A Novel Method for Synthesis of Water-Soluble Polypyrrole with Horseradish Peroxidase Enzyme

Mohammad Reza Nabid[1] and Ali Akbar Entezami[2]

[1]*Department of Chemistry, Faculty of Science, Shahid Beheshti University, Evin, Tehran, Iran;*
[2]*Faculty of Chemistry, Tabriz University, Tabriz, Iran*

ABSTRACT: A novel water-soluble, conducting polypyrrole was synthesized by horseradish peroxidase (HRP), in the presence of sulfonated polystyrene, as a polyanionic template. The HRP is an effective catalyst for the oxidative polymerization of pyrrole in the presence of hydrogen peroxide at room temperature. The reaction is sensitive to solution pH and it is performed in pH 2 aqueous solutions. Polymerization of pyrrole by this biological route produced a conducting water-soluble polypyrrole for the first time. The reaction is benign and in one pot, and the product requires minimal purification. The reversible redox activity of the polypyrrole displays a hystersis loop with pH changes. FT-IR, UV-vis absorption spectroscopy ,and cyclic voltammetry are used in the characterization of the synthesized polypyrrole. These studies confirm the electroactive and conducting form of polypyrrole, similar to that which has been traditionally synthesized, chemically and electrochemically. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 254–258, 2004

Key words: polypyrrole; water-soluble polymers; conducting polymers; horseradish peroxidase; enzymatic polymerization

INTRODUCTION

Over the past 2 decades the field of π-conjugated polymers has attracted the attention of many scientists due to the interesting electrical and optical properties of these compounds.[1] There has been increasing interest in soluble conducting polymers because of their potential applications, such as soluble ion-exchangers, energy-storage materials, corrosion-resistant coatings, and catalysts.[2–4] Conducting polymers are insoluble in most solvents and decompose without melting when heated, although many of the applications require solution processability.

Synthesis of polymers through enzymatic polymerization has been extensively developed.[5] This method is recognized as a new area of precision polymer synthesis. In enzymatic polymerization, the polymer product can be obtained under mild reaction conditions without using toxic reagents. Horseradish peroxidase (HRP) is capable of catalyzing the polymerization of anilines and phenols in the presence of hydrogen peroxide to generate their respective free radicals. These free radicals undergo coupling to produce dimmers. Successive oxidation and coupling reactions result in the polymer formation.[6]

Usually, the peroxidase-catalyzed polymerization produces low-molecular-weight products. The use of the anionic polyelectrolyte as a template in polymerization of aniline led to the first enzymatic synthesis of conducting polyaniline.[7] The role of template in this approach is critical. Strongly acidic polyelectrolyte, such as sulfonated polystyrene (SPS), is the most favorable template because it provides a lower and local pH environment and preferentially aligns the monomers through electrostatic and hydrophobic interactions to promote the desired head-to-tail coupling.[8] An extension of this work then included a template-assisted, HRP-catalyzed polymerization to synthesize a water-soluble and processable form of conducting polymer under environmentally mild conditions. We have recently reported the enzymatic synthesis and characterization of a water-soluble, conducting poly (*o*-toluidine) and poly (2-ethylaniline).[9,10]

Polypyrrole is one of the most stable of the conducting polymers and also one of the easiest to synthesis. It displays a good conductivity in combination with high stability in its oxidized form. A polypyrrole that is polymerized either electrochemically or chemically is known to be insoluble. Electrochemical polymerization on a metal electrode results in a good quality film,[11] while chemical polymerization yields fine conducting powders.[12] The lack of solubility of polypyrrole limited their characterization, processability, and applications. Attempts are being made to solubilize the polypyrrole to improve their properties.[13,14] Solu-

Correspondence to: A. Akbar Entezami (aaentezami@yahoo.com).

ble polypyrrole was obtained by chemical polymerization of the pyrrole monomer in the presence of ammonium persulfate as an oxidant and dodecylbenzene sulfonic acid as a dopant.[15] The protonic acid with a large size reduces the molecular interactions of the polymer chains by being incorporated in it as a dopant, which may improve the polymer's solubility.[16]

In this study, we report the enzymatic polymerization of pyrrole in the presence of template to yield a conducting water-soluble polypyrrole that is being doped by SPS. The synthesis is simple and carried out at room temperature in pH 2 aqueous solutions by the addition of hydrogen peroxide. The formation of a conducting and electroactive form of the polypyrrole/SPS complex was confirmed through UV-vis and FT-IR absorption spectroscopy and cyclic voltammetry.

EXPERIMENTAL

Materials

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

Polymer synthesis

The polymerization of pyrrole in presence of SPS was catalyzed by HRP at room temperature in the presence of hydrogen peroxide under ambient conditions. A total of 37.1 mg (0.18 mmol) of SPS (based on the molecular repeat unit) was dissolved in 10 mL of water (pH 2, adjusted with concentrated HCl). This was followed by the addition of 12.7μL (0.18 mmol) pyrrole and 1.0 mg HRP to this solution. To commence the polymerization, 9 mL of 0.02 M hydrogen peroxide was added in increments. The reaction was stirred for 4 h to complete the polymerization. The final solution was dark black. This solution was transferred to individual regenerated tube and dialyzed (cut off molecular 3000) for 48 h against acidic water solution to remove any unreacted monomers and oligomers.

Characterization

All UV-vis spectra were obtained using a Shimadzu UV-2100 spectrophotometer. FT-IR measurements were carried out on a BOMEM MB-Series FT-IR spectrometer in the form of cast a film of the polypyrrole/SPS complex solution in AgBr crystal. The cyclic voltammetry measurements were performed using a Metrohm Polarograph Model 746 VA trace analyzer. Cyclic voltammograms were recorded at room tem-
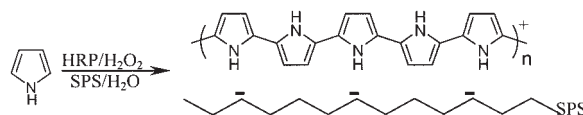

Figure 1 Mechanism of the enzymatic polymerization of the pyrrole.

perature using a three-electrode cell with platinum as an auxiliary electrode, an Ag/AgCl electrode as the reference electrode, and a Pt foil (0.2 cm^2 surface area) as the working electrode. The cyclic voltammograms were obtained in 1.0 M HCl electrolyte solutions and scanned between -0.2 and 1.0 V at a scan rate between 20 and 200 mV/s.

RESULTS AND DISCUSSION

Polypyrrole can be synthesized by an oxidative polymerization of the corresponding monomer. This may be accomplished either electrochemically or with a chemical oxidizing agent. In this work, the enzymatic method was used to synthesize water-soluble polypyrrole. A major limitation of the enzymatic oxidation, however, was that as soon as polymer begins to form, it precipitates out and only the very-low-molecular-weight polymers are produced. A variety of modified enzymatic polymerization reactions were investigated to improve processability.[17–19] Although these modifications improved the molecular weight and processability, the enzymatic reaction typically results in a mixture of branched polymeric structures and limits the degree of conjugation and hence the electrical and optical properties of the resulting polymer.

Polyelectrolyte-assisted chemical polymerization was used to form a water-soluble and doped conducting complex simultaneously. Figure 1 shows the mechanism of an enzymatic polymerization of pyrrole in the presence of SPS. This approach is based on a preferential electrostatic alignment of monomer onto an anionic template to minimize branching and to promote a linear chain growth. SPS was found to be a suitable template in enzymatic polymerization. In this approach, pyrrole monomers are preferentially dispersed in a SPS template under aqueous conditions.

Figure 2 shows the UV-vis spectra of the solution containing a 1:1 M ratio of pyrrole to SPS and 1.0 mg enzyme (diluted 10 times with water, pH 2) before (Fig. 2a) and after (Fig. 2b) the addition of hydrogen peroxide. The peak at 270 nm in Figure 2a corresponded to SPS, and the absorbance of this peak increased after the addition of H_2O_2 and it is assigned to polypyrrole in a doped state.[20] In Figure 2b, also one absorption peak appears at 450 nm and there is a high-intensity tail extending into the near IR region. The former was identified as a $\pi-\pi^*$ transition while
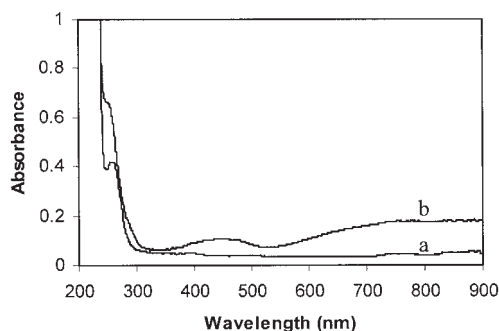
Figure 2 UV-vis spectra of the mixture of reaction: (a) before and (b) after the addition of H_2O_2.

Figure 4 The absorption spectra of the polypyrrole/SPS complex synthesized in different pH: (a) 1.5, (b) 2, and (c) 3.

the latter is characteristic of an oxidized polypyrrole since it is not observed in neutral polypyrrole before exposure to O_2.[21] This spectrum is similar to the spectrum of the pristine film that is characterized by a band at 486 nm.[22] The differences between the two spectra are due to the presence of an electron-withdrawing sulfonic group in the complex and therefore the transition band is observed at a lower wavelength.

The absorption of the polaron band is strongly dependent on the molecular weight of the polymer.[23] As seen in Figure 3, as the reaction time increases, the polaron band at 450 nm shifts to longer wavelengths and an increase in the absorbance of this band is also noticed. This indicates that an increase in the reaction time leads to the formation of a chain with higher molecular weight. The peak intensity reaches a maximum after a 2-day reaction time at pH 2.

The solubility of the polypyrrole/SPS complex is very much dependent on the composition of polypyrrole and SPS in the solution. Since the solubility of the complex is due to the anionic charges on the template, it is essential that sufficient charges should remain so as to keep the complex in solution. It was expected that if a sufficient number of charged sites on the SPS were neutralized by polypyrrole, then the precipitation of the complex would occur. Therefore, in an attempt to optimize the conditions required for maintaining the complex in a soluble form, a matrix of
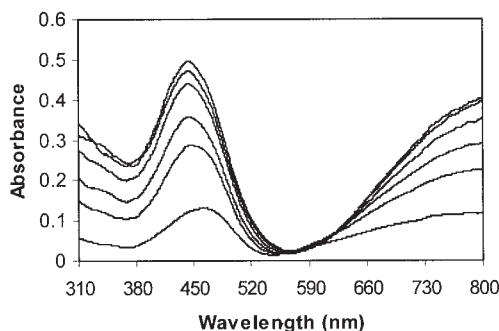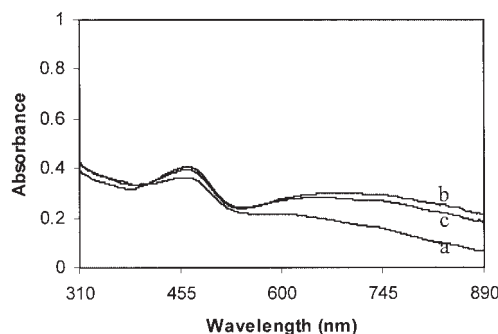
reaction systems was set up. In these experiments, the molar ratio of pyrrole to SPS in the reaction medium was varied from 1:1 to 10:1, while the amount of enzyme was kept constant in all cases. It was found that this complex would precipitate at a specific stoichiometric ratio of pyrrole to SPS. The precipitation was observed when the ratio of pyrrole to SPS was taken higher than 8:1 over a time period of 7 days.

Effect of pH

The pH of the reaction medium dictates the extent of dissociation of the template polyelectrolyte and it influences the solubility as well as the nature of the polypyrrole formed in the reaction. To study the effect of pH, the polymerization was carried out at different pH′s from 1.5 to 4. The absorption spectrum of the polypyrrole/SPS complex synthesized in this pH range is given in Figure 4. The absorbances of the polaron bands at different pH′s demonstrate that pH 2 is the best condition for polymerization. The tests show that no polymerization occurred higher than pH 4 (results not shown). This result shows that the polymerization of pyrrole is strongly pH-dependent, and optimal pH is needed to provide the conducting forms of polypyrrole.

Redox reversibility

Figure 5 displays the reversible redox behavior of the polypyrrole/SPS complex. The synthesized complex showed reversible reduction/oxidation behavior in the absorption spectra with varying pH. The evaluations of absorption spectra were studied in aqueous solution with increasing pH from 2 to 10 by titration with NaOH (1 N). The complex is in the doped state at pH 2 by the presence of a polaron band at 450 nm. As seen in Figure 5a, when the pH is increased from beyond 2, the polaron band gradually disappears and a new peak at 600 nm emerges. At pH 10 polypyrrole is fully dedoped. The dedoped form can be redoped by titration of polypyrrole with 1 N HCl to yield the
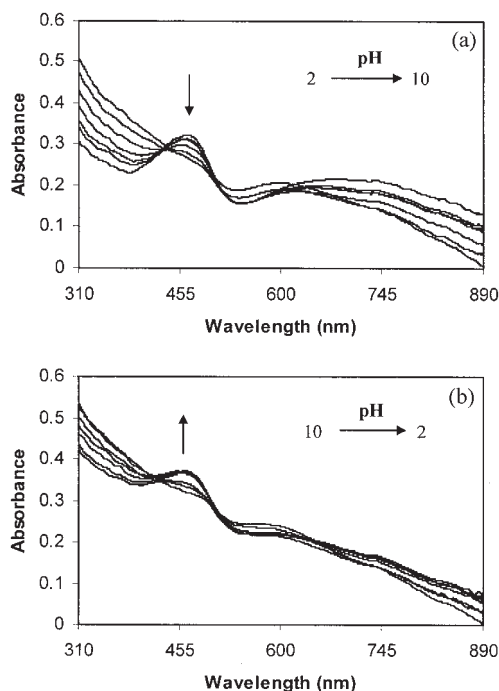

Figure 3 Effect of reaction time on the polaron band.

Figure 7 Cyclic voltammograms of a cast film of polypyrrole/SPS complex in 1.0 M HCl at 100 mV/s scan rate.

broad band at 3300 cm^{-1}, is assigned to O–H stretching vibration. The peak at 1710 cm^{-1} is believed to be due to the carbonyl group. Another feature in the FT-IR spectrum is the skeletal stretching of the pyrrole ring, which occurs in the 1550 cm^{-1} region.[25] While the polypyrrole was doped by a certain anion the N-H band of the pyrrole ring was not observed in the FT-IR spectrum (in 3400 cm^{-1}).[26] Also, the peaks observed at 1008 and 1036 cm^{-1}, corresponded to symmetric and asymmetric S=O stretching and a band at 670 cm^{-1} which was attributed to the -SO$_3$ group confirmed the presence of SPS in the complex. These data indicate that the polymer consists of carbonyl and hydroxyl groups.

Cyclic voltammetry

Conjugated polymers, such as polypyrrole, can be oxidized and reduced in a way that is in principle similar to the redox polymers.[27] During reduction, anionic sites are formed that require cations for charge compensation (or anion expulsion). Upon oxidation cationic sites, such as polarons (PPy$^+$) and bipolarons (PPy^{2+}), are normally formed on the polypyrrole chains as follows:[27]

$$PPy + X^- \rightarrow PPy^+X^- + e^-$$

$$PPy^+ + 2X^- \rightarrow PPy^{2+}(X^-)_2 + e^-$$

where X^- is an anion.

Cyclic voltammetry was carried out to determine the electrochemical nature of the polypyrrole/SPS complex. Cyclic voltammograms are obtained as a cast film of the complex on a Pt electrode and also as an aqueous solution complex by using three electrodes in a 1.0 M HCl electrolyte solution. The results are in good agreement with each other. The cyclic voltammogram of the polypyrrole/SPS complex as a cast film, recorded at 100 mV/s, is shown in Figure 7. As can be seen from Figure 7, two sets of redox peaks are

Figure 5 UV-vis spectra of polypyrrole/SPS during titration with base and acid in the pH range of (a) 2 to 10 and (b) 10 to 2.

conducting form again. Figure 5b shows that these dedoping/redoping processes are reversible. This pH-induced redox reversibility demonstrates that the electroactive polypyrrole was enzymatically synthesized.

FT-IR

The IR spectrum of the polypyrrole/SPS complex was measured in transmission mode. Figure 6 shows the FT-IR spectrum of the complex as a cast film in the AgBr crystal. FT-IR spectroscopy indicates that this complex is an oxidized structure with covalently bonded oxygen, which differs from native polypyrrole that was synthesized under oxygen-free conditions previously.[24] The absorption peak, observed as a
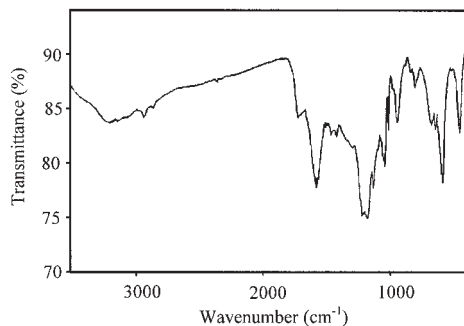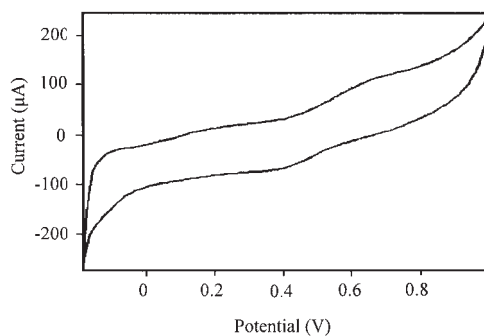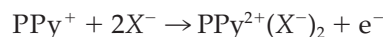

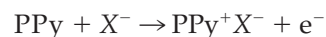**Figure 6** FT-IR spectra of polypyrrole/SPS complex by casting a film on an AgBr crystal window.
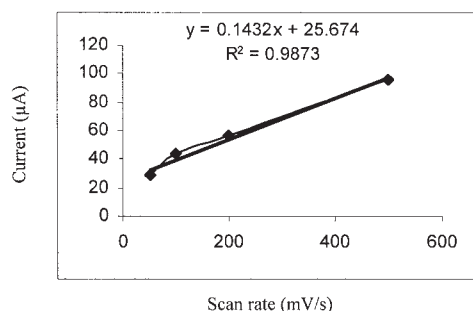
Figure 8   Plot of anodic peak current versus scan rates for the polypyrrole/SPS complex.

observed at $E_{1/2} = 0.17$ and $E_{1/2} = 0.50$ V. An appreciable change in the anodic peak current was observed when we recorded the cyclic voltammograms at different scan rates. A good linear relationship between the anodic peak current and scan rates for polypyrrole/SPS complex is shown in Figure 8. This result indicates the electroactivity of the complex and this polymer complex shows a convenient electroactivity. Similar results also were observed for the solution of polypyrrole/SPS complex.

CONCLUSION

A biological route for the synthesis of water-soluble conducting polymer is presented. The synthesis of an electroactive polypyrrole was accomplished using an environmentally friendly process. Polypyrrole was enzymatically synthesized in aqueous solution for the first time. The polymerization product required minimal separation and purification. The solubility of the polymer makes it a suitable material for self-assembly into organized structures with biological macromolecules such as enzymes for fabrication of biosensors. Varying the pH, polyelectrolyte concentration, and time of reaction may optimize the final polymer structure and properties.

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