

Synthesis and Characterization of Conducting Polyaniline Prepared by Laccase-Catalyzed Method in Sodium Dodecylbenzenesulfonate Micellar Solutions

Alexander V. Streltsov,¹ Olga V. Morozova,¹ Nataliya A. Arkharova,² Vera V. Klechkovskaya,² Irina N. Staroverova,³ Galina P. Shumakovich,¹ Alexander I. Yaropolov¹

¹Laboratory of Chemical Enzymology, A.N. Bakh Institute of Biochemistry, RAS, Moscow 119071, Russia

²Laboratory of Electron Diffraction, A.V. Shubnikov Institute of Crystallography, RAS, Moscow 119333, Russia

³Department of Biophysics and Physics, K.I. Skryabin Moscow State Academy of Veterinary, Medicine and Biotechnology, Moscow 109472, Russia

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ABSTRACT: Enzymatic polymerization of aniline was performed in micellar solutions of the anionic surfactant sodium dodecylbenzenesulfonate (SDBS). High-redox-potential oxidase-laccase isolated from the fungi, *Trametes hirsuta*, was used as a catalyst in oxidative polymerization of aniline, the atmospheric oxygen serving as an oxidizing agent. It is shown that the laccase-catalyzed reaction of monomer polymerization is kinetically controllable and its mechanism is very distinctly different from that of chemical polymerization.

The synthesized PANI/SDBS complexes were characterized using Fourier transform infrared and UV-vis spectroscopy, cyclic voltammetry, thermogravimetry, transmission electron microscopy, and electron diffraction. The antistatic properties of the prepared PANI/SDBS composite were also studied. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 114: 928–934, 2009

Key words: conducting polymers; polyaniline; laccase; radical polymerization; micelles

INTRODUCTION

Intrinsically conducting polymers have attracted high attention over recent years due to their unique electrical and optical properties. Among these conducting polymers, polyaniline (PANI) has been most extensively studied because of its wide potential for many technological applications, such as organic lightweight batteries, microelectronics, optical displays, antistatic coatings, and electromagnetic shielding.^{1–3}

PANI is commonly synthesized by chemical or electrochemical methods in strongly acidic media by aniline oxidative polymerization.⁴

In the course of the synthesis, the emeraldine base of PANI is formed. Emeraldine salt is usually obtained from the emeraldine base via protonation of its imine sites with sufficiently strong acids (Scheme 1). The reaction is far from being environmentally friendly because of high acid and ammonium persulfate concentrations, and the use of these

chemicals requires further steps of final product purification. Moreover, the reaction of chemical polymerization of the monomer is not kinetically controllable, and the synthesized conducting PANI is not processable due to its poor solubility in common solvents. Thus, biochemical reactions are more attractive as an alternative route for the synthesis of conducting PANI.^{5,6} The enzymatic approach is environmentally benign and kinetically controllable. The peroxidase enzymes, such as horseradish peroxidase (HRP) or soybean peroxidase, are widely used for aniline polymerization.^{7,8} The reaction is carried out in the presence of hydrogen peroxide as oxidizing substrate. It should be noted that peroxidases are very sensitive to hydrogen peroxide concentration and at concentrations above 1 mM lose their initial activity, which requires stepwise addition of diluted hydrogen peroxide to the reaction medium.

To address this issue, a high-redox-potential fungal laccase, which can catalyze the oxidation of aniline by atmospheric oxygen, has been introduced in the synthesis of conducting PANI.

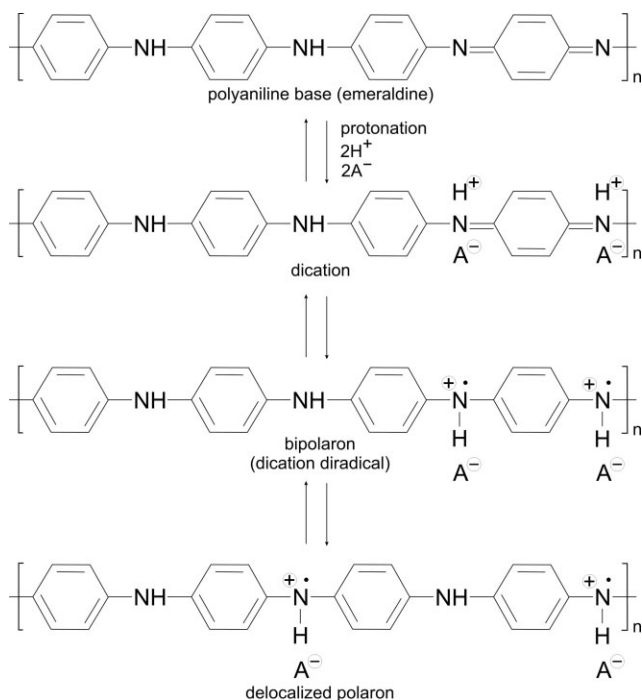
Laccase (benzenediol: oxygen oxidoreductase; EC 1.10.3.2), a multicopper enzyme belonging to the family of blue oxidases, catalyzes the oxidation of a wide variety of inorganic and organic (including aniline) substrates with the concomitant four-electron reduction of oxygen to water.^{9–11} Laccases are attractive, industrially relevant enzymes that can be used for a number of different applications, e.g., biofuel

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Correspondence to: A. I. Yaropolov (yaropolov@inbi.ras.ru).

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Scheme 1 Chemical structures of nonprotonated/protonated PANI.

cells, labeling in immunoassays, bioremediation, green biodegradation of xenobiotics, green organic synthesis.^{11–15}

However, even after enzyme-catalyzed synthesis of conducting PANI there are still some problems due to a poor processability of the polymer and the possibility of generating ortho- and para-directed units. These two kinds of units can form a cross-linked or branched chain. To avoid these disadvantages, several strategies have been proposed. One of them involves the addition in the reaction medium of a water-soluble sulfonated polyelectrolyte, which serves as a linear template, dopant, and steric stabilizer.⁸ Sulfonated polystyrene and poly(2-acrylamido-2-methyl-1-propanesulphonic acid) have been used as such templates.^{8,16} The same effect can be reached with sodium dodecylsulfate (SDS), sodium dodecylbenzenesulfonate (SDBS), and dodecylbenzenesulfonic acid (DBSA). These chemicals aggregate to form micelles over a critical micellar concentration (CMC); thus, providing a suitable local environment that facilitates the para-directed coupling of the monomer and leads to the formation of conducting PANI. Moreover, the long alkyl chains of the dodecylbenzene functional group contribute to solubility in common solvents, such as toluene, xylene, chloroform, etc.¹⁷

The purpose of the given work was (i) to perform laccase-catalyzed polymerization of aniline in SDBS water solutions; (ii) to compare the mechanisms of PANI synthesis at the initial stages of aniline poly-

merization by laccase-catalyzed and traditional chemical methods; (iii) to study some physico-chemical properties of the PANI/SDBS complex obtained.

EXPERIMENTAL

Chemicals and materials

Homogeneous preparations of laccase from the fungus *Trametes hirsuta* were obtained as described in the work.¹⁸ Aniline was purchased from Chem. Med. (Russia) and purified by double distillation in vacuum. SDBS and NaOH were acquired from Fluka (Germany), (1S)-(+)-10-camphorsulfonic acid (CSA) from Aldrich (Germany), Na₂HPO₄ from Merck (Germany), citric acid and ammonium persulfate from ICN (Great Britain). All the reagents, except aniline, were used without further purification. The other chemicals and solvents were of analytical grade or better, and also used as received. All solutions were prepared with deionized water.

Laccase activity

The activity of laccase was measured spectrophotometrically, using 10 mM catechol as a chromogenic substrate ($\lambda = 410 \text{ nm}$, $\epsilon = 740 \text{ M}^{-1}\text{cm}^{-1}$), at 24°C in 0.1M Na-citrate-phosphate buffer, pH 4.5. One unit of activity is defined as the amount of laccase oxidizing 1 μm of substrate per min. The specific activity of the enzyme preparation was about 160 units/mg of protein.

Enzymatic and chemical polymerization

In the case of enzymatic polymerization, equimolar quantities (typically 10 mM) of the surfactant template SDBS and aniline were dissolved into 10 mL of 50 mM Na₂HPO₄-citric acid buffered solution (maintained at pH 3.5) at room temperature. A 34.9 mg sample of SDBS was added first to the buffered solution, followed by the addition of aniline with constant stirring. Then, the pH was adjusted to 3.8 with H₃PO₄. Micelles formed spontaneously when the concentration of the surfactant in the solution was higher than the CMC. The known CMC of SDBS is 1.6 mM. The reaction was initiated by the addition of 16 μL of stock laccase solution (with concentration protein $\sim 74.3 \mu\text{M}$) under vigorous magnetic stirring. The final activity of laccase in the reaction medium was about 13.3 units. It is noteworthy that on aniline enzymatic polymerization atmospheric oxygen serves as oxidation agent and laccase is the catalyst.

The chemical synthesis of PANI/SDBS complex was carried out under the conditions similar to those of the enzymatic synthesis. The reaction was

initiated by dripping 0.5 mL of 0.1 M ammonium persulfate solution in 50 mM Na_2HPO_4 -citric acid buffered solution. The final volume of the reaction mixture was 10 mL, and aniline and SDBS concentrations were 10 mM.

In both cases after initiation, the reactions were left under stirring for >24 h to complete the polymerization process and UV-vis spectra of the reaction products were constantly being recorded at certain intervals.

Precipitation, washing, and drying of PANI

A double amount of ethanol was added into the PANI/SDBS dispersion to collapse the micelles and precipitate PANI by breaking the hydrophilic-lyphophilic balance of the system. The precipitate was collected by centrifugation and washed two times each with 10 mL ethanol and 10 mL 50% (v/v) ethanol/water mixture to remove the unreacted chemicals, aniline oligomers and SDBS. The final purified polymer complex was dried in oven at 37°C for 72 h for further characterization.

Characterization methods

UV-vis spectra of the PANI complexes were recorded by using a Hitachi-557 spectrophotometer (Japan) in the range of 300–900 nm. In each measurement, buffer solution was used as control. The polymerization kinetics was monitored by changes in the absorption of samples at 750 nm.

Fourier transform infrared (FTIR) spectra were obtained using KBr pellets on a Nicolet (Atkinson, NH) Magna-750 spectrophotometer.

Cyclic voltammetry was performed using a three-electrode system connected to a potentiostat (BAS CV-50W Electrochemical analyzer, Bioanalytical System, West Lafayette, IN). A glassy carbon electrode (BAS) was used as the working electrode for electrochemical measurements. The reference and counter electrodes were Ag/AgCl (BAS) and platinum wires, respectively. All cyclic voltammograms were obtained at room temperature.

The electric conductivity of PANI/SDBS samples was measured on pressed pellets at ambient temperature using the four-probe technique.

The morphology and structure of PANI thin films were studied by transmission electron microscopy (Tecnai G-2, accelerating potential 300 kV) and electron diffraction using a special electron diffraction camera ESR-102 (Ukraine).

The thermogravimetric analysis (TGA) of the synthesized PANI complexes was carried out using a Netzsch TG 209 F1 thermogravimetric analyzer (Germany) in argon atmosphere at a heating rate of 20°C/min. Before the thermogravimetric measure-

ments, the obtained PANI particles were dried in an oven at 100°C for 2 h.

Antistatic properties of PANI/SDBS complexes were investigated by measuring and evaluating the electrostatic charge dissipation from cotton fabric samples (total surface area 200 cm²). The samples were treated with conducting PANI/SDBS complexes and SDBS as control. The treated cotton fabrics were charged positively or negatively through their contact with an electrode at an applied electrical tension of (+) or (–) 18 kV. The electrostatic charge density on the surface of the fabric samples and the rate of charge dissipation were calculated taking into consideration the resistance (*R*) of samples.

RESULTS AND DISCUSSION

Enzymatic polymerization of aniline in the presence of SDBS

In our earlier article,¹⁹ we proposed the method of the synthesis of PANI/SDBS complexes based on laccase-catalyzed reaction of oxidation aniline polymerization.

By mixing aniline and SDBS in water buffer solution at pH 3.5, we obtained insoluble SDBS/anilinium cation complexes, whose structure is shown in Figure 1. As can be seen, the structure is far from being spherical. When the complexes took shape, we added laccase to initiate the reaction of aniline polymerization.

The evolution of UV-vis absorption spectra of the obtained PANI dispersions with time is shown in Figure 2(1). In contrast to the enzymatic synthesis on which conducting PANI salt formed immediately, on the chemical synthesis we observed accumulation of cation radicals with time. An advantage of the enzymatic synthesis of PANI/SDBS is the absence of a

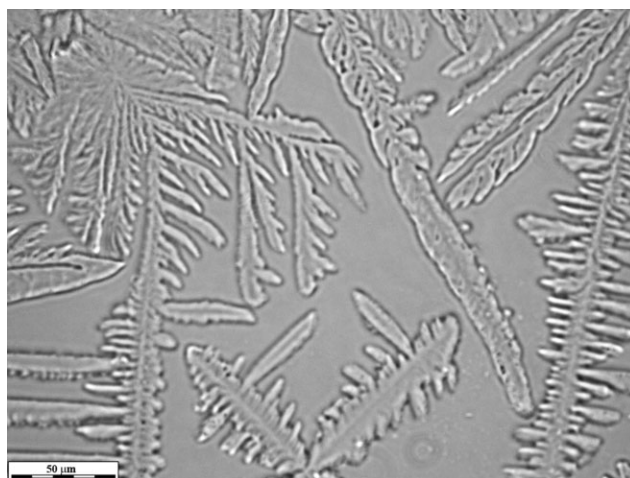


Figure 1 Optical image of the structure of SDBS/anilinium cation complexes.

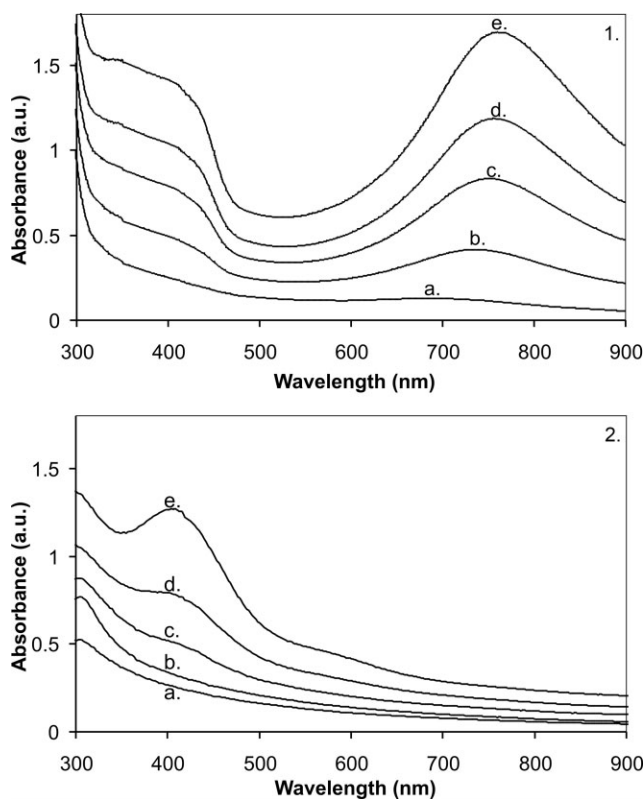


Figure 2 Evolution of UV-vis spectra of the products of enzymatic (1) and chemical (2) PANI syntheses with reaction time: (a) 6 min, (b) 40 min, (c) 100 min, (d) 160 min, and (e) 240 min. The experimental conditions: 50 mM Na-citrate-phosphate buffer; pH of reaction medium 3.8; [aniline] = [SDBS] = 10 mM; [laccase] = 120 nM; $[(\text{NH}_4)_2\text{S}_2\text{O}_8] = 5 \text{ mM}$.

long induction period, which in the case of the chemical synthesis can last 3.5 h at 20°C [Fig. 2(2)]. The final UV-vis spectra of PANI/SDBS complexes are shown in Figure 3.

For the conducting form of PANI (PANI/SDBS), the UV-vis spectra show three characteristic absorption bands at 320–360, 400–420 and 740–800 nm

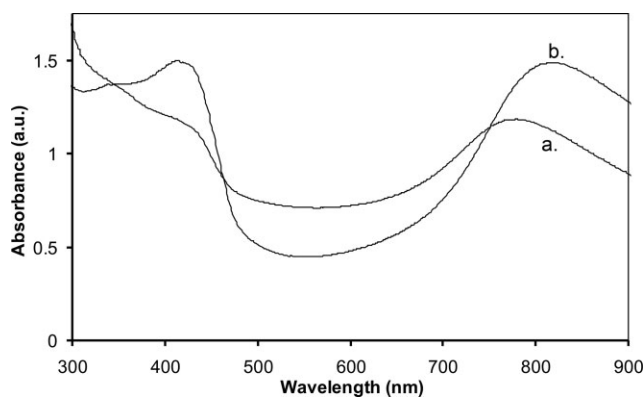


Figure 3 The final UV-vis absorption spectra of PANI/SDBS complexes in aqueous media prepared by enzymatic (a) and chemical (b) methods.

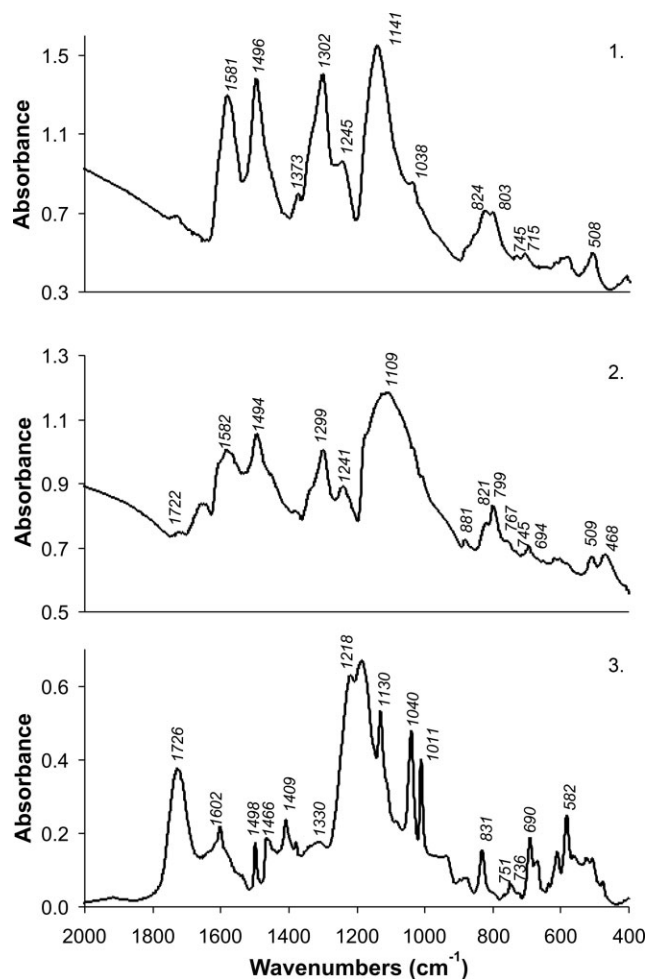


Figure 4 FTIR spectra of chemically synthesized PANI doped by CSA (1); purified PANI/SDBS complexes (2); and original PANI/SDBS complexes (3).

wavelengths. The first absorption band arises from π - π^* electron transition within benzenoid segments. The first two bands are often combined into a flat or distorted single peak with a local maximum between 360 and 420 nm. The second and third absorption bands are related to the anilinium radical cation and formation of polaron, respectively.

FTIR analysis

FTIR spectra for two kinds of PANI/SDBS powder samples are shown in Figure 4. The samples were prepared by two different methods: (2) the original PANI/SDBS composite was first precipitated with an excess amount of ethanol, washed with ethanol, and finally dried at 37°C for 72 h (as described in Precipitation, washing, and drying of PANI section); (3) the original PANI/SDBS composite was directly dried at 37°C for 72 h to get solid PANI cake. Both spectra are quite similar to that of chemically synthesized PANI doped by (1S)-(+)-10-camphorsulfonic acid [Fig. 4(1)]. They have the characteristic bands of

PANI, such as the ring stretching of the quinonoid diimine and the benzenoid diamine units at the regions $1581\text{--}1600\text{ cm}^{-1}$ and $1494\text{--}1500\text{ cm}^{-1}$, respectively.⁷

The FTIR spectra of the investigated PANI samples showed a relative small number of ortho-directed units as compared with para-directed ones. The ortho-substituted aromatic ring absorbs between 750 and 700 cm^{-1} , whereas the para-substituted aromatic ring absorbs between 850 and 800 cm^{-1} .²⁰

Both samples of PANI/SDBS complexes exhibit a peak in the region $1123\text{--}1130\text{ cm}^{-1}$ assigned to S=O stretching mode of sulfonic acid, which indicates the presence of SDBS.²¹ However, unpurified original samples show sharp peaks from 2854 to 2958 cm^{-1} due to aliphatic C—H stretching mode on the long alkyl tail of SDBS (data not shown). The presence of the peak at *ca.* 1300 cm^{-1} characteristic of C—N_{aromatic} stretching vibration confirms that PANI in the complexes studied is in doped form.

Electron diffraction and electron microscopy

A drop of water dispersion of the original PANI/SDBS complex synthesized at pH 3.5 was placed on an electron microscopy grid. PANI fibers make up a porous network. The fiber diameter is $0.5\text{--}3\text{ }\mu\text{m}$ (Fig. 5). The fibers have a crystalline structure. The electron diffraction pattern contains ring reflections with interlace distances d : 4.37 , 3.10 , 2.64 , 2.10 , 1.78 , 1.26 , 1.07 , 0.82 , and $0.79\text{ }\text{\AA}$ (Fig. 6). In this pattern, we can also see diffuse rings with $d \approx 2.0$; $1.1\text{ }\text{\AA}$. These rings are characteristic of the diffraction by an amorphous portion of PANI backbone.

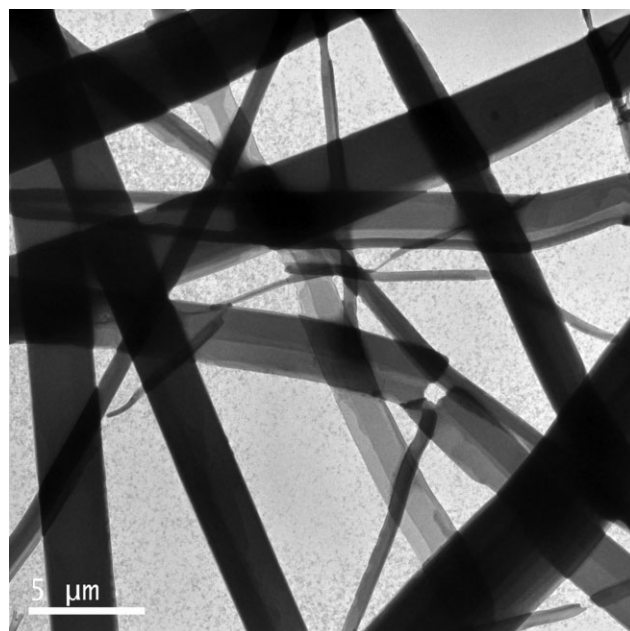


Figure 5 TEM micrograph of the PANI/SDBS complex.

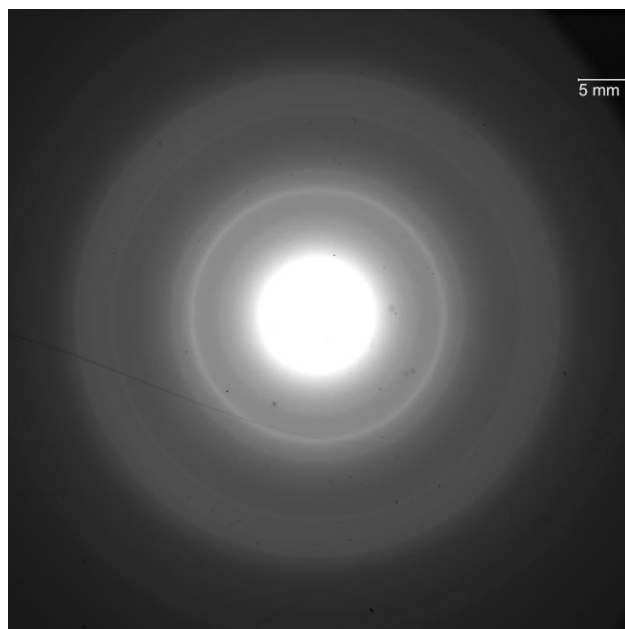


Figure 6 Electron diffraction pattern of the PANI/SDBS complex.

Thermal stability

Figure 7 shows TGA curves for the PANI/SDBS complexes synthesized by the laccase-catalyzed method (a,b) and for pure SDBS (c). In TGA profile for the original PANI/SDBS complex, there are two major stages of weight loss. The first stage of mass decrease occurs within a temperature range of $177\text{--}211^\circ\text{C}$ mainly due to the removal of excess SDBS from the polymer structure ($\sim 38\%$ weight loss). The second degradation process is observed between 470 and 508°C with $\sim 15\%$ weight loss assigned both to the detaching of the strongly bound SDBS and to the structural decomposition of the polymer.

Gradual weight loss over the wide temperature range between 100 and 520°C for the ethanol-

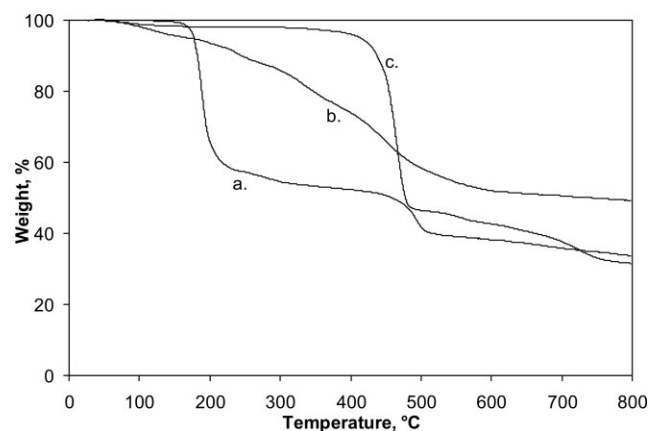


Figure 7 TGA curves: (a) original PANI/SDBS complex; (b) ethanol-purified PANI/SDBS complex; and (c) pure SDBS.

purified PANI/SDBS complex might be attributed to both the removal from the polymer of dopant molecules that are not so firmly bound to the main PANI chain (which might be proved by the bends on the curve of weight loss) and decomposition of the polymer main chain.

Antistatic properties

The enzymatically synthesized PANI/SDBS complex is an attractive antistatic agent. The values of half-lives of dissipation for positive ($\tau_{1/2}^+$) and negative ($\tau_{1/2}^-$) charges were significantly lower for the samples of cotton fabrics treated with PANI/SDBS complex than for others (Table I).

Electrochemical characterization of PANI/SDBS complexes

Because aniline is an electroactive compound and can be electropolymerized on an electrode, the synthesized dispersion of SDBS-doped PANI was treated with ethanol and PANI precipitate was washed several times with ethanol and ethanol/water mixture to eliminate the unreacted monomer, oligomers, and buffer components. The enzymatically produced PANI/SDBS complexes were dissolved in DMSO and then placed on a glassy carbon electrode. Cyclic voltammetry was used to confirm the electroactive nature of PANI in the PANI/SDBS complex. Figure 8 shows the cyclic voltammograms (CVs) of a cast film of the complex at two different pH. Only one well-defined quasireversible redox couple is observed on both CVs with the middle point potentials at 345 mV for pH 2.0 and 235 mV for pH 3.5. Increasing pH of the working solution led to the growth of difference between the potentials of cathodic and anodic peaks. Similar results were reported in several works.^{22,23} It should be noted that PANI electrochemically synthesized on a platinum electrode in strong acidic solution exhibits three sets of redox peaks between -200 and 800 mV.⁸

TABLE I
Antistatic Characteristics of Cotton Samples

Parameter	Samples		
	Control (cotton)	Cotton-SDBS	Cotton-PANI/SDBS
R, Ohm	6×10^{12}	9×10^{11}	1.2×10^8
$\tau_{1/2}^+$, s	200	154	3.8
$\tau_{1/2}^-$, s	135	114	5.8

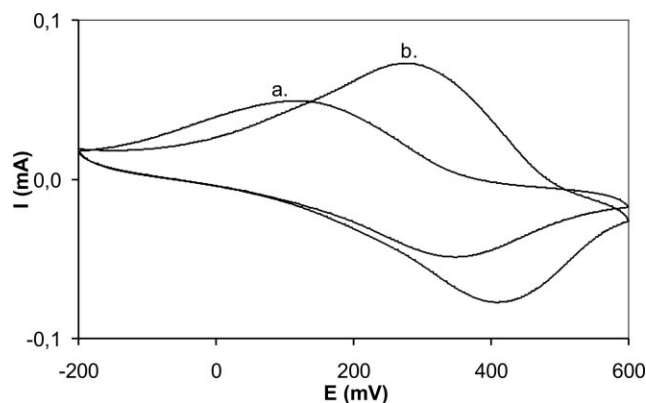


Figure 8 Cyclic voltammograms of PANI/SDBS thin films deposited from DMSO onto a glassy carbon electrode in 50 mM Na-citrate-phosphate buffer at pH 3.5 (a) and 2.0 (b). The experimental conditions: scan rate, 100 mV/s; start potential, -200 mV. PANI/SDBS complexes were synthesized at pH 3.8 and aniline/SDBS ratio of 1 : 1.

CONCLUSION

A stable dispersion of electroactive PANI/SDBS complexes was successfully prepared from aqueous SDBS micellar solution using an environmentally friendly process. The atmospheric oxygen served as an oxidizing agent in the reaction of aniline oxidative polymerization. The high-redox-potential oxidase-laccase from the fungi *Trametes hirsuta* was used as a catalyst for the synthesis of PANI. The laccase-catalyzed PANI synthesis has been efficiently carried out with SDBS to improve solubility and processability of the polymer. The laccase-catalyzed reaction of aniline oxidative polymerization has a reduced induction period in contrast to chemical oxidation. Because laccase belongs to industrial enzymes, this method of aniline polymerization is very promising for industrial production of conducting PANI. Laccase-catalyzed synthesis of conducting PANI has remarkable advantages as compared with both peroxidase-catalyzed and chemical methods due to the use of air oxygen as oxidant in the reaction of aniline polymerization.

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