


Laccase 촉매 작용에 의한 아닐린 중합에 Soft Template이 미치는 영향

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(2017년 4월 5일 접수, 2017년 9월 2일 수정, 2017년 11월 5일 채택)

Laccase-catalyzed Polymerization of Aniline with Different Soft Templates

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(Received April 5, 2017; Revised September 2, 2017; Accepted November 5, 2017)

Abstract: A comparison of laccase-catalyzed polymerization of aniline in the presence of two different “soft” templates - micelle forming surfactant sodium dodecylbenzenesulfonate (SDBS) and sulfonated natural polyelectrolyte calcium lignosulfonate (LGS) - was investigated to check the influences of different templates on the kinetics of the polymerization and on the characteristics of the complexes obtained. Under respective optimal conditions, pH 4.5 for SDBS and pH 3.5 for LGS, the reaction with LGS was much slower than that in SDBS emulsion. The conditions for laccase-catalyzed synthesis of emeraldine salt of polyaniline (PANI-ES) in both cases were optimized using UV-vis spectroscopy. It was found that PANI-ES obtained with LGS template had a higher degree of oxidation with a higher amount of benzene rings than PANI-ES obtained in SDBS emulsion. Since the protonation of PANI occurred on its imine sites, the doping effect of LGS-doped PANI-ES, confirmed by XPS, was thus better than that of SDBS-doped one.


Keywords: laccase-catalyzed polymerization, emeraldine salt of polyaniline, soft templates, sodium dodecylbenzenesulfonate, calcium lignosulfonate.

Introduction

Polyaniline (PANI) is one of the most popular conducting polymers thanks to low price of the monomer, simple preparation, good stabilities and variable optoelectronic properties.¹⁻⁵ The general formula of PANI is $[(\text{-B-NH-B-NH-})_x(\text{-B-N=Q=N-})_{1-x}]_n$, in which B and Q refer to the benzenoid and quinoid rings respectively. PANI varies in oxidation from fully oxidized state (pernigraniline, $x=0$), through half oxidized state (emeraldine, $x=0.5$) to fully reduced state (leucoemeraldine, $x=1$). Emeraldine salt (PANI-ES) is the only one that can be conducting, because PANI gets its conductivity through the protonation of imine nitrogens ($=\text{N-}$), which is also referred as “doping”.¹ The structural changes of PANI according to different redox conditions and protonated/deprotonated states are shown in Scheme 1. Since polymer chains of PANI are very

long, detailed structural analyses of PANI, such as mass spectrometry and nuclear magnetic resonance spectrometry, are very difficult.

In case of traditional chemical and electrochemical synthesis of PANI in strongly acidic media, intermolecular hydrogen bonds in the polymer chains result in the poor solubility of PANI and limit its practical application.^{6,7} In order to improve the solubility and expand practical application of the polymer, “hard” or “soft” templates are usually used in aniline polymerization. “Hard” templates refer to silicon, various carbon and plastic materials, as well as metals and metal oxides. “Soft” templates can be negatively charged assemblies of amphiphiles, like micelles⁸⁻¹³ and vesicles,¹⁴⁻¹⁶ or negatively charged polyelectrolytes, including synthetic polymers, like sulfonated polystyrene (SPS),¹⁷⁻²² poly(amic acid)²³ and poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS),^{24,25} and natural polymer derivatives, like lignosulfonate (LGS).²⁶⁻²⁹ Compared to pure PANI, complexes composed of these templates and PANI are more promising for practical application.^{1,2} The mechanism of anionic template influence on the

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polymerization of aniline was quite clear now.^{8-10,14-17} These templates have three main functions: (1) they bind and align the aniline cation, thus resulting in a preferential head-to-tail coupling and therefore leading to the formation of mainly a linear structure of PANI; (2) the template molecules are attached to the backbone of PANI and play the role of a dopant which leads to an increase in the conductivity of the polymer;¹⁰ (3) the template help PANI to disperse in water and the suspension obtained are very stable for many months without precipitation and notable color changes. Such suspension can even be used as ink with an inkjet printer.¹⁶

Compared with traditional chemical methods, enzymatic catalysis of PANI, usually using “soft” templates, provides an alternative “green” method toward the formation of soluble and processable conducting PANI because of the milder reaction conditions and the simplified purification process of final products.^{8-12,14-22} Oxidative polymerization of aniline has been conducted for a century and enzymatic syntheses of conducting PANI have been investigated since the 1990s.^{30,31} By enzymatic syntheses stable suspension of complex, composed of PANI and templates, was obtained. The complex was so called as “water-soluble polyaniline”. Actually, PANI does not real dissolve in water, but disperse in water with the help of soft templates.^{8-12,14-22} The soft templates also increase the solubility of PANI in some organic solvents.^{14,21,32}

From our previous work, we realized that soft templates are very important for laccase-catalyzed polymerization of aniline and the complexes obtained differ in many properties, based on the templates used.^{11,32,33} The aim of our present work was to check the influences of different “soft” templates on the kinetics of the polymerization and the characteristics of the complexes obtained. The templates used were typical negatively charged assemblies of amphiphiles (emulsion of sodium dodecylbenzenesulfonate, SDBS) and sulfonated natural polyelectrolyte (calcium lignosulfonate, LGS), respectively. The reaction was monitored and optimized using UV-Vis spectroscopy, FTTR, Raman spectra and XPS analyses of the complexes demonstrated that LGS-doped PANI-ES had a higher degree of oxidation with a higher amount of benzene rings compared with SDBS-doped PANI-ES. Since the results of cyclic voltammetry and thermal analyses proved that the electroactivity and thermal stability of LGS-doped PANI-ES was better than that of SDBS-doped PANI-ES, we believe that LGS could be a promising dopant in enzymatic syntheses of conducting PANI-ES.

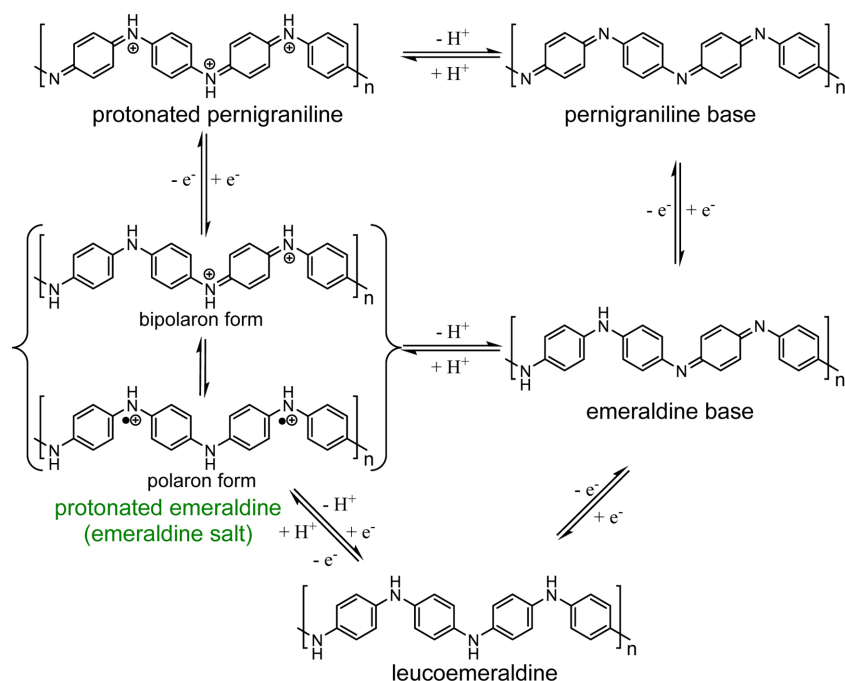
Experimental

Materials. Laccase from *Aspergillus* was supplied by Novozymes (Shanghai, China) with an enzyme activity of 60 U/g. One unit of laccase referred to the amount of laccase required to oxidize 1 μmol ABTS²⁻ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) per minute at room temperature. LGS (calcium lignosulfonate, M_n 2500, M_w 18000) was purchased from Sigma-Aldrich. Aniline, SDBS (sodium dodecylbenzenesulfonate) and all other reagents used were analytical grade from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) without further purification. All solutions were prepared with deionized water.

Laccase-catalyzed Polymerization of Aniline in SDBS Emulsion. A 69.7 mg SDBS sample was dissolved into 20 mL of 50 mM Na₂HPO₄-citric acid buffered solution, followed by the addition of aniline with constant stirring. The stirring was stopped when aniline was completely dissolved. After adjustment of pH to 4.5 with citric acid, the reaction was initiated by the addition of laccase (0.5 U/mL). Briefly, the optimal reaction conditions were: 10 mM SDBS, 10 mM aniline, pH 4.5, 0.5 U/mL laccase; the typical reaction time was 3 days at 5 °C.³³

Acetone at an equal volume with reaction solution (20 mL) was added to the dispersion to induce precipitation by adjusting the hydrophilic-hydrophobic balance of the system. The precipitate was collected by centrifugation and washed twice with 20 mL of acetone and 100 mL of 50% (v/v) acetone/water mixture each time to remove the unreacted chemicals and aniline oligomers. The final purified complex, abbreviated as PANI-ES-SDBS, was dried in an oven at 60 °C for 24 h for further characterization.

Laccase-catalyzed Polymerization of Aniline in LGS Template System. A 100 mg LGS-Ca sample was dissolved into 20 mL of 50 mM Na₂HPO₄-citric acid buffered solution, followed by the addition of 35 mM aniline with constant manually stirring. The stirring was stopped when the monomer was completely dissolved and then the pH was adjusted to 3.5 with citric acid. The reaction was initiated by the addition of laccase (1.2 U/mL). The initial concentrations in the reaction mixture were as follows: 5 mg/mL LGS, 35 mM aniline, pH 3.5, 1.2 U/mL laccase.³² It took 6 days to complete the polymerization at 5 °C and the precipitation was obtained by the same way described above. The dried complex was abbreviated as PANI-ES-LGS.



Scheme 1. Structural changes of polyaniline according to different redox conditions and protonated/deprotonated states.

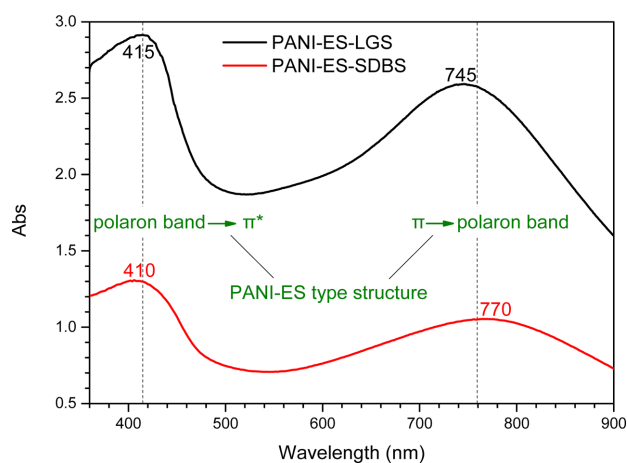


Figure 1. Full UV-vis spectra of PANI-ES-LGS and PANI-ES-SDBS. Conditions for PANI-ES-LGS: [LGS]=5 mg/mL, [aniline]=35 mM, 1.2 U/mL laccase, 6 days at pH 3.5 and 5 °C. Conditions for PANI-ES-SDBS: [SDBS]=10 mM, [aniline]=10 mM, 0.5 U/mL laccase, 24 h at pH 4.5 and 5 °C.

UV-vis Spectra Analysis. UV-vis spectra of the complexes were recorded using a UV-2808S UV-vis spectrophotometer (Unicosh, China) in the range of 190–900 nm. Full UV-vis spectra of the suspensions were shown in Figure 1. Two absorption peaks around 420 and 770 nm indicated the formation of polarons, typical in PANI-ES type structure (Scheme 1 and Figure 1). The polymerization was monitored by mea-

suring the changes of maximal absorption of the complexes ($\lambda_{\text{max}} \approx 770$ nm).^{1-5,32,33} In the case of LGS, all the suspensions were diluted with buffer (1:10) before recorded due to the high concentration of PANI-ES-LGS complex.

FTIR and Raman Spectroscopy. FTIR spectroscopy was performed by the conventional procedure. Complexes of about 1 mg were finely ground with 200 mg KBr and pellets were pressed and measured using a Nicolet iS10 infrared spectrophotometer (Thermo Nicolet, USA). Raman spectra of the complexes were measured using a Raman spectrometer coupled to a microscope (Renishaw, UK).

XPS Characterizations. XPS measurements of the complexes were carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer, USA) using aluminum as the source of radiation at 14 kV. Both the entire range spectra (0–1100 eV) and the narrow range spectra of all the elements were recorded using a RBD 147 interface (RBD Enterprises, USA) using an AugerScan 3.21 software.

Cyclic Voltammetry Tests. Cyclic voltammetry tests of the complexes were performed using a CHI 660D electrochemical workstation (CH Instruments, Inc., Austin, USA) to confirm the electrochemical activity of the complexes. Before the measurement, the complexes were dissolved in dimethyl sulfoxide (DMSO) with the some concentration (60 mg/mL), applied to the glassy-carbon electrode (10 μ L), and dried again to obtain

a film. It's important to note that the solubility of PANI-ES-LGS in DMSO was about 110 mg/mL,³² much higher than that of pure PANI with a poor solubility of less than 20 mg/mL in DMSO.²¹ In addition, Guo *et al.*¹⁴ found that the PANI-ES isolated by freeze-drying method with more soft templates was much more "soluble" in some organic solvent than the PANI-ES isolated with acetone precipitation method.

Thermal Analysis. Thermogravimetric analysis (TGA) measurements of PANI-ES-SDBS and PANI-ES-LGS were performed under N₂ atmosphere in the range of 30–900 °C at a heating rate of 20 °C·min⁻¹ using a TA/TGA Q500 thermogravimetric analyzer (TA Co., Ltd., USA).

XRD Measurements. XRD measurements of the complexes were performed on a Bruker D8-Advance XRD instrument (Bruker AXS Inc., Germany) with a scanning angular range of 5–40° (2θ) and a scanning rate of 2°/min.

Results and Discussion

Comparison of Optimal Conditions and Influences of Conditions on the Polymerization of Aniline. The laccase used in this paper has an optimal pH of 5.0 and will be inactivated with a pH value less than 2.5. As other conditions were fixed, the influence of pH on laccase-catalyzed polymerization of aniline in SDBS and LGS template systems were shown in Figure 2. The appropriate pH values for laccase-catalyzed synthesis of PANI-ES complexes, in SDBS and LGS template systems, were 4.5 and 3.5, respectively. In SDBS emulsion, the high absorbance around 785 nm at pH 5.0 was mainly from the turbidity of the reaction suspension (scattered light which did

not reach the detector) and some branched aniline oligomers as side-products, e.g., phenazines, since the monomers cannot be protonated or absorbed onto the surface of negatively charged templates at a pH higher than the pK_a of aniline, 4.6.³³ In the field of polymerization of aniline, low pH is conducive for doping and conductivity of the polymer. However, for enzymatic polymerization of aniline, low pH will decrease the activity of enzyme. Therefore, the conductivity of PANI produced by enzymatic catalysis methods under a relatively higher pH (pH=3–5) environment tends to be two orders of magnitude lower than that of PANI obtained from traditional chemical methods in strongly acidic media (pH<2).^{8,9,25,34} Similarly, although *Aspergillus* laccase catalyzed the polymerization of aniline in SDBS micellar solution to form PANI-ES-SDBS complex under milder conditions than in LGS template system, the degree of protonation of PANI-ES-SDBS may be lower than that of PANI-ES-LGS, since PANI-ES-LGS was produced under a lower pH environment.

Since laccase-catalyzed reactions run with atmospheric O₂ only, oxidation of chain ends during the polymerization could not occur with H₂O₂ as in the case of peroxidase-based oxidative polymerization. Under the premise that the other conditions were optimal, effect of reaction time on laccase-catalyzed polymerization of aniline in different templates was shown in Figure 3. In the case of SDBS template system, the absorbance around 785 nm increased linearly in the first 24 h. In the meantime, the color of the suspension changed from white to emerald and got darker gradually before it showed completely dark-green. As for LGS template system, there was no absorbance peak at 785 nm in the first 48 h and the color

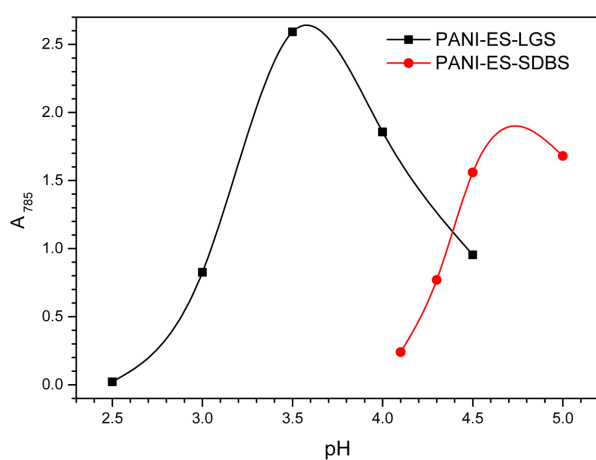


Figure 2. Effect of pH on laccase-catalyzed polymerization of aniline in SDBS (●) and LGS template (■) systems.

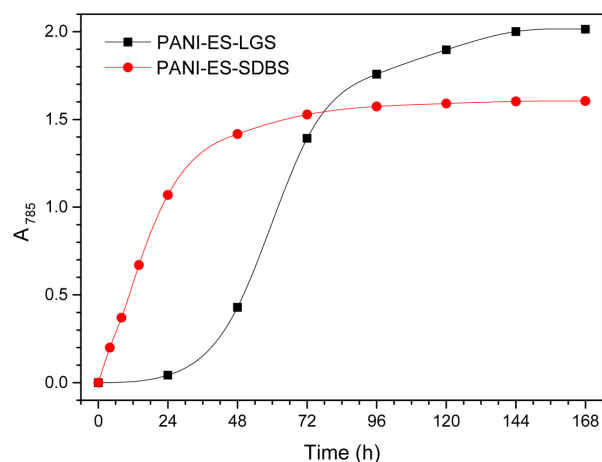


Figure 3. The time evolution of the absorption at $\lambda=785$ nm during laccase-catalyzed polymerization of aniline in SDBS (●) and LGS template (■) systems.

of the suspension changed from orange yellow to brown during this period. The absorption band around 785 nm appeared in the third day with the suspension color turning to dark-green. Since the chemical structure of LGS is much more complicated than that of SDBS, the poor regularity of LGS might be the drawbacks of LGS as a template for enzymatic reaction. On the other hand, the polymerization of aniline in the presence of LGS, which was also the substrate of laccase,³⁵⁻³⁸ was rather complex. Although it is not immediately clear why no PANI-ES was produced in the first 48 h and the effect of laccase-catalyzed oxidation of LGS on the polymerization of aniline is also not clear at the moment, they are taken as interesting preliminary observation for further more detailed investigations toward a better understanding of enzyme-catalyzed LGS-assisted polymerization. It might be due to the steric hindrance of LGS or the incipient degradation and the subsequent polymerization of LGS induced by laccase,³⁵⁻³⁸ which postponed the polymerization of aniline. It seems that, the oxidation of LGS did not affect its function as anionic templates in the long term, since the SO_3^- groups existing in LGS could not be oxidized by laccase. After the reaction, the complexes obtained in both cases remained homogeneously dispersed in aqueous solution for weeks thanks to the sulfo groups existed in the templates.

Compared with chemical methods, enzymatic synthesis of conducting PANI was always carried out with a much lower monomer concentration and therefore had a relatively lower polymer yield.¹⁴⁻¹⁶ In our case, the influence of monomer concentration, as the other conditions were fixed, on laccase-catalyzed polymerization of aniline in different template systems was shown in Figure 4. The optimal aniline concentration for the polymerization in SDBS and LGS template was 10 and 35 mM, respectively. In LGS template system, although LGS exhibited weaker template effect than SDBS at low aniline concentration (aniline polymerization began at higher aniline concentrations), relatively more monomers took part in the polymerization in the end. Since LGS contains an unknown amount of sulfo-groups, the exact comparison of the number of adsorption sites in these two template systems would be very difficult. However, we still provide reasonable speculation based on the properties of LGS and the low pH environment in LGS template system: Compared to SDBS emulsion templates, LGS might provide more adsorption sites (sulfo groups), which could absorb more aniline monomers to participate in the template-oriented polymerization, and the lower pH in LGS template system is conducive to the absorption of

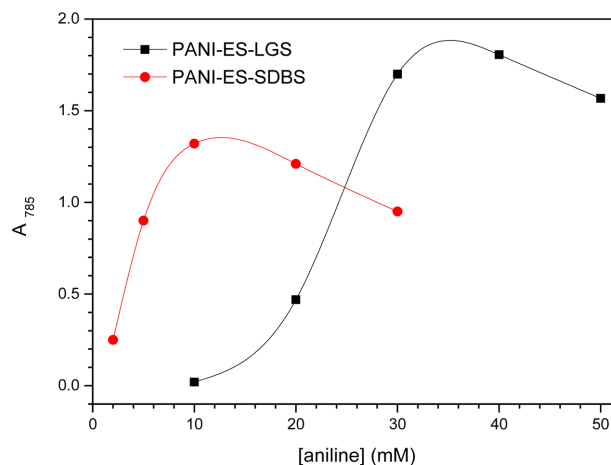


Figure 4. Effect of aniline concentration on laccase-catalyzed polymerization of aniline in SDBS (●) and LGS template (■) systems.

positively charged aniline cations onto negatively charged templates.

Comparison of the Oxidation Degree and Doping Level of the PANI-ES Obtained in Different Template Systems. FTIR spectra of the suspensions of PANI-ES-SDBS and PANI-ES-LGS are shown in Figure 5(A). In the spectrum of PANI-ES-LGS, the peak at 3450 cm^{-1} was mainly attributed to $-\text{OH}$ stretching in LGS. This peak could be also found in the spectrum of PANI-ES-SDBS as a result of trace water that existed in the complex. In the spectrum of PANI-ES-SDBS, the vibration bands around 2925 and 2850 cm^{-1} were much stronger than that of PANI-ES-LGS, because there were more $-\text{CH}_3$ and $-\text{CH}_2$ groups in SDBS than in LGS. The bands around 1585 and 1498 cm^{-1} in both cases were assigned to quinone and benzene ring deformation respectively.³⁹ Compared with PANI-ES-LGS, the peak at 1498 cm^{-1} was much stronger than the peak at 1585 cm^{-1} in PANI-ES-SDBS, which means a lower oxidation degree of PANI chains and more leucoemeraldine existed in SDBS-doped PANI-ES. As the protonation of emeraldine base occurred on its imine sites (Scheme 1), the doping effect of LGS-doped PANI-ES, which had more quinone rings, might be better than that of SDBS-doped PANI-ES. This indeed turned out to be the case (see XPS analysis below for details). The peak at 1300 cm^{-1} , typical for C-N aromatic bond, and the peak at 1131 cm^{-1} , corresponding to $\text{S}=\text{O}$ stretching vibration, indicated the presence of SO_3^- doped PANI in both samples.⁴⁰ The peak at 824 cm^{-1} indicated the head-to-tail coupling of the monomers, which led to the formation of linear polymeric chains.⁴¹

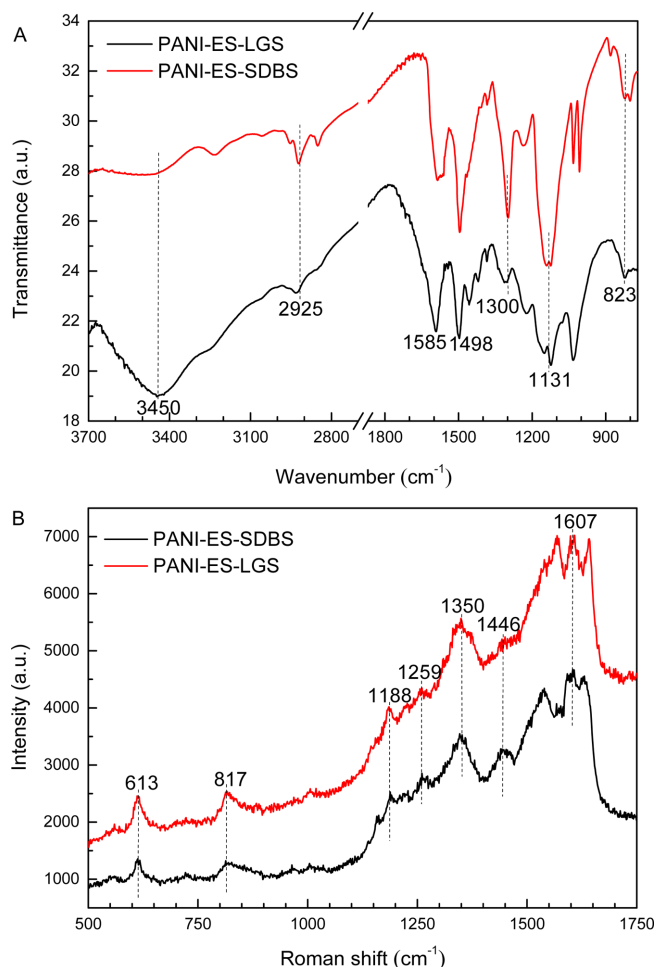


Figure 5. FTIR (A); Raman (B) spectra of PANI-ES-SDBS and PANI-ES-LGS.

Raman spectra of the solid powders of PANI-ES-SDBS and PANI-ES-LGS were quite similar (Figure 5(B)). The intense band around 1607 cm^{-1} was attributed to the C-C stretching modes of semiquinone units.⁴² The peak of C=N stretching vibrations at about 1537 cm^{-1} of PANI-ES-SDBS was weaker than that of PANI-ES-LGS, indicating a relatively lower oxidation level of PANI chains. The band at 1259 cm^{-1} was due to the C-N stretching in polaronic units. The strong C-N stretching vibrations at about 1350 cm^{-1} signified semiquinone radical structures in both samples.^{42,43}

As a tool for the analysis of the structure of material surface,

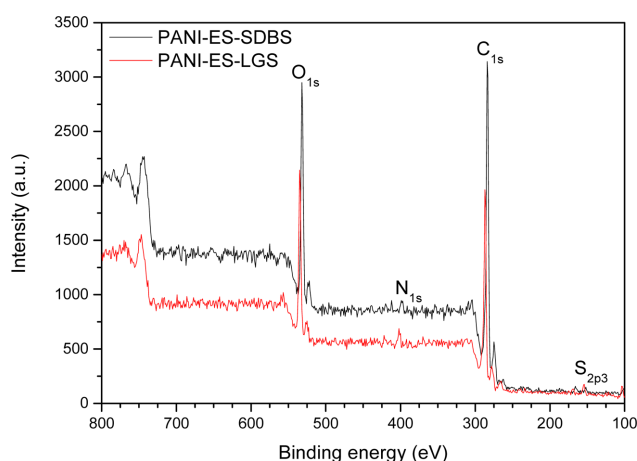


Figure 6. XPS wide scan spectra of PANI-ES-SDBS and PANI-ES-LGS.

X-ray photoelectron spectroscopy (XPS) is very useful for the quantitative evaluation of various redox states of PANI.⁴⁴ The relative surface composition (Table 1) of the complexes obtained from the results of quantitative XPS analysis was in accordance with XPS wide scan (Figure 6). Relatively large amounts of nitrogen and sulfur elements were detected on PANI-ES-LGS surface and the ratio of sulphur atom (from sulfo groups in the dopants) to nitrogen atom (from PAN) on PANI-ES-LGS surface (30.56) was also higher than that of PANI-ES-SDBS (27.41), indicating higher doping level of PANI-ES-LGS than PANI-ES-SDBS.

The XPS N1s core level spectra of the complexes are shown in Figure 7. Three main contributions in both samples could be considered as amine nitrogen (-NH), imine nitrogen (-N=) and N^+ species.⁴⁴⁻⁴⁶ In the case of PANI-ES-LGS, the peak from N^+ was relatively stronger (Figure 7(B)) than the one from PANI-ES-SDBS (Figure 7(A)). According to the contribution of nitrogen groups resulting from XPS (Table 1), the N^+/N ratio of PANI-ES-LGS (30.45) is higher than that of PANI-ES-SDBS (24.82), indicating again higher doping level of PANI-ES-LGS. This was in accordance with the results of FTIR and Raman spectra, since PANI-ES-LGS had more imine sites than PANI-ES-SDBS and the protonation of PANI-ES occurred on its imine sites.

Table 1. Composition of the Complexes and Contribution of Nitrogen Groups Resulting from XPS

Samples	Relative surface composition (%)					Intensity of N1s (%)		
	C1s	O1s	N1s	S2p	S/N	N^+/N	-NH-/N	-N=/N
PANI-ES-SDBS	77.92	19.58	1.97	0.54	27.41	24.82	48.82	26.36
PANI-ES-LGS	73.44	23.28	2.52	0.77	30.56	30.45	41.85	27.70

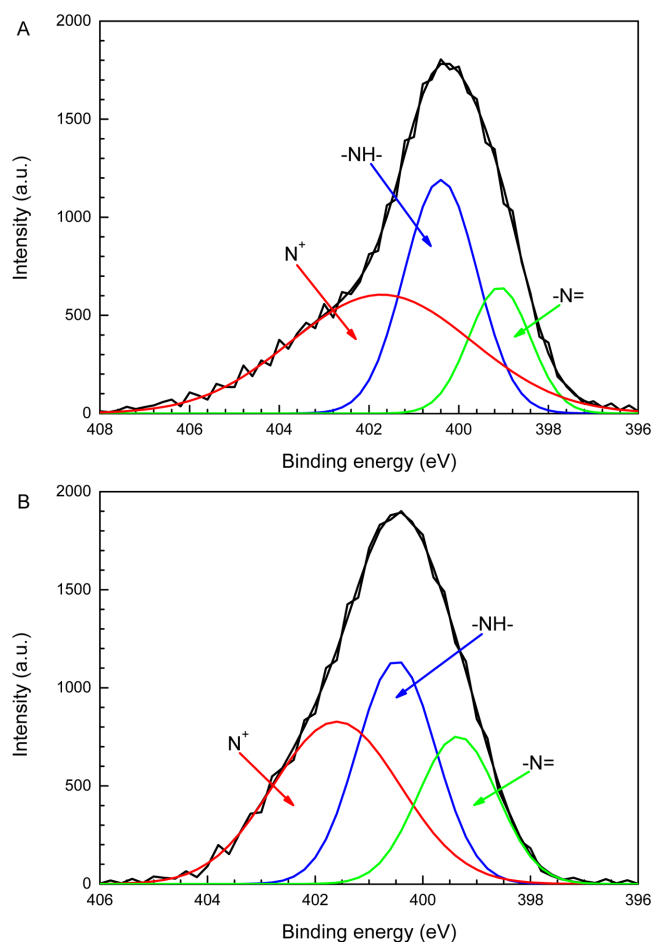


Figure 7. XPS N1s core level spectra of PANI-ES-SDBS (A); PANI-ES-LGS (B).

Comparison of the Properties of the Complexes Obtained.

It is generally accepted that higher electroactivity of PANI can be reached at low pH and all oxidation peaks shift to less positive potentials with increasing pH.⁴⁷ In our case, the recorded cyclic voltammograms (Figure 8) showed that PANI-ES obtained in the presence of SDBS or LGS was redox active up to pH 4 (50 mM Na₂HPO₄-citric acid buffered solution). For both samples, two pairs of reversible symmetrical redox peaks were detected, which confirmed that these complexes possessed a certain degree of electrochemical activity and indicated the potential for using these complexes in electrodes, coated with a conductive organic material.⁴⁸⁻⁵²

The cyclic voltammogram of PANI-ES-LGS resembles that of PANI, with two anodic peaks observed at 283 and 552 mV, and the corresponding cathodic peaks found at 17 and 420 mV. Commonly, the couple located at lower potentials is ascribed to the redox transformations of fully reduced chains of the leu-

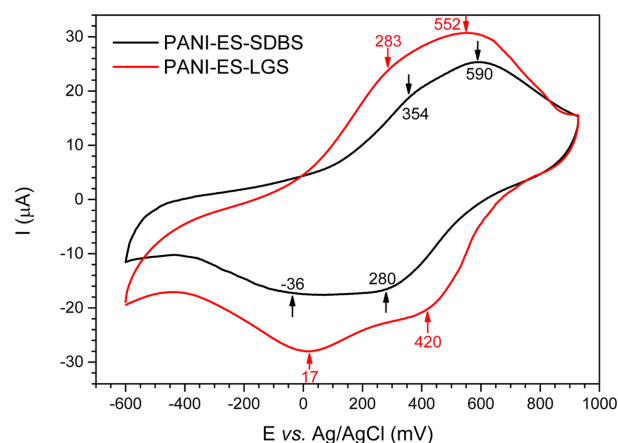


Figure 8. Cyclic voltammograms of PANI-ES-SDBS and PANI-ES-LGS.

coemeraldine form of PANI to the half-oxidized chains of the PANI-ES form; while the second couple found at higher potentials is due to the interconversion of PANI-ES to the fully oxidized pernigraniline form of PANI.^{53,54} The curve of PANI-ES-LGS was also compared with other works, in which PANI-ES-LGS was obtained using different catalysts.^{26,27} The cyclic voltammogram of PANI-ES-LGS obtained in this work was quite similar to that of PANI-ES-LGS obtained from hematin-catalyzed polymerization of aniline,²⁶ indicating that the electrochemical property of PANI-ES-LGS obtained in this work was equivalent to PANI-ES-LGS obtained using other catalysts.

The cyclic voltammogram of PANI-ES-SDBS had two anodic peaks at 354 and 590 mV, and the corresponding cathodic peaks at -36 and 280 mV. With lower values of current, the cyclic voltammogram of PANI-ES-SDBS revealed lower electrochemical activity than PANI-ES-LGS. In addition, it's worth noting that the distance between oxidation and reduction peaks of PANI-ES-LGS was relatively shorter than that of PANI-ES-SDBS. The reason for this difference and what does this phenomenon mean is not clear yet.

TGA curves of PANI-ES-SDBS and PANI-ES-LGS were presented in Figure 9. For PANI-ES-LGS a bigger initial loss, probably attributed to the loss of water and LGS, was found between 50 and 200 °C, although both samples started to decompose at around 200 °C. In the curve of PANI-ES-SDBS, a 52.0 wt% loss took place in the range of temperature between 300 and 500 °C and the polymer maintains 37.6 wt% at 500 °C. In comparison, for PANI-ES-LGS a 21.9 wt% loss occurred in this range and the polymer maintains 59.8 wt% at 500 °C, which was almost the same with others' work.²⁸ No

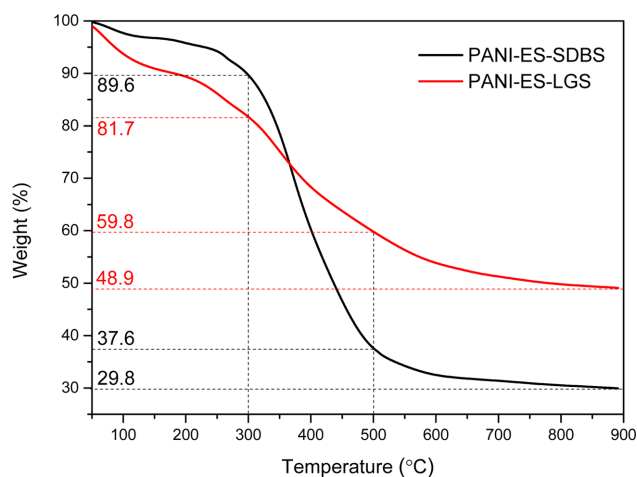


Figure 9. TGA curves of PANI-ES-SDBS and PANI-ES-LGS.

any polymers exist at temperatures up to 900 °C and only carbon skeleton gave the remaining 48.9 and 29.8% in PANI-ES-LGS and PANI-ES-SDBS, respectively. In brief, compared to PANI-ES-SDBS, although PANI-ES-LGS had higher mass loss at low temperatures, it seems more stable at high temperatures with lower mass loss.

The content and composition of templates definitely make a big different of the thermal stability of the complexes obtained. It is known that PANI is characterized by a high thermal stability and makes the complexes more stable. On the contrary, the templates are rather unstable and this may lead to the higher mass loss of PANI-ES-LGS at low temperatures, since PANI-ES-LGS had a higher amounts of templates (S/N=30.56%, Table 1). The lower mass loss of PANI-ES-LGS at high temperatures might be due to the formation of complicated bonds between PANI chains and the functional groups in LGS. Beside the main force, electrostatic adsorption, 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.^{28,46} All these bonds improved the stability of the complex in different extent.

In the end, PANI-ES-SDBS and PANI-ES-LGS were analyzed by XRD to investigate whether there are differences in the crystallinity of these PANI-ES products (Figure 10). It is known that degree of ordering for conducting PANI form is higher than for a nonconducting one (neutral PANI) because

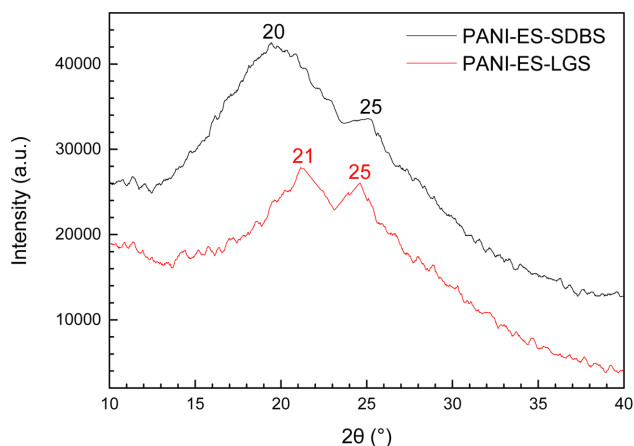


Figure 10. XRD spectra of PANI-ES-SDBS and PANI-ES-LGS.

the first form contains more rigid molecules due to longer conjugation length and it has been reported that the degree of crystallinity of HCl-doped PANI increases with the doping level.⁵⁵ In our case, the crystallinity of the complexes seemed not ideal, since no clear crystal peak was shown in both cases. However, we still find some literatures with similar spectrum and try to explain our case:⁵⁶⁻⁵⁸ For PANI-ES-LGS, the diffraction peak at $2\theta=25^\circ$ was much stronger than that of PANI-ES-SDBS, which was in accordance with the literatures reporting that neutral PANI has a major broad diffraction peak at $2\theta=20^\circ$ and doped PANI-ES has two broad diffraction peaks at $2\theta=20^\circ, 25^\circ$.⁵²⁻⁵⁴ In summary, the result of XRD analysis indicated that PANI-ES-LGS might have relatively higher crystallinity and better doping effect than PANI-ES-SDBS.

Conclusions

In this study, PANI-ES were prepared using *Aspergillus* lacase as a biocatalyst in SDBS and LGS template systems, respectively. Under respective optimal conditions, relatively more aniline monomers were absorbed to LGS to participate in the template-oriented polymerization. This could be an improvement for enzymatic synthesis of PANI-ES, which was generally carried out with a lower monomer concentration compared with chemical synthesis and therefore had a lower polymer yield. It's explicit that, PANI-ES-LGS, produced under a lower pH, had a higher oxidation degree with a higher amount of benzene rings than PANI-ES-SDBS and the doping effect of LGS-doped PANI-ES, confirmed by XPS, was also better than that of SDBS-doped one. Cyclic voltammetry and thermal analyses indicated that PANI-ES-LGS exhibited

higher electrochemical activity and better thermal stability than PANI-ES-SDBS. In conclusion, our research showed expansive application prospects of LGS in the field of enzymatic reaction.

Acknowledgments: Financial supports by Fundamental Research Funds for the Central Universities (JUSRP51717A), the Programme for Changjiang Scholars and Innovative Research Teams at University (IRT_15R26), and the Graduate Student Innovation project (KYLX_1140) are greatly appreciated.

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