$ spectrum of 4-aminophenol (Figure 1, curve a) was also measured. 4-Aminophenol exhibited four kinds of protons in its $^1\text{H-NMR}$ spectrum, and these four kinds of protons were marked as A, B, C, and D, respectively. This is shown in Figure 1. Peak A in curve a corresponded to the hydroxyl protons in 4-aminophenol, and the chemical shift of peak A shifted from a δ value of 8.35 ppm to a value of 9.28 ppm, and the shape of peak A became the special steamed-bread peak of a polymer. The chemical shifts of protons B and C in 4-aminophenol also shifted to $\delta = 7.08$ ppm and $\delta = 6.78$ ppm, respectively. It was worth noting that the NMR spectrum of the D protons of 4-aminophenol could be split into two peaks of its polymer with an approximate ratio of 1:1. Because of the NMR data, we supposed that poly(4-aminophenol) was probably composed of three constitutional units, as shown in Figure 1.

Silver-Ion Adsorption by the Poly(4-aminophenol) with Different Conditions

Figure 2 illustrates the adsorption of silver ions as a function of the pH value on the polymer. The pH was adjusted with 0.5 and 5×10^{-3} mol/L HNO_3 solutions. We observed that when the pH value was low, the ability of the polymer to complex Ag(I) decreased. When the concentration of H^+ was high, the

Table IV. $A\%$ and q Values in Different Concentrations of Ion Solution

Entry ^a	C_0 (mg/L)	A (%)	q (mg/g)
1	100	100	37.8
2	200	99.7	75.5
3	400	80.5	122.9
4	600	71.0	158.9
5	800	60.7	180.5
6	1000	59.5	220.4

^aThe temperature was 20°C. The reaction time was 12 h, and the pH value was 6.

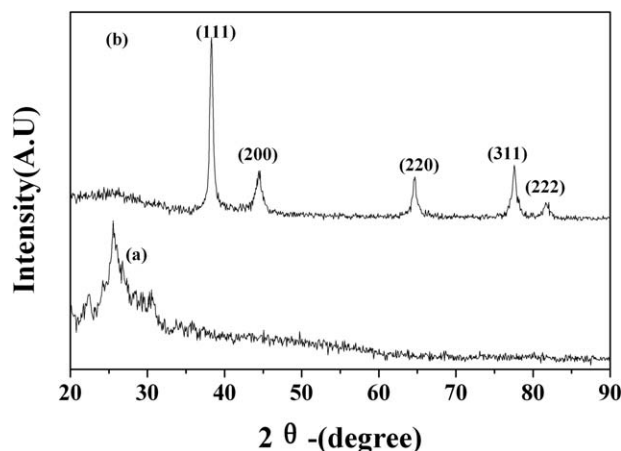


Figure 4. XRD patterns of poly(4-aminophenol) (a) before and (b) after the adsorption of silver ions.

amino group of the polymer was combined with H^+ , and it took the form of a positively charged ion, which made it hard for the silver ions to get close to the chain of the polymer. From Figure 2, when the pH value was 6, the adsorption of silver ions could reach approximately 100%. The results indicate that the poly(4-aminophenol) showed a good capacity to adsorb the silver ions. The late adsorption reactions were carried out in the solution with a pH value of 6.

As shown in Figure 3, the results reveal that the silver-ion adsorption monotonously increased with time until it reached equilibrium. The effects of the temperature are also shown in Figure 3 at various temperatures: 0, 10, 20, 30, 40, and 50°C. When the temperature was 0°C, its adsorption grew with time, and its adsorption speed was very slow, and its adsorption was only 55% after 12 h. When the temperature was 10°C, it took 12 h to get about 98%. However, the reaction needed 9 h to reach 100% when the temperature was 20°C. If the temperature was 40°C, it reached 100% only in 6 h. We might safely draw a conclusion that the adsorption was controlled by the temperature and time, and if we wanted to get a higher adsorption, we could raise the temperature or extend the adsorption time. In this study, the optimum temperature was 40°C, and the optimum time was 6 h, but to emphasize the influence of other conditions, we chose 20°C and 12 h to eliminate the influence of the temperature and time in the further research.

For concentrations of metal-ion solutions ranging from 100 to 1000 ppm, it was obvious that $A\%$ decreased, but q grew with the concentration. When the concentration was 1000 ppm, we could achieve the highest q of 220 mg/g (entry 6 in Table IV). After adsorption, we found a silvery white powder form, and we inferred that it might have been elemental silver. To prove this, XRD, XPS, and TEM were used to investigate the characteristics of the silvery white powder.

Characterization of Poly(4-aminophenol) Before and After the Adsorption of Ag(I)

The SNPs were characterized by XRD to confirm the particles as silver and to acquire the structural information. Figure 4(a) shows the poly(4-aminophenol) before adsorption. One broad

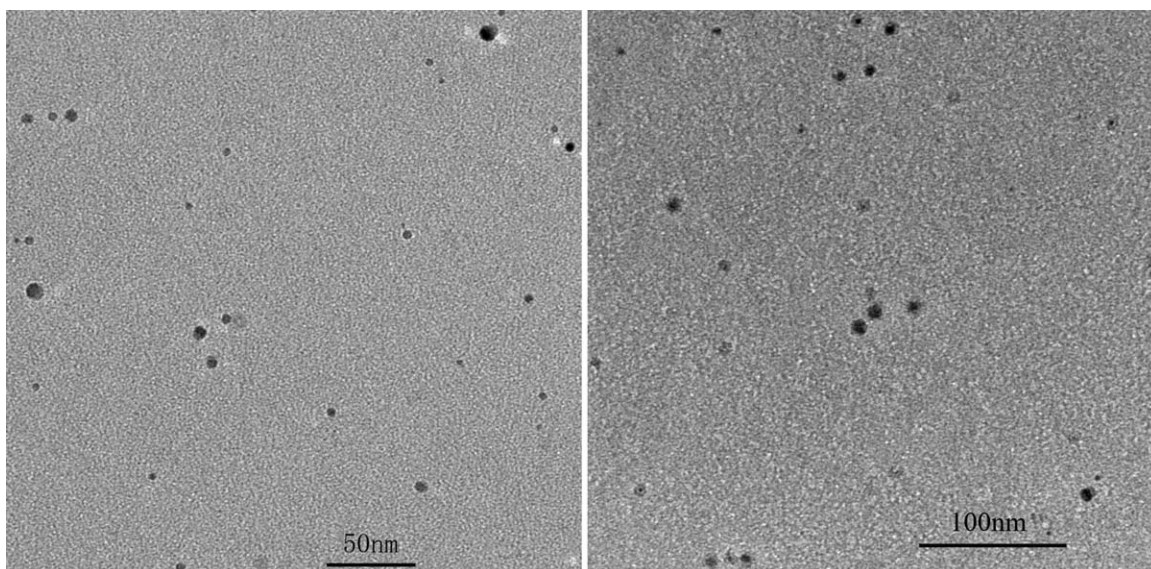


Figure 5. TEM images of the polymer after the adsorption of silver ions.

peak around a 2θ value of 26° was observed; this indicated that the structure of the poly(4-aminophenol) was orderly, but the peak of the polymer was reduced because the strength of the hydrogen bond was weakened after adsorption. Figure 4(b) shows the polymer after the adsorption of silver ions, and five specific peaks of silver crystalline were observed at 2θ values of 38.19 , 44.36 , 64.76 , 77.56 , and 81.64° corresponding to the (111), (200), (220), (311), and (222) planes, respectively (JCPDS file number 87-0720) with the majority of particles showing the (111) plane with a face-centered cubic structure. We observed that the adsorption of the silver ions was mainly achieved by a reduction reaction.

After silver-ion adsorption (entry 6 in Table IV), the TEM images of the polymer showed the formation of silver particles, as shown in Figure 5. The resulting images showed that the size of silver particles was about 10 nm, and the size of the silver particles obtained through the Scherrer equation from the XRD pattern was 15 nm.

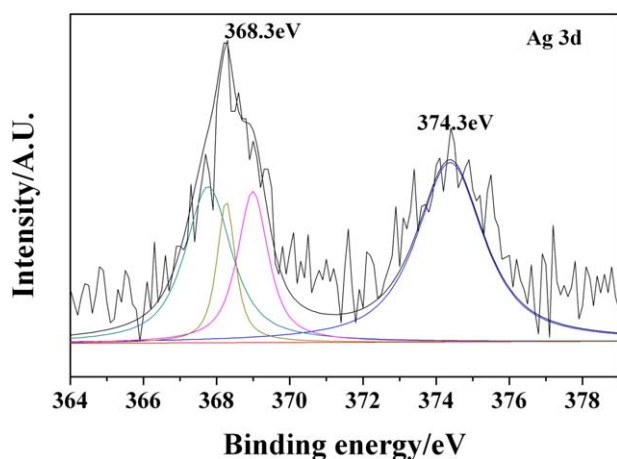


Figure 6. XPS spectra of the Ag3d in the polymer after adsorption. Ag 3d = 3d electron orbital binding energy of Ag. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The result of the XPS spectrum of the Ag is shown in Figure 6. The binding energy of Ag 3d_{5/2} in the poly(4-aminophenol) was 368.3 eV; this suggested that the chemical state of silver was elemental silver. This also proved that the adsorption of the silver ions was compiled by a redox reaction again.

We also observed a change in the functional groups after the adsorption from the FTIR spectra. As shown in Figure 6(a), the broad bands around 3200 and 3380 cm^{-1} were ascribed to the vibration of hydroxyl and amino groups, and those at 1570 and 1520 cm^{-1} were assigned to the benzene ring. In addition, the peaks at 1230 and 1030 cm^{-1} were due to the C—O—C or C—OH linkage. After adsorption, the C—N linkage shifted to 1385 cm^{-1} from 1371 cm^{-1} . The area of the broad band at 3200 cm^{-1} was reduced, as shown in Figure 7(b). This was caused by the decrease in hydrogen bonding, and this was also proven by the XRD pattern.

The thermal stability of the poly(4-aminophenol) before and after the adsorption of Ag(I) (entry 6 in Table IV) was

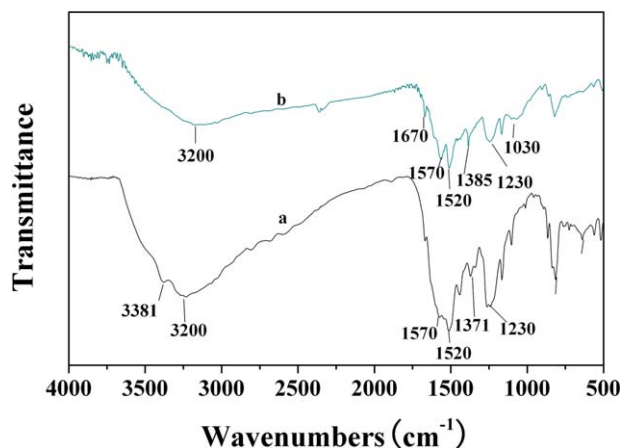


Figure 7. FTIR spectra of poly(4-aminophenol) (a) before and (b) after the adsorption of silver ions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

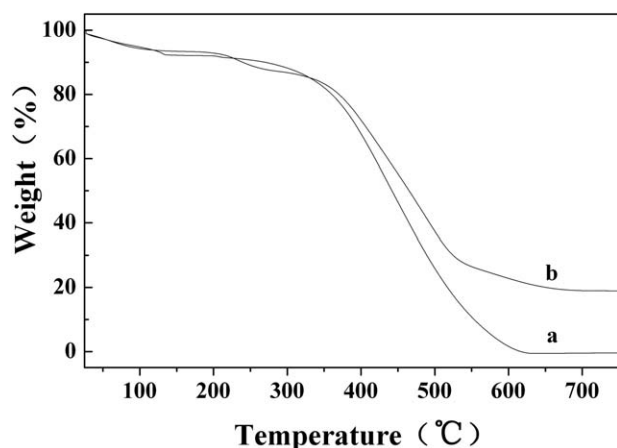


Figure 8. TGA of the poly(4-aminophenol) (a) before and (b) after the adsorption of Ag(I) ions under air.

evaluated by thermogravimetry under air. The obtained thermogravimetry curves are shown in Figure 8. There was about a 15% weight loss around 400°C. The polymer before adsorption was completely decomposed at 600°C, but after adsorption, as shown in curve b in Figure 8, the temperature arrived at 680°C, and there was still 22% weight, which was in accordance with a q of 220 mg/g (entry 6 in Table IV).

CONCLUSIONS

In this study, poly(4-aminophenol) was successfully prepared by an enzymatic reaction with HRP as a catalyst under mild reaction conditions, and the effects of the temperature and pH value on the yield of poly(4-aminophenol) were also investigated. When the pH value ranged from 4 to 7, there was a high yield in both the citrate–phosphate and phosphate buffer solutions, especially when the pH values were 6 and 7 in the citrate–phosphate buffer solution. Then, the yield could be up to 100%. In addition, the polymer was used for silver-ion adsorption because of the vast number of amino and hydroxyl groups. The adsorption capacity increased with increasing concentration of Ag(I), and when the concentration of silver ion was 1000 ppm, the maximum q was as much as 220 mg/g. The XRD pattern, XPS spectra, and TEM image of the polymer after adsorption showed that adsorption of the silver ions was mainly achieved by a redox reaction, and the size of the SNPs was about 10 nm.

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