

# Synthesis of Water-soluble and Conducting Poly(2-ethylaniline) by Using Horseradish Peroxidase

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Received 18 December 2002; accepted 14 June 2003

## ABSTRACT

Enzymatic synthesis has been used to prepare a water-soluble and conducting poly(2-ethylaniline) in the presence of sulphonated polystyrene (SPS). The polymerization of 2-ethylaniline was performed with horseradish peroxidase (HRP) as a catalyst to form conducting poly(2-ethylaniline)/SPS complex with moderate electrical conductivity. The polymerization is simple and can be carried out at room temperature in pH 4.0 phosphate buffered solution. This electrochemically active polymer shows strong pH dependence of absorption and other characteristics. The formation of conducting and electroactive form of poly(2-ethylaniline)/SPS complex was confirmed through UV-vis, FTIR and NMR spectroscopy. Cyclic voltammetry studies show only one set of redox peaks over the potential range of -0.2 to 1.0 V.

### Key Words:

conducting polymers;  
horseradish peroxidase;  
water-soluble polymers;  
poly(2-ethylaniline);  
enzymatic polymerization.

## INTRODUCTION

Electroactive polymers have attracted a considerable amount of attention due to potentialities for their applications to various fields ranging from sensor and light-weight batteries to printing circuit boards in micro-circuit boards [1-3].

Polyanilines have been one of the most widely studied conducting polymers but are limited by poor thermal processability and solvent solubility [4], due to the stiffness of its backbone. Recent investigations have been focused on exploring

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methods to augment the electrical properties, processability and solubility. Several chemical [5,6] and electrochemical [7] methods have been reported for obtaining the polymer in both a conducting and processable form. Recently polymeric templates have also been used for the synthesis of soluble and electroactive polyanilines [8].

Enzymatic polymerization has been explored as an alternative approach to the synthesis of electronically and optically active polymers. Horseradish peroxidase (HRP) is a single-chain  $\beta$ -type hemoprotein that catalyzes the decomposition of hydrogen peroxide at the expense of aromatic proton donors. This enzyme has been extensively used for oxidative polymerization of phenols and anilines in the presence of hydrogen peroxide [10]. Various aniline derivatives also have been polymerized by peroxidase enzyme catalyst [11,12].

The oxidation of aniline derivatives by hydrogen peroxide catalyzed by HRP and sulphonated polystyrene (SPS) was used as a template. An extension of this work was then included a template-assisted, HRP-catalyzed polymerization of aniline to synthesize a water-soluble and processable form of conducting polyaniline under environmentally mild condition [13]. A wide range of polyelectrolytes have been investigated and provides a preferential local environment that facilitates the para directed coupling of the monomers, provides the counter ions for doping the polyaniline, and remains complexed to the polyaniline, imparting water solubility [14].

Recently we have reported the enzymatic synthesis and characterization of a water-soluble and doped conducting poly(o-toluidine) [15]. In the present work we report the enzymatic polymerization of 2-ethylaniline in the presence of a polyelectrolyte that serves as a matrix within which the monomers align and preferentially react to form water-soluble, electrically active poly(2-ethylaniline). The synthesis is simple, and the conditions are mild in that the polymerization can be carried out in a pH 4.0 of 0.1 M sodium phosphate buffer solution with stoichiometric amount of hydrogen peroxide and a catalytic amount of enzyme.

The progress of the reaction was monitored spectroscopically. UV-vis absorption, FTIR and NMR analysis all confirm the electroactive form of poly(2-ethylaniline).

The pyrolysis mass spectrometry with a normal

heatable probe was used. Because of the presence of the polyelectrolyte SPS system, that inhibits a precise mass spectrum, it was unsuccessful.

## EXPERIMENTAL

### Materials

Poly(sodium 4-styrene sulphonate) (MW of 70,000), used in this study was purchased from Aldrich Chemical Co. (Milwaukee, WI) and it was used without any further purification. Horseradish peroxidase (EC 1.11.1.7) (about 170 units/mg), 2-ethylaniline (assay > 98%) and hydrogen peroxide (30 wt %) were obtained from Merck.

### Polymerization Reaction

The synthesis of water-soluble polyaniline by a biological route using an aqueous solution reported by Liu et al. [13]. The polymerization of 2-ethylaniline was carried out at room temperature in 0.1 sodium phosphate buffer at pH 4.0 with equimolar amount of monomer and polyelectrolyte (12 mM). To 30 mL buffer solution were added 0.0742 g SPS (based on the molecular repeat unit) and 45.2  $\mu$ L 2-ethylaniline under constant stirring. Then the catalytic amount of the enzyme was added (2 mg HRP). To initiate the reaction, a stoichiometric amount of diluted hydrogen peroxide (0.02 M) was added dropwise under vigorous stirring over a period of 1.5 h. The reaction was then left stirring for at least 1 h at room temperature. The final solution was dark green and was dialyzed (cut off molecular 3,000) for 20 h to remove any unreacted monomers, oligomers and phosphate salts.

### Characterization

All UV-vis spectra were obtained using a Shimadzu UV-2100 spectrophotometer. FTIR measurements were carried out on a BOMEM MB-Series FTIR spectrometer in the form of cast film of the poly(2-ethylaniline)/SPS complex solution in on AgBr crystal. The NMR spectra were obtained using a Varian FT-80A spectrometer. The cyclic voltammetry (CV) measurements were performed using a Metrohm Polarograph model 746 VA Trace Analyzer. Cyclic voltammograms were recorded at room temperature by using a three-electrode cell with platinum as an auxiliary electrode,

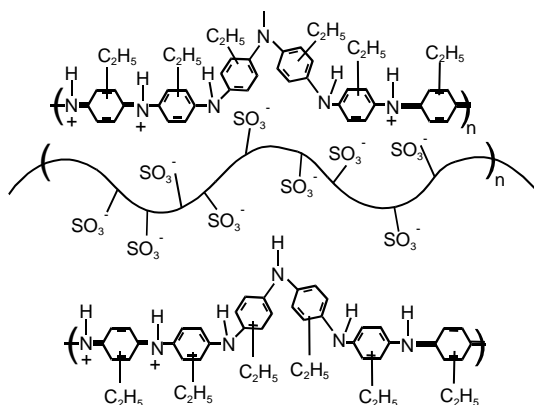
an Ag/AgCl electrode as the reference electrode and Pt foil (0.2 cm<sup>2</sup> area surface) as the working electrode. The cyclic voltammograms were obtained in 1.0 M HCl electrolyte solutions and scanned between -0.2 to 1.0 V at a scan rate of 100 mV/s.

## RESULT AND DISCUSSION

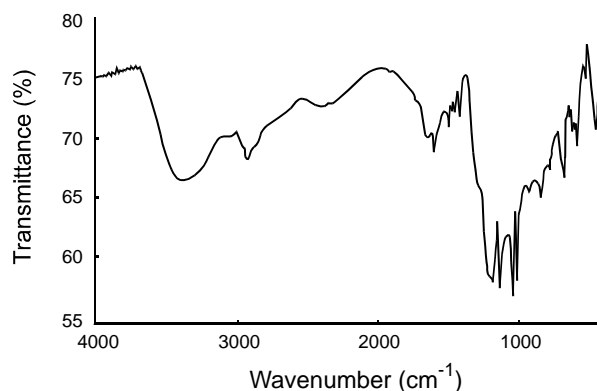
Previous studies on the alkyl ring-substituted PANIs shown that in general the substituted polymers have lower intrinsic oxidation states, i.e. lower imine/amine ratio than their unsubstituted emeraldine counter part [16]. These compounds suffer of low molecular mass and lower conductivity in comparison to PANI. The conductivity decreases by increasing the length of the side alkyl chain [17]. The present work is essentially intended to study of the enzymatic polymerization of poly(2-ethylaniline). The schematics of the poly(2-ethylaniline) synthesized in the presence of the SPS is shown in Figure 1 wherein SPS acts as the template and polymerization was catalyzed by using HRP.

### FTIR

Figure 2 shows the FTIR spectrum of poly(2-ethylaniline)/SPS which is in agreement with the spectra already reported [18]. This spectrum was obtained as a cast film on AgBr crystal and in the region from 4000 to 400 cm<sup>-1</sup>. The N-H stretching can be detected at 3380 cm<sup>-1</sup>, followed by the aromatic C-H stretching at 3040 cm<sup>-1</sup> and the aliphatic stretching at 2930 cm<sup>-1</sup>. The rings stretching of quinoid and benzenoid forms are observed at 1610 cm<sup>-1</sup> and 1505 cm<sup>-1</sup>, respectively.



**Figure 1.** Proposed schematic for the formation of the molecular complex poly(2-ethylaniline)/SPS.



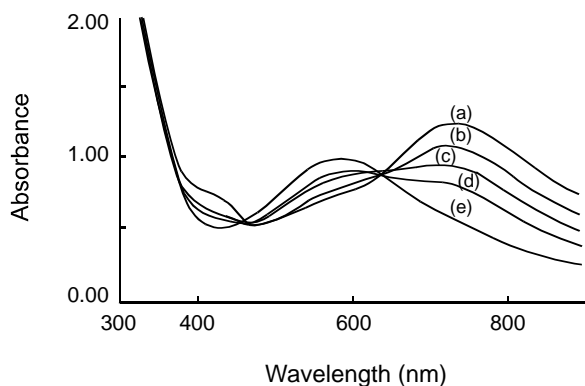
**Figure 2.** FTIR spectra of poly(2-ethylaniline)/SPS complex by casting a film on a AgBr crystal window.

The imine band C=N is observable at 1310 cm<sup>-1</sup>. The 1130-1250 cm<sup>-1</sup> regions are characteristic of the C-H stretching. The peaks at 790 cm<sup>-1</sup> and 860 cm<sup>-1</sup> are due to a 1,2,4-substitution pattern indicating that the monomeric unit is linked to para position of the ring and a head-to-tail coupling of 2-ethylaniline occurs during the polymerization. In addition, the presence of asymmetric and symmetric S=O stretching bands at 1040 and 1014 cm<sup>-1</sup> confirms the presence of SPS in the complex.

### Effect of pH

The enzymatic template polymerizations of PANIs are strongly pH dependent. The local environment in the vicinity of the template has been known to have a profound effect on the ability to charge and preferentially align the monomers on the template prior to polymerization [14]. Therefore, the pH of the reaction medium dictates the extent of dissociation of the polyelectrolyte and affects the solubility as well as the nature of polyanilines in the reaction. To study the effect of the pH, the poly(2-ethylaniline) was enzymatically synthesized at different pH from 4.0 to 10.0. The absorption spectrum of the poly(2-ethylaniline)/SPS complex in this pH range is given in Figure 3. As shown in this Figure, at pH 4.0, the polymer have strong absorption bands at 735 nm and 415 nm, which are due to polaron band transitions and confirm the presence of conductive form of poly(2-ethylaniline) (emeraldine salt).

As it is noticed, when the pH of the solution is increased, the intensity of the polaron bands decreased and a new peak at 570 nm appears which is assigned to the formation of branched polymer [19]. At higher pH

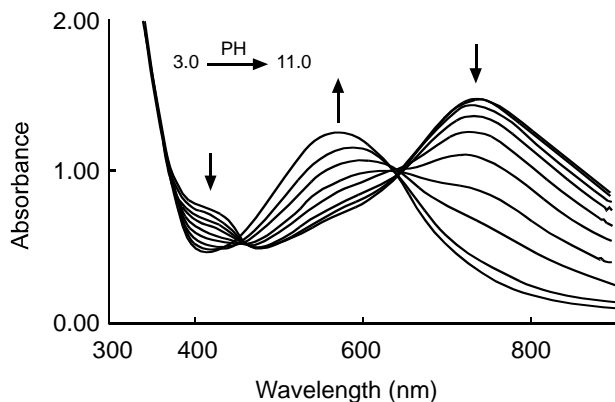


**Figure 3.** UV-vis spectra of poly(2-ethylaniline)/SPS obtained by polymerization of a 12 mM 2-ethylaniline and 12 mM SPS at different pH (a) 4.0, (b) 5.5, (c) 7.0, (d) 8.5, (e) 10.0.

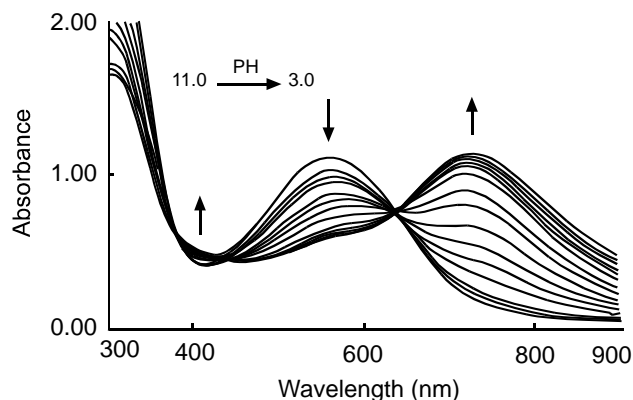
the polaron bands completely disappeared and only one peak at 570 nm is observed. Since HRP polymerization is known to be both ortho- and para-directed [20], the *ortho* coupling may become more dominant at pH 6.0 or higher and result in a more highly branched, insulating form of poly(2-ethylaniline). Therefore, at pH lower than 5.0 the electrically conducting polymer was obtained.

### Doping-dedoping Reversibility of Poly(2-ethylaniline)/SPS Complex

Poly(2-ethylaniline)/SPS was used for studying the reversible redox behaviour of the polymer in the complex, and the results are presented in Figures 4 and 5. At pH 4.0, the poly(2-ethylaniline) is in the doped state and reflected by the presence of the polaron bands. Figure 4 gives the shift in absorption spectrum of the complex with increasing of the pH from 3.0 to 11.0 by titration with 1N NaOH. As the pH is increased, the



**Figure 4.** Dedoping of poly(2-ethylaniline)/SPS complex (pH 3.0-pH 11.0).



**Figure 5.** Redoping of poly(2-ethylaniline)/SPS complex (pH 11.0-pH 3.0).

polaron bands gradually disappear with the emergence of a new peak at 570 nm due to the exciton transition. This is also accompanied by a colour change in the complex from green to blue and finally to a purple colour.

Figure 5 shows that these doping/dedoping processes are completely reversible. Dedoped complex can be redoped by titration with 1N HCl, and a reversible colour change is observed. This pH induced redox reversibility was indicated the presence of the electroactive form of poly(2-ethylaniline) in the complex. Three isobestic points at 640, 445 and 370 nm are also observed clearly.

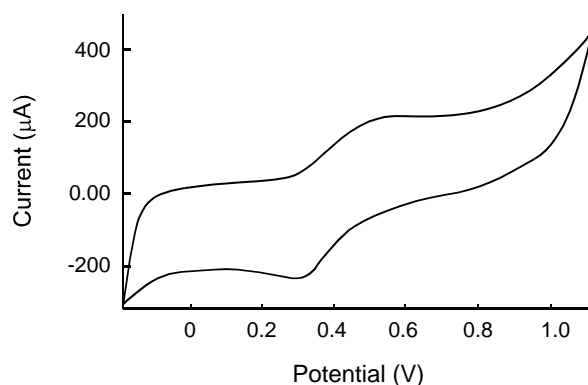
### Electrochemical Properties

It was already shown that the presence of an alkyl substituent in the benzene ring causes the shift of redox couple to more positive potential [21]. This fact can be explained on the basis of steric and electronic effects of the ethyl group. Two redox peaks were previously observed for electrochemical synthesis of poly(2-ethylaniline).

Cyclic voltammogram of the poly(2-ethylaniline)/SPS complex recorded at 100 mV/s was shown in Figure 6. This complex displays only one set of redox peaks at  $E_{1/2} = 0.57$  V over a potential of -0.2 to 1.0 V. Similar results also have been observed for POT/SPS complex at our previous work [15]. The absence of the second redox process is believed to be due to the exceptional resistance of the poly(2-ethylaniline) to oxidation to the pernigraniline state [13].

### <sup>1</sup>H NMR

<sup>1</sup>H NMR spectrum of the poly(2-ethylaniline)/SPS complex in D<sub>2</sub>O are given in Figure 7. As it is seen,

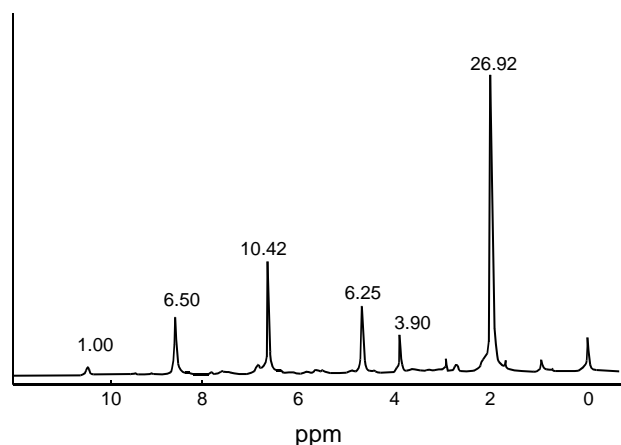


**Figure 6.** Cyclic voltammograms of a solution cast film of poly(2-ethylaniline)/SPS complex in 1M HCl with a scan rate of 100mV/s.

this spectrum has good agreement with complex structure. A more detailed look at this spectrum shows that the peaks at about 2.0 ppm are attributed to  $-\text{CH}-$  and  $-\text{CH}_2$  of polystyrene sulphonate and  $-\text{C}_2\text{H}_5$  of poly(2-ethylaniline). The peak at 6.7 ppm corresponds to quinoid form and the peak at 8.5 ppm is belonged to benzenoid form of polymer and also the peak at 3.9 ppm is attributed to N-H group of polymer.

### Mass

Recently, Liu et al. has reported the molecular weight distribution of PANI/SPS complex by using gel permeation chromatography (GPC) [13]. The results were shown that, because of the complex nature of the PANI/SPS system, it is not possible to determine the molecular weight of the PANI alone. Pyrolysis-mass spectrometry analysis with a normal heatable probe



**Figure 7.**  $^1\text{H}$  NMR spectrum of poly(2-ethylaniline)/SPS in  $\text{D}_2\text{O}$ .

was attempted to obtain the molecular weight of the polymer. However, because of the complexity of the SPS structure, it was impossible to determine the mass spectrum of the poly(2-ethylaniline).

### CONCLUSION

The enzymatic synthesis of a conducting water-soluble macromolecular complex of poly(2-ethylaniline)/SPS is presented. The polymerization was accomplished in an aqueous buffer at pH 4.0, and the electroactive complex was characterized by spectroscopy. This process is simple (one step), chemically mild, environmentally benign and requires minimal separation and purification. Horseradish peroxidase enzyme polymerization is known to be both *ortho*- and *para*-directed and *para* (head-to-tail) coupling may become more dominated with control of the pH. This approach is general and various monomers and templates may be interchanged to produce important electroactive polymers.

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