

Laccase-catalyzed synthesis of conducting polyaniline-lignosulfonate composite

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ABSTRACT: Enzymatic polymerization of aniline was first performed in lignosulfonate (LGS) template system. High-redox-potential catalyst laccase, isolated from *Aspergillus*, was used as a biocatalyst in the synthesis of conducting polyaniline/lignosulfonate (PANI-ES-LGS) complex using atmospheric oxygen as the oxidizing agent. The linear templates (LGS), also serving as the dopants, could facilitate the directional alignment of the monomer and improve the solubility of the conducting polymer. The process of the polymerization was monitored using UV-Vis spectroscopy, by which the conditions for laccase-catalyzed synthesis of PANI-ES-LGS complex were also optimized. The structure characterizations and solubility of the complex were carried out using corresponding characterization techniques respectively. The PANI-ES-LGS suspensions obtained was used as coating for cotton with a conventional padder to explore the applications of the complex. The variable optoelectronic properties of the coated cotton were confirmed by cyclic voltammetry and color strength test. The molecular weight changes of LGS treated by laccase were also studied to discuss the mechanism of laccase catalyzed aniline polymerization in LGS template system. © 2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2016**, *133*, 42941.

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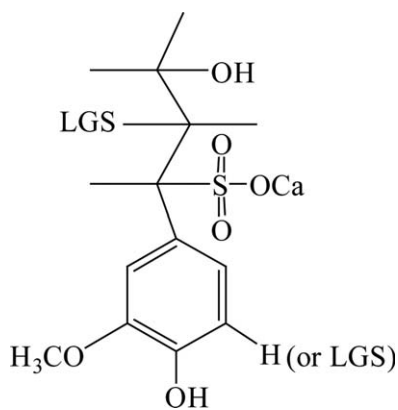
INTRODUCTION

Polyaniline (PANI) is one of the hot topics in the study of conducting polymers, ascribed to its structural diversity, special doping mechanism, low monomer price, favorable electrical properties and high thermal and chemical stability.¹ It is widely used in many applications such as biosensors,² organic lightweight batteries,³ and antistatic coatings.⁴ Unprotonated PANI has three typical oxidation states: leucoemeraldine, pernigraniline, and emeraldine base, contributing to the disparate conjugation mechanism of PANI.¹ Emeraldine salt (PANI-ES), protonated form of emeraldine base, is the one that can be conducting.

The well-known methods for the synthesis of conducting PANI are either chemical or electrochemical oxidation polymerization of aniline monomer.⁵ The reaction conditions are harsh with extreme pH, strong oxidants, and highly toxic solvents. The synthesized PANI is usually postpolymerization-treated with fuming sulfuric acid to improve its solubility and processability. By contrast, enzymatic catalysis, having no induction period,⁶

provides an alternative method of a “green process” toward the formation of soluble and processable conducting polymers because of the milder conditions and the simplified purification process of final products. Laccases, multicopper oxidoreductases, can catalyze the oxidation of many substrates and laccase-catalyzed reactions avoid using H₂O₂, since the oxidizing agent is atmospheric oxygen.^{7,8}

LGS-Ca, an inexpensive byproduct from pulp processing, is a kind of water-soluble anionic polymeric surfactant (Scheme 1). If the electrical conductivity could be given to LGS, the application fields of this natural polyelectrolyte could be further widen, far more than anticorrosion protection.⁹ Dong and Shen¹⁰ found that LGS-Ca had higher surface free energy than LGS-Na and LGS-Mg, which facilitated the electron delocalization of PANI and then enhanced its crystallization. In our experiment, laccase-catalyzed polymerization of conducting PANI-ES-LGS complex was performed in LGS template system using atmospheric oxygen as the



Scheme 1. A typical structure of LGS-Ca

oxidizing agent. The linear templates (LGS), which also served as the dopants, could not only facilitate the head-to-tail coupling of the monomer but also improve the solubility of the conducting polymer. As the polymerization of aniline in the presence of LGS was rather complex due to the degradation and polymerization of LGS induced by laccase, the molecular weight changes of LGS treated by laccase were studied using gel permeation chromatography.

Although enzymatic synthesis of conducting PANI has been studied for many years,¹ the application of PANI complex is still a problem due to its comparatively low conductivities (about 10^{-4} to 10^{-3} S/cm) in a pressed pellet form without any external dopants. However, it was confirmed in our previous work that the antistatic property and electromagnetic shielding effect of cotton could be enhanced by laccase-catalyzed PANI in situ polymerization.¹¹ In this article, the variable optoelectronic properties of PANI were given to cotton using a conventional padder. According to cyclic voltammetry and color strength test, the optoelectronic properties of coated cotton responded to pH and ammonia, which might give researchers some enlightenment for the application of the complex in the field of multifunctional textiles.

EXPERIMENTAL

Materials

Laccase, isolated from *Aspergillus* (EC1.10.3.2), was supplied by Novozymes (Shanghai, China). The enzyme activity of laccase, which was about 60 U/g, had been measured in previous work¹² and one unit of laccase was defined as the amount of enzyme required to oxidize 1 μ mol of ABTS per minute at room temperature. Nafion, a perfluorosulfonate linear polymer, and LGS-Ca (M_n 7000, M_w 52,000) were purchased from Sigma-Aldrich. Aniline and all other reagents used were analytical grade from SCR Co. Ltd. (Shanghai, China) without further purification. All solutions were prepared with deionized water.

Laccase-Catalyzed Synthesis of Conducting PANI-ES-LGS Complex

A 0.10 mg LGS-Ca sample was dissolved into 20 mL of 50 mM Na_2HPO_4 -citric acid buffered solution (pH 3.0), followed by the addition of 35 mM aniline with constant manual stirring. The stirring was stopped when the monomer was completely dis-

solved. The pH value of the reaction system increased to about 3.9 under the influence of LGS-Ca and aniline, and then was adjusted to 3.5 with citric acid. The reaction was initiated by the addition of laccase (1.2 U/mL). The reaction vessels were put into a freezer at 5°C for 144 h to complete the polymerization process. The same amount of acetone (20 mL) was added to the dispersion to collapse the micelles and precipitate the complex. The precipitate was collected by centrifugation and was thoroughly washed with acetone and 50% (v/v) acetone/water mixture to remove unreacted monomer and any oligomeric products. The final purified polymer was dried in an oven at 60°C for 24 h for further characterization.

UV-Vis Spectra Analysis

UV-Vis spectra of the complex were recorded using a UV-2808S spectrophotometer (Unicos, China) in the range of 200 to 1100 nm. The absorption bands at 430 and 775 nm indicate the formation of polaron in PANI structure,¹³ which make PANI conducting. The polymerization kinetics was monitored by changes in absorption of samples at 775 nm. The solutions were diluted with buffer (1:10) before recorded.

Fourier Transformed-Infrared Spectroscopy

FT-IR spectroscopy was performed by the conventional procedure using KBr pellets on an iS10 FT-IR spectrophotometer (Nicolet).

Elemental Analysis

Elemental analyses of LGS-Ca and PANI-ES-LGS complex were carried out using an Elementar Analysensysteme GmbH VarioEL III.

Solubility Test

Since the liquid conductivity is proportional to the concentration of the complex, the solubility of the complex was described by measuring the liquid conductivity by dissolving the complex in different solvents using a S230 conductivity meter (Mettler Toledo, Switzerland).

Cyclic Voltammetry Tests of Coated Cotton

Cyclic voltammetry tests of coated cotton were performed using a CHI 660D electrochemical workstation (Austin) at room temperature to confirm the electroactive nature of the complex and explore its potential applications in the field of textiles. Before the measurement, a small piece of coated cotton sample (about 0.2 cm²) was fixed to the glassy-carbon electrode by drying Nafion emulsion (1.5 wt %).

Color of Coated Cotton

The color strength of coated cotton samples (K/S) was evaluated from 360 nm to 700 nm using a 7000A reflectance measuring apparatus (Gretagmacbeth). K/S is the Kubelka-Munk relationship, where K is an adsorption coefficient, and S is a scattering coefficient.¹⁴

Gel Permeation Chromatography

To characterize the change of LGS molecular weight distribution, the solutions of LGS-Ca and LGS-Ca treated by laccase with different time were injected into a HPSEC system (Summit HPLC system, Dionex) using Shodex OHpak SB-804 and SB-802.5 columns (Showa Denko, Tokyo, Japan). The column

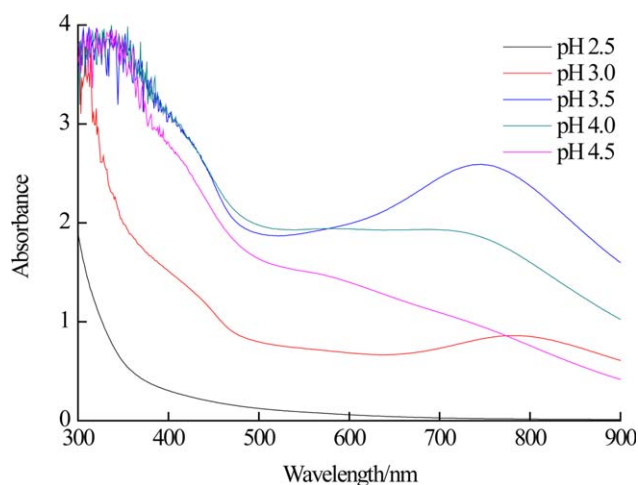


Figure 1. UV-Vis spectra of the polymerization product catalyzed by laccase at different pH. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

temperature was 50°C and the temperature of RI detector was 30°C. The elution was performed using the HPLC grade water at a flow rate of 1.0 mL/min.

RESULTS AND DISCUSSION

Optimal Reaction Conditions for the Polymerization

Under the premise that the other conditions were optimal, UV-Vis absorption spectra of laccase-catalyzed aniline polymerization at different pH value were shown in Figure 1. The absorption band at 775 nm was maximum when the polymerization was carried out at pH 3.5, which indicated that the polymer had a high degree of conjugation and high conductivity under the condition and laccase from *Aspergillus* catalyzed the polymerization of aniline in LGS template system to form conducting PANI under milder conditions than traditional chemical. In other studies on the template-assisted laccase catalyzed polymerization of aniline an optimal pH value of 3.5 was also found.¹⁵ The maximum absorption peak of the polaron moved to the long wavelength part of the spectrum at pH 3.0 compared with the maximum absorption peak at pH 3.5, which revealed that the linear arrangement of positively charged aniline ($pK_a = 4.6$) along negatively charged LGS was better at a lower pH. Although a low pH was beneficial for the crystallization and conductivity of PANI-ES, the activity of laccase and the yield of the complex would be reduced. When the pH value was less than 3.0, laccase was inactivated and no aniline polymerization was observed. The absorption band at 775 nm disappeared and brown byproduct increased, when the pH value increased to 4.0.

Compared with chemical methods, enzymatic synthesis of conducting PANI was often carried out with a very low monomer concentration and therefore had relatively lower polymer production. To evaluate the effects of aniline concentration on the reaction course and the properties of the products formed, laccase-catalyzed polymerization of aniline was carried out with different aniline concentration at 5°C (Figure 2). The absorption band at 775 nm was maximum when the polymerization was

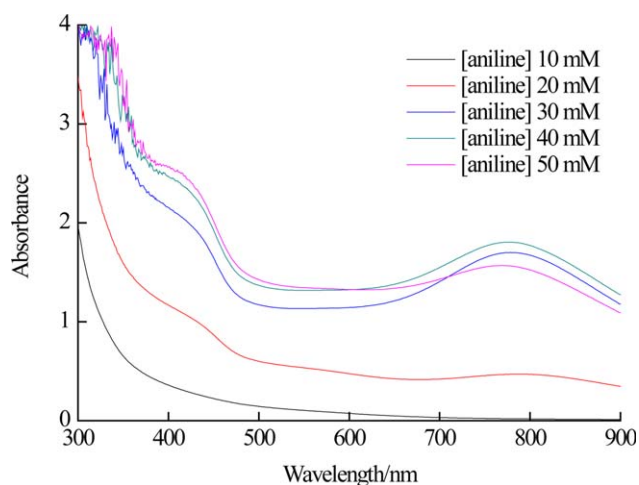


Figure 2. Effect of concentration of aniline on the polymerization catalyzed by laccase. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

carried out with 35 mM aniline with a yield of about 68%. When the concentration of aniline was more than 35 mM, the absorption band at 775 nm was weakened and brown byproduct increased. We speculated that there was a mass of dissociative monomer that was not arranged along the negatively charged LGS in the reaction system and the dissociative monomer would polymerize promiscuously under the effect of laccase. Therefore, the concentration of aniline optimal for laccase-catalyzed polymerization of aniline was 35 mM.

In the experiment, LGS was indispensable in the synthesis of conducting PANI and no green product was produced without LGS. The effect of LGS-Ca concentration on laccase-catalyzed polymerization of aniline was shown in Figure 3. The absorption band at 775 nm was maximum when the polymerization was carried out with 5 mg/mL LGS. About When LGS-Ca concentration exceeded 5 mg/mL, the maximum absorption peak of the polaron moved to the long wavelength part of the spectrum with a gradually reduced peak value, which revealed that

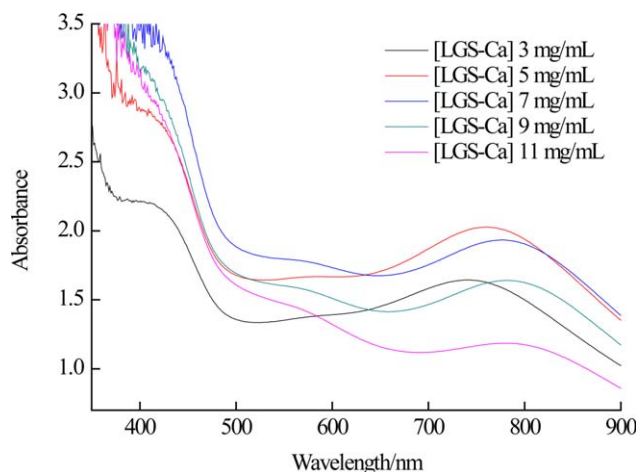


Figure 3. Effect of concentration of LGS-Ca on the polymerization catalyzed by laccase. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

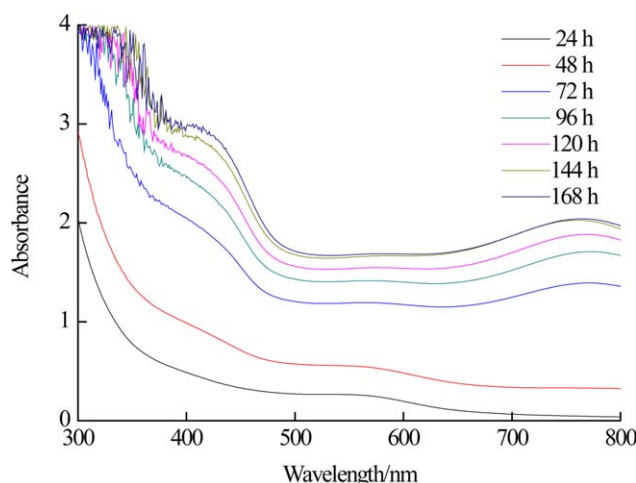


Figure 4. Effect of reaction time on the polymerization catalyzed by laccase. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the increase of LGS-Ca concentration was conducive to the conductivity of the complex, but would reduce the yield of the complex. We speculated that, the adsorption amount of aniline monomer on each unit of template was relatively reduced, although the monomer was arranged along LGS regularly. Furthermore, there was not enough dissociative monomer in the reaction system to ensure that the polymerization of aniline was kept running in the later stage of the reaction.

The effect of reaction time on laccase-catalyzed polymerization of aniline was shown in Figure 4. In the first 48 h, the solution color changed from orange yellow to brown and no absorption band was observed at 775 nm. The absorption band at 775 nm, appearing and enhancing after 72 h, got the maximum and became stable after 144 h. Since laccase-catalyzed reactions run with atmospheric O_2 only, oxidation of chain ends during the polymerization could not occur with H_2O_2 as in the case of peroxidase-based oxidative polymerization.¹⁶ On the other hand, laccase-catalyzed reactions avoid the over oxidation of the PANI chains and the formation of pernigraniline, which may happen in the case of peroxidase-based oxidative polymerization when H_2O_2 is present in excess.¹⁶ At the end of the reaction, the complex formed remained homogeneously disperse in the aqueous solution thanks to the presence of LGS and no precipitation occurred for weeks.

Structure Characterizations of LGS-Ca and PANI-ES-LGS Complex

FT-IR spectra of LGS-Ca and PANI-ES-LGS complex were shown in Figure 5. The peak at 3436 cm^{-1} was mainly attributed to O-H stretching in LGS. The vibration bands around 2935 and 2850 cm^{-1} originated from $-CH_3$ and $-CH_2$ groups in LGS. The bands around 1592 and 1499 cm^{-1} were due to quinone and benzene ring deformation. Two bands appear at 1123 and 1034 cm^{-1} , in both LGS-Ca and PANI-ES-LGS spectra, were due to the asymmetric and symmetric stretching of SO_3^- , indicating the presence of LGS in the complex.¹⁷ The head-to-tail coupling of the monomer, which led to the formation of

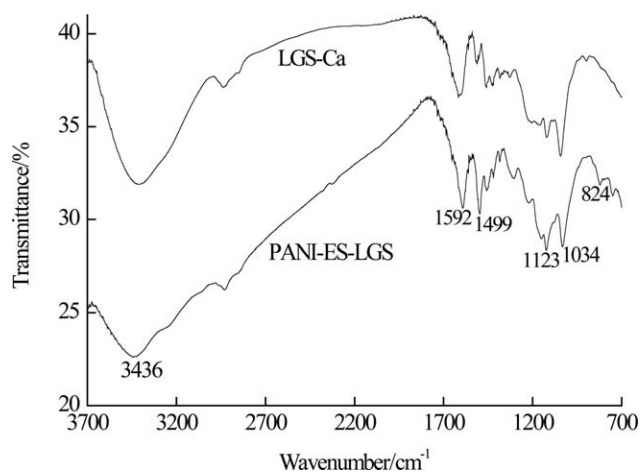


Figure 5. FT-IR spectra of LGS-Ca and PANI-ES-LGS complex.

linear polymeric chains, was indicated by the peak at 824 cm^{-1} .¹⁸

To obtain an estimate of the relative ratios of PANI-ES to LGS in PANI-ES-LGS complex, elemental analyses of LGS-Ca and PANI-ES-LGS were carried out. The element composition of PANI-ES-LGS complex was quite similar to those of chemical methods.¹⁰ There was almost no nitrogen present in LGS-Ca as indicated in Table I. On the basis of the ratio of nitrogen to sulfur obtained, the ratio of aniline unit to LGS unit in PANI-ES-LGS complex was calculated to be 7:13, implying that approximately 13 repeat units of LGS were combined with every 7 repeat units of aniline. Meanwhile, it was evident that PANI-ES was doped with LGS.

Solubility of PANI-ES-LGS Complex

It is generally accepted that PANI is insoluble in most common organic and aqueous solvents^{19,20} and low processability, due to its stiffness and the hydrogen bonding interactions between the imino and amino groups, limits the applications of this polymer.²¹ In contrast to pure PANI, the solubility of PANI-ES-LGS complex increased significantly due to the strong polarity group (SO_3^-) existed in LGS. The measurement of liquid conductivity (Figure 6) reflected the relationship between the solvent and related concentration which was useful for application. The solubility of the complex in dimethyl sulfoxide (DMSO) and *N,N*-dimethylformamide (DMF) were about 11 and 8 g/100 mL respectively. By contrast, pure PANI was slightly soluble in these two solvents with a solubility of no more than 2.0 g/100 mL.¹⁰ It was important to emphasize that, although the product formed could homogeneously disperse in the aqueous solution for a very long time, it was difficult for the complex to “dissolve” in water again after precipitation. Guo *et al.*¹⁶ found

Table I. Elemental Analysis of LGS-Ca and PANI-ES-LGS Complex

Samples	Element composition (%)			
	C	H	N	S
LGS-Ca	38.304	5.746	0.072	5.813
PANI-ES-LGS	58.410	5.883	6.209	4.337

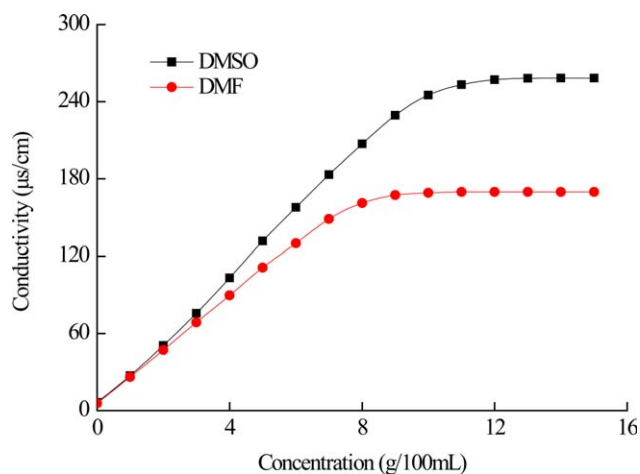


Figure 6. The conductivity vs. concentration of PANI-ES-LGS in DMSO and DMF. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that the PANI-ES isolated by the freeze-drying method with more soft templates was much more “soluble” in some organic solvent than the PANI-ES isolated with the acetone precipitation method, although a real molecular PANI-ES solution could not be obtained by the freeze-drying method.

Optoelectrical Characteristics and Applications of PANI-ES-LGS Complex

As can be seen in Figure 7, cotton coated with the suspensions was conducting in 50 mM Na_2HPO_4 -citric acid buffered solution (pH 3) and lost its conductivity in buffered solution with a pH value great than 6. This reversible change demonstrated the reversible electrical properties of the complex and the potential for using common textiles in electronic devices. Figure 8 showed the K/S values of coated cotton treated by ammonia with different concentration. When the ammonia concentration and pH value increased, the color of the coated cotton changed from emerald green to bluish violet. This color variation was also reversible, indicating the potential for using common tex-

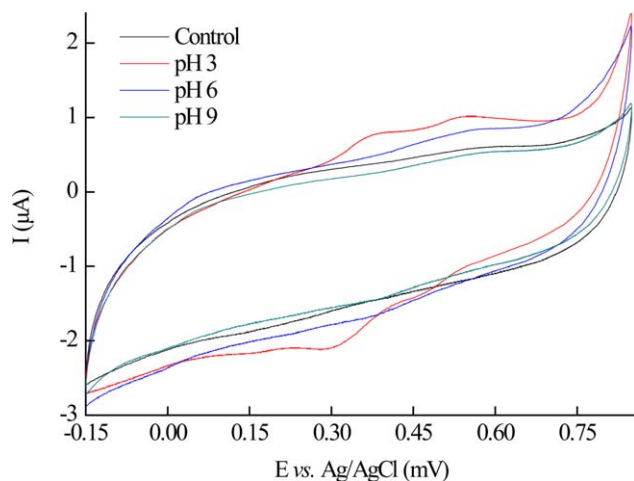


Figure 7. Cyclic voltammograms of the coated cotton recorded in buffered solution with different pH. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

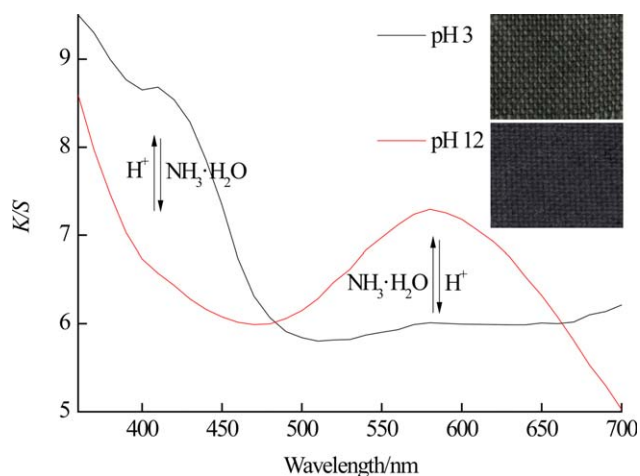


Figure 8. K/S data of cotton coated by PANI-ES LGS suspensions in ammonia with different concentration. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tiles in the fields of color changing textiles and gas sensing fibers, which could detect ammonia and some toxic acid and alkaline gases. Meanwhile, the coated cotton exhibits an electrochromic behavior. At pH 3 in Figure 7, for instance, the color of coated cotton took a reversible variation from emerald green to blackish green when the applied potential changed from -0.15 to 0.85 V, indicating that, besides pH values, the color variations of the coated cotton could also be triggered by applied electric field.²²

Degradation and Polymerization of LGS in the Reaction System

Laccases are polyphenol oxidoreductases which are capable of reducing oxygen to water and eliminating one electron from phenolic compounds to form phenoxy radicals in the meantime. On this account, phenolic compounds and their polymers (such as LGS) could be polymerized via laccase-catalyzed oxidation. The polymerization of aniline in the presence of LGS, which was also the substrate of laccase, was rather complex. To study the molecular weight changes of LGS treated by laccase, the HPSEC profiles of LGS and LGS treated by laccase with different time were discussed (Figure 9). The peaks of untreated LGS exhibited a wide bimodal distribution and shifted toward a lower molecular weight region after the LGS was treated by laccase for 24 h. It was interesting to note that the peaks shifted back to a higher molecular weight region after 96 h. It was evident that the molecular weight of LGS treated by laccase decreased in the early stage of the reaction and increased in the later stage. It could also be seen that, after treated by laccase, the molecular weight distribution of LGS was much more concentrated than before.

The HPSEC profiles of LGS treated by laccase were consistent with previous reports,^{23–26} which indicated that laccases were able to promote both polymerization and degradation of LGS. In the beginning, demethylation by laccase oxidation decreased the molecular weight of LGS, accompanied by the increase of the content of phenolic group. With the increase of incubation time, the content of phenolic group decreased and the molecular weight

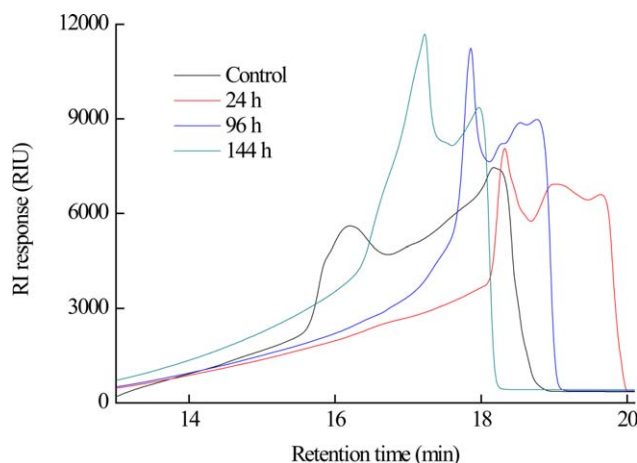


Figure 9. HPSEC profiles of LGS treated by laccase with different time and control (LGS untreated by laccase). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of LGS increased, which indicated that the polymerization of LGS was more predominant than the degradation of LGS.²³

In order to check how the degradation and polymerization of LGS affect the polymerization of aniline, UV-Vis spectra of LGS treated by laccase with and without aniline were studied in Figure 10. Compared with control LGS, the absorption band near 278 nm of laccase treated LGS enhanced a lot, confirming the polymerization of LGS. By comparing the UV-Vis spectra of LGS treated by laccase with and without aniline, a conclusion could be drawn that LGS could still act as templates and dopants in aniline polymerization after its degradation and polymerization induced by laccase, because the SO_3^- groups existing in LGS could not be oxidized by laccase. In other words, although the degradation of LGS might delay the synthesis of conducting PANI-ES in the early stage of the reaction (in the first 48 h, no conducting PANI-ES was produced), the molecu-

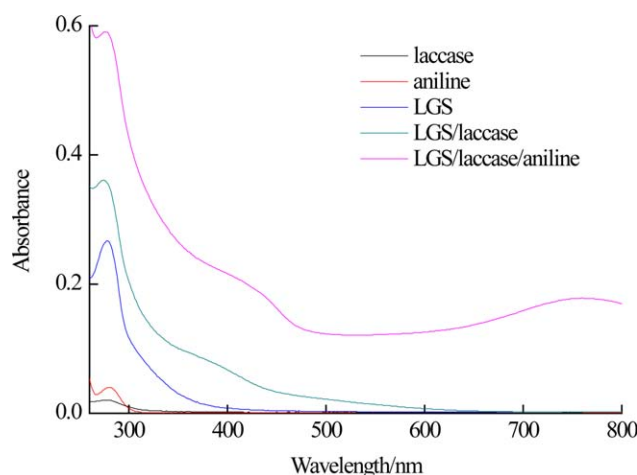


Figure 10. Effect of LGS degradation and polymerization on aniline polymerization (All the solutions were diluted with buffer (1:100) before recorded). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

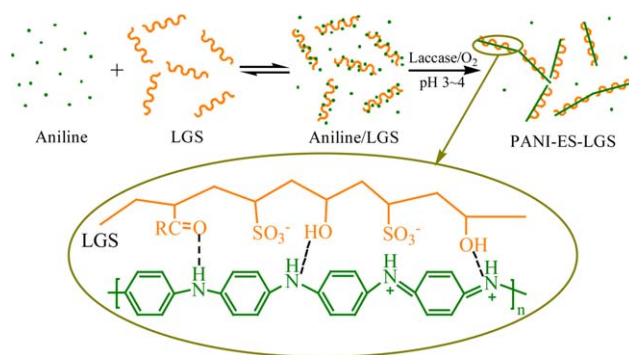


Figure 11. Schematic diagram of laccase catalyzed polymerization of aniline in LGS template system. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

lar weight changes of LGS did not affect its function in aniline polymerization and the role of laccase for aniline polymerization in the long term. On the contrary, the polymerization of LGS might provide aniline polymerization with a bigger template, which was conducive to the directional alignment of the monomer along the linear templates, and then the increase of the conductivity of the polymer.

Interactions between LGS And PANI-ES

Positively charged aniline monomer was fixed along negatively charged LGS before aniline polymerization and this electrostatic adsorption was the main force between PANI-ES chains and LGS. We speculated that the hydrogen bonds between PANI-ES chains and LGS were also easy to form because hydroxyl and carbonyl groups are the most abundant two functional groups existing in LGS. In addition, the protonation of the PANI-ES imine nitrogens through interactions with the hydroxyl groups in LGS could also strengthen the interactions between PANI-ES chains and LGS.^{27,28} All these bonds improved the stability of the complex in different extent. Based on above analysis, the schematic diagram of laccase catalyzed polymerization of aniline in LGS template system was drawn, as shown in Figure 11.

CONCLUSIONS

In this study, conducting PANI-ES-LGS complex was prepared using laccase as a biocatalyst at pH 3.5. LGS, serving as linear templates and dopants, promoted the synthesis and processability of conducting polyaniline. This method had remarkable advantages as compared with both chemical and peroxidase-catalyzed methods due to the use of atmospheric oxygen as the oxidant. The variable optoelectronic properties of PANI were given to natural textile materials by immobilizing the complex on the surface of cotton. Cyclic voltammetry of the coated cotton indicated a high electrochemical activity of LGS-doped PANI and the potential for using common textiles in electronic devices. As the color of the coated cotton responded to ammonia, the applications of common textiles in the fields of gas sensing fibers and color changing textiles might also be realized utilizing the PANI-ES-LGS suspensions. As for the mechanism, the polymerization of aniline in the presence of LGS, which was also the substrate of laccase, was rather complex due to the degradation of LGS might delay the synthesis of conducting PANI

in the early stage of the reaction. However, the degradation and polymerization of LGS did not affect aniline polymerization in the long term.

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