Enzymatic Oxidation of Alkoxyanilines for Preparation of Conducting Polymers

Mohammad Reza Nabid,[1] Roya Sedghi,[1] Ali Akbar Entezami[2]

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

Received 26 November 2005; accepted 24 July 2006
DOI 10.1002/app.25208
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A biomimetic route for synthesis of a conducting molecular complex between polyalkoxyanilines and a polyelectrolyte, poly (sodium 4-styrenesulfonate) (SPS) is presented. Horseradish peroxidase (HRP) was used to catalyze the polymerization of alkoxyanilines. A few of water-soluble ring-substituted polyalkoxyanilines have been enzymatically synthesized with variation of groups, such as ortho-methoxy, meta-methoxy, ortho-ethoxy, and meta-ethoxy to form polyalkoxyanilines/SPS complexes. The presence of alkoxy substituents affects the polymerization reactions. These enzymatic oxidation reactions occur in a different potential range to that observed for the chemical polymerization. Similar electrochemical and optical properties were obtained for every pair of ortho- and meta-alkoxy substituted polyanilines. For comparing, polyalkoxyanilines were also prepared by chemical polymerization in the presence of SPS. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 3724–3729, 2007

Key words: enzymatic polymerization; polyalkoxyanilines; conducting polymers; horseradish peroxidase

INTRODUCTION

The development of new and efficient catalysts plays a central role in chemical research. Polymerization catalyzed by an enzyme is a new methodology for polymer synthesis.[1] Polymers with well-defined structures can be prepared by enzyme-catalyzed process in an environmentally benign process that has attracted much attention in recent years.[2,3] Furthermore, enzymatic polymerization enabled the production of polymers having complicated structures, which would have otherwise been difficult to synthesize. The key for enzymatic polymerization to proceed lies in the natures of the monomer and the enzyme. The monomer has to be recognized and activated by the enzyme.

Peroxidase-catalyzed synthesis of polyphenol and polyaromatics involves a reaction mechanism that results in a direct ring-to-ring coupling of phenol and aniline monomers.[4] Dordick and coworkers reported the enzymatic synthesis of polyphenols using horseradish peroxidase (HRP) for the first time.[5] Also aniline monomers have been enzymatically polymerized to yield a wide range of soluble polyanilines.[6] Since then, a variety of modified enzymatic reactions have been investigated to optimize these reactions for polyphenol and polyaromatic synthesis.[7–9] Samuelson and coworkers reported a new conducting water-soluble complex with applying template techniques.[10] Laccase-catalyzed synthesis of conducting polyaniline was also introduced in the presence of SPS.[11] Palm tree peroxidase have used for the synthesis of polyaniline (PANI) in the presence of template,[12] and Ma et al. synthesized polyaniline nanowire by using fully stretched DNA as growing templates and HRP as a catalyst.[13] The enzymatic polymerization of some aniline derivative[14] and pyrrole[15] were recently reported by using HRP as a catalyst, in our lab.

Polyaniline is a conducting polymer that presents good stability and interesting electrochemical and optical properties.[16] However, high-conductivity and good processability are not readily compatible. Thus, recent researches on conducting polymers have targeted improving processability of these polymers by the polymerization of ring-substituted aniline monomers. PANI derivatives present similar physical properties as the parent PANI, but show an improved solubility. Lack of solubility for polyaniline may be attributed to the stiffness of its main chain because of the existence of a strongly conjugated π electron system. Flexible groups induce distortions in the polymer chain and the presence of polar group increased the polarity of the polymer chain which result better solubility.[17]

Previously, Shan and coworkers polymerized aminophenols, methoxy anilines, and 1-naphthyl amine via the catalysis of horseradish peroxidase in dioxane by using hydrogen peroxide as the oxidant.[2] To study the influence of alkoxy groups at the ortho and meta positions on the polymerization and prop-

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

erties of the resulting polymers, here we wish to report the preparation of alkoxy ring-substituted polyaniline by enzymatic oxidation. SPS as a template and HRP as a catalyst were used for synthesis of water-soluble, conducting poly(*o*-anisidine) (POA), poly (*o*-phenetidine) (POP), poly(*m*-anisidine) (PMA), and poly(*m*-phenetidine) (PMP). Results of enzymatic polymerization with chemical polymerization of alkoxyanilines are compared in the presence of SPS.

EXPERIMENTAL

Materials

Poly(sodium 4-styrene sulfonate) (M_W of 70,000), was purchased from Aldrich Chemical (Milwaukee, WI) and was used without any further purification. Horseradish peroxidase (EC 1.11.1.7) (about 170 units/mg), hydrogen peroxide (30 wt %), ammonium persulfate, and alkoxyanilines were obtained from Merck.

Polymerization reactions

The enzymatic polymerizations of *o*-anisidine, *m*-anisidine, *o*-phenetidine, and *m*-phenetidine were carried out in 0.1*M* sodium phosphate buffer at pH 4 at room temperature. An equimolar amount of each monomer and template (12 m*M*) were used. Typically, 0.0371 g (12 m*M*) SPS and 20.8 μL (12 m*M*) *o*-anisidine were added to 15 mL buffer solution under constant stirring, followed by the addition of catalytic amount of the enzyme (2 mg HRP). To initiate the reaction, a stoichiometrtic amount of diluted hydrogen peroxide (0.02*M*) was added dropwise under vigorous stirring over a period of 2 h. The reaction was then left to stir 2 h at room temperature at least. The dark violet resulted solution was transferred to individual regenerated cellulose tube and was dialyzed (cut off molecular 3000) for 20 h. All the other monomers were polymerized in the same manner. The final solutions of the meta isomers were purple brown. The UV-vis spectrum of the samples diluted with buffer solution (1:10 V/V) for ortho isomers and (1:1 V/V) for meta isomers were then measured. The unreacted monomers concentrations in the dialysis solutions were determined by measuring their λ_{max} absorbance in 1*M* HCl. On the basis of the concentration of unreacted *o*-anisidine and *m*-anisidine, the percentage yields of polymers were calculated to be about 80 and 10%, respectively.

Polyalkoxyanilines were also synthesized by chemical oxidation using ammonium persulfate in 0.1*M* HCl aqueous solution. In a 100 mL flask, 6 mmol SPS (based on monomer repeat unit) and equimolar amounts of each monomer were dissolved in 15 mL HCl and the solution was cooled to below 5°C by using an ice bath. A prechilled solution of 0.06 mol of ammonium persulfate in 15 mL 0.1*M* HCl was added dropwise under vigorous stirring. The resulting solution was left in the ice bath for 1.5 h. The final solutions were dark green for ortho and purple brown for meta isomers. These solutions were then dialyzed for 20 h. The UV-vis spectrum of the polymers were obtained with dilution (1 : 100 V/V) for ortho and (1 : 10 V/V) for meta isomers. The yield of chemical polymerization based on unreacted *o*-anisidine and *m*-anisidine in the dialysis solutions were calculated to be over 90 and about 15%, respectively.

Analytical techniques

FTIR measurements were carried out on a BRUKER IFS 66/S FTIR spectrometer in the form of dried polyalkoxyanilines/SPS complexes using KBr pellets. UV-vis spectra were obtained using a Shimadzu UV-1601PC spectrophotometer. The cyclic voltametry (CV) measurements were performed using a Metrohm Polarograph model 746 VA trace analyzer. Cyclic voltammograms were recorded at room temperature by employing a three-electrode cell with platinum as an auxiliary electrode, an Ag/AgCl electrode as the reference electrode, and a Pt foil (1 cm^2 surface area) as the working electrode. The cyclic voltammograms were obtained in 1.0*M* HCl electrolyte in polymers solutions and scanned between 0 and 1.0 V at scan rate of 100 mV/s.

RESULTS AND DISCUSSION

Aniline derivatives possess different oxidation potentials than aniline. The oxidation potentials of aniline derivatives correlate with their protonation, which takes place in the first step of the oxidative polymerization. Earlier studies on the alkyl ring-substituted PANIs have shown that the substituted polymers have lower intrinsic oxidation state i.e., lower imine/amine ratio than their unsubstituted emeraldine counter part.[18] The steric hindrance and electronic effects of the substituents play an important role in this kind of polymerization.[19] The electron-donor properties of alkoxyanilines depend on the localization of electron density on the nitrogen atom of the electroactive amino group. Koval'chuk et al. have shown the potential of the first oxidation of the isomeric anisidins,[20] which can be ordered according to the values of the first oxidation potential:

m-anisidine > *p*-anisidine > *o*-anisidine

Different results in enzymatic polymerization of ortho- and meta-isomers of alkoxyanilines are obtained in this work. The polyalkoxyanilines/SPS complexes were conveniently soluble in water. Thus, homogeneous solutions are obtained after synthesis and dialysis of these complexes. The solutions remain stable

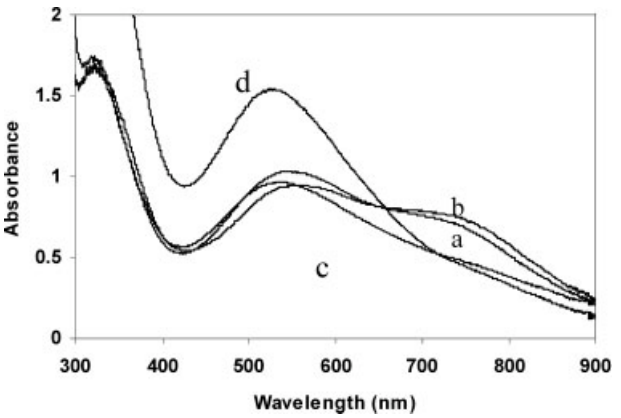

Figure 1 UV-vis spectra of POA/SPS complex obtained by enzymatic polymerization at different pH (a) 3, (b) 4, (c) 6, and (d) 7.

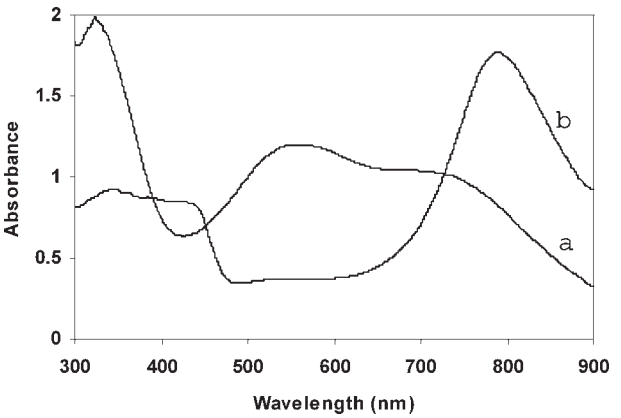

Figure 3 UV-vis spectra of (a) enzymatic and (b) chemical synthesis of POA/SPS complex.

for a long period of time and no precipitate observed at the 1:1*M* ratio of polyalkoxyanilines to SPS. The polymerization also performed at a higher molar ratio up to 4:1 of polyalkoxyanilines to SPS and no precipitate was observed after several months. It is therefore derived that the synthesized polymer has good solubility in water.

To optimize the reaction condition, the enzymatic polymerization of *o*-anisidine was typically carried out at different pH ranging from 3 to 7. The absorption spectra of the POA/SPS complex in this pH range are indicated in Figure 1. From the figure, it is clear that the best results were obtained at pH 4. As the pK_a of *o*-anisidine is 4.52, a pH of 4 is sufficient to provide the necessary cationic charges. Thus, it is expected that as the pH of the solution increases, the degree of protonation is justified by decreasing and probably the degree of branching increases. This judgment is the intensities of the bands at 760 and 580 nm, and production of a new peak at 520 nm emerged, which the latter is assigned to the formation of branched polymer. In general, the presence of multiple branch in the polymer structures were indicated by appearance of an absorption band at ~500 nm regions.[6]

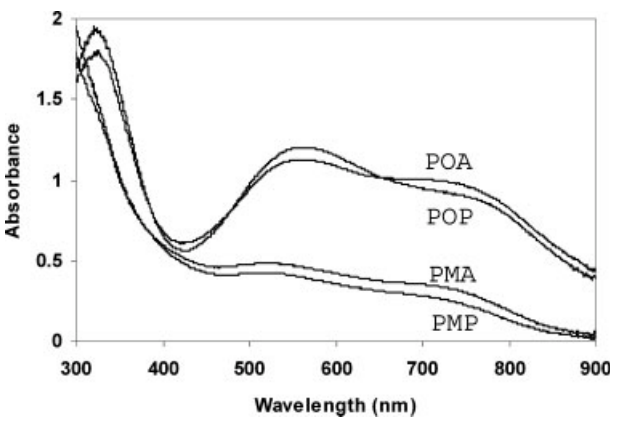

Figure 2 UV-vis spectra of enzymatic synthesis polyalkoxyanilines/SPS complexes.

Figure 2 shows the absorption spectra of aqueous solutions of polyalkoxyanilines/SPS complexes at pH 4. As can be seen, the polymerization of ortho isomers gives relatively high yields, while for meta isomers, the yields are low. Three strong bands at 320, 560, and 735 nm in the spectra of ortho isomers and two weak bands at 520 and 730 nm for meta isomers were observed. These UV-vis spectra were completely different with that obtained from chemically synthesized polyalkoxyanilines. The results of enzymatic polymerization of alkoxyanilines with chemical polymerization of *o*-anisidine in the presence of SPS were compared in Figure 3. This figure shows the UV-vis spectra of POA/SPS complex synthesized by enzymatic [Fig. 3(a)] and chemical [Fig. 3(b)] methods. Three strong bands were observed at 340, 420, and 790 nm for chemically synthesized POA/SPS complex, and obviously the results of these two methods were completely different. Our earlier works have shown that the results of enzymatic polymerization of alkyl substituted aniline[14,21] and *N*-alkyl substituted aniline[22] were in good agreement with the chemically synthesized polymers. However the changes observed here are probably because of the different interaction of enzyme and chemical oxidants with alkoxy-substituted anilines and also difference in the potential of oxidation of enzyme and chemical oxidants.

The polyalkoxyanilines/SPS complexes produced at pH 4.0 were studied in detail. The shifts in UV-vis spectra of these complexes with varying pH were considered. For ortho-isomers, when the pH of the solution increased, great changes were observed, while for meta-isomers, no considerable changes were seen. The shift in UV-vis spectra of POA/SPS complex was typically shown in Figure 4. As the pH

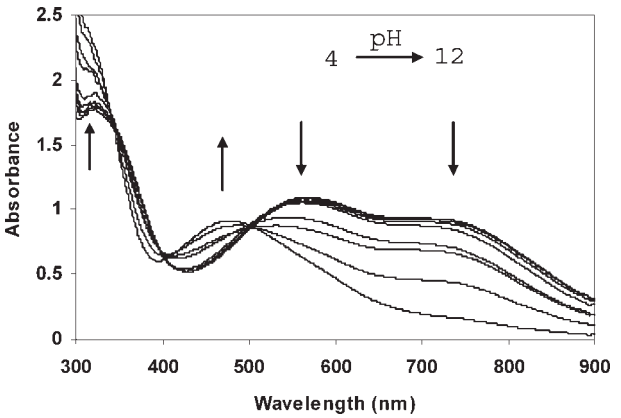

Figure 4 Absorption spectra of enzymatically synthesized POA/SPS during titration with NaOH in the pH range of 4–12.

is increased from 4 to 12, the bands at 745 and 570 nm gradually disappear with the emergence of a new peak at 480 nm, and at the same time, the band at 320 nm increased. At pH 4, the color of solution is dark violet and as the pH increased with addition of 1*N* NaOH, the color has changed to blue at pH 8 and purple at pH 12. At pH 12, the POA/SPS complex is completely dedoped and the absorption spectrum is comparable to chemically synthesized POA/SPS base complex. This process shows reversible reduction/oxidation behavior in the absorption spectra. When the pH of the solution is decreased from 12 to 4 by using HCl, a reverse process was observed, and the solution color changed from purple to violet. Figure 5 proves that this process is completely reversible. This pH-induced redox reversibility demonstrates that the electroactive poly (*o*-anisidine) was enzymatically synthesized.[7] The same results were obtained for POP/SPS complex, but for PMA/SPS and PMP/SPS complexes, we did not see considerable change and redox reversibility.

The FTIR spectra of SPS and polyalkoxyanilines/SPS complexes are presented in Figure 6. In FTIR

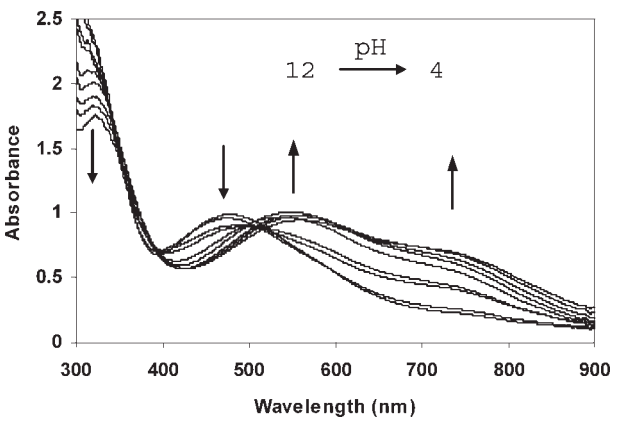

Figure 5 Absorption spectra of enzymatically synthesized POA/SPS during titration with HCl in the pH range of 12–4.

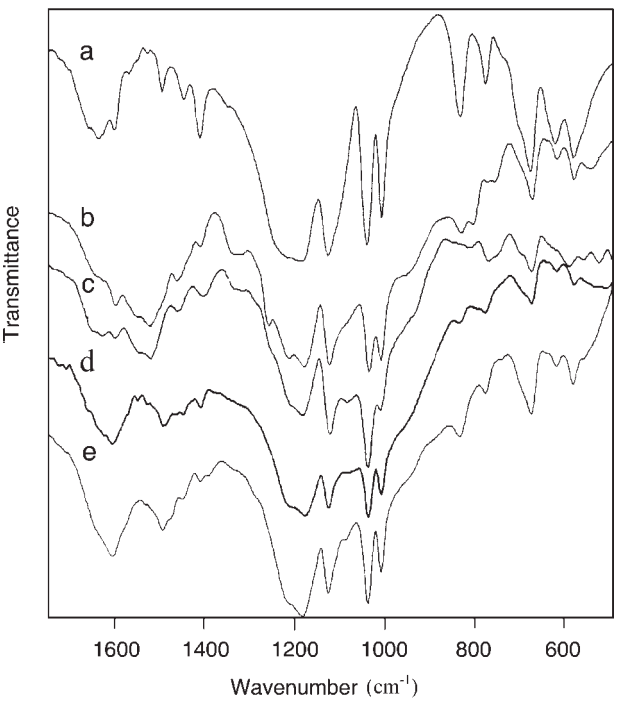

Figure 6 FTIR spectra of (a) SPS (b) POA/SPS (c) POP/SPS (d) PMA/SPS, and (e) PMP/SPS complexes.

spectra of ortho isomers, some characteristic bands for polyaniline derivatives are observed. The peak at 1600 cm^{-1} is assigned to ring stretched of quinoid form and other peak at 1502 cm^{-1} is assigned to the benzenoid ring.[23,24] A weak peak at 1408 cm^{-1} is attributed to the C—N stretching vibration in quinoid imine units.[25] The band at 1290 cm^{-1} can be assigned to C—N stretching vibrations of the groups with partially double bond order.[26] Two other peaks at 835 and 758 cm^{-1} are related to the C—H out-of-plane bending of 1, 2, 4-trisubstituted benzene ring. In the FTIR spectra of the meta isomers, some weak peaks characteristic of polyalkoxyanilines are seen. These results confirm the UV-vis spectra and show that the position of substitutions is very important in the polymerization. For a pair of ortho- and meta-substituted isomers, the ortho-substituted aniline always gives a higher polymerization yield. These observations indicate that polyalkoxyanilines/SPS complexes result mainly from head-to-tail coupling and enzymatic polymerization of alkoxyanilines proceeds via formation of — C—N=C— and —C—NH—C— bonds.

The FTIR spectra of poly(*o*-anisidine) obtained by enzymatic and chemical polymerizations in the presence of SPS were shown in Figures 7(a) and 7(b), respectively. Enzymatic polymerization of *o*-alkoxyanilines yields violet solutions, whereas chemical polymerization yields green solutions. As it can be seen, these two spectra show some differences to each other. Looking at the band ratio between the

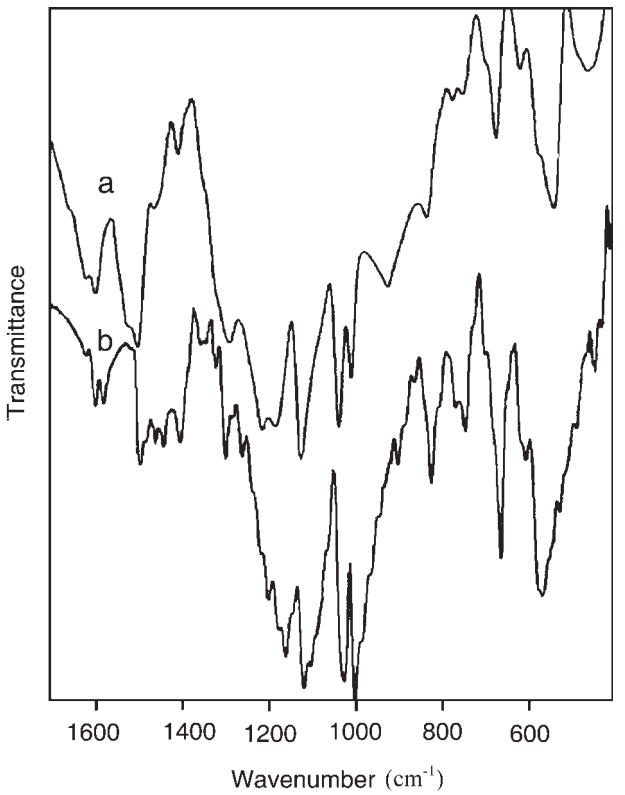

Figure 7 FTIR spectra of POA/SPS complex synthesized by (a) enzymatic and (b) chemical polymerization.

quinoid and benzenoid bands in the FTIR spectra, it shows that the relative intensity of quinoid (1600 cm^{-1}) to benzenoid (1502 cm^{-1}) bands is greater for enzymatic polymerization. We can estimate that in the case of enzymatic oxidation, we have higher quinoimine structure in the polymer chain relative to chemical oxidation. These observations confirmed the results obtained by UV-vis spectra.

The electrochemical nature of ortho-substituted PANIs in the complexes synthesized by different methods was measured by cyclic voltammetry, and all of them were electroactive. Earlier studies on PANIs have shown that the cyclic voltammograms of the substituted polyanilines exhibit two oxidation peaks.[27] Figure 8 shows the cyclic voltammograms of enzymatically prepared POA/SPS [Fig. 8(a)] and POP/SPS [Fig. 8(b)] complexes. Two anodic and two cathodic peaks were observed at these voltammograms. The anodic peak current (E_{pa}) values of POA/SPS, POP/SPS, PMA/SPS, and PMP/SPS complexes at a scan rate of 100 mV/s are shown in Table I. As it is evident, the values of E_{pa} show a positive shift from POA to PMA. These results are in agreement with the earlier works, although in enzymatic method, the second anodic peak is very weak.[20] Figure 9 indicates the voltammograms of poly(*o*-alkoxyanilines) obtained by chemical polymerization in the presence of SPS. Two anodic signals are observed at 0.29 and 0.46 V for POA/SPS complex [Fig. 9(a)] and at 0.31 and 0.45 V for POP/SPS complex [Fig. 9(b)]. However, the oxidation potentials are dependent upon the nature of the substituents. Electron-donating substituents lower the potential of oxidation and thus are confirmatory of more easily oxidized species. In contrast, we have seen a positive shift to lower potential for the first anodic peaks when compared with unsubstituted polyanilines. It seems that

TABLE I
Cyclic Voltammetry Data for Polyalkoxyanilines/SPS Complexes in 1.0*M* HCL at a Scan Rate of 100 mV/s

	Anodic peaks (V vs Ag/AgCl)	
Polyalkoxyanilines/SPS	E_{pa1}	E_{pa2}
POA/SPS	0.30	0.72
POP/SPS	0.34	0.68
PMA/SPS	0.46	–
PMP/SPS	0.47	–

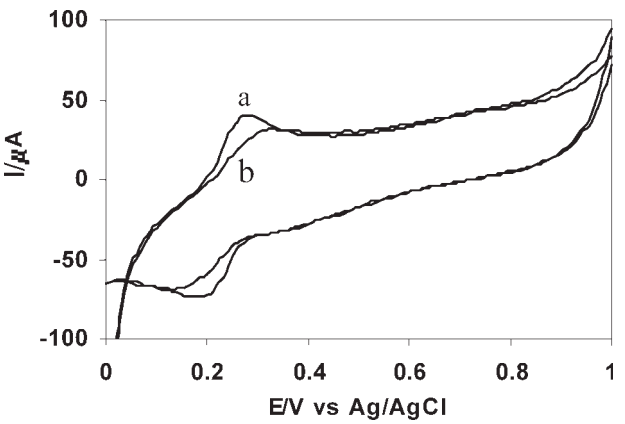

Figure 8 Cyclic voltammograms of enzymatically synthesized (a) POA/SPS and (b) POP/SPS complexes obtained at 100 mV/s.

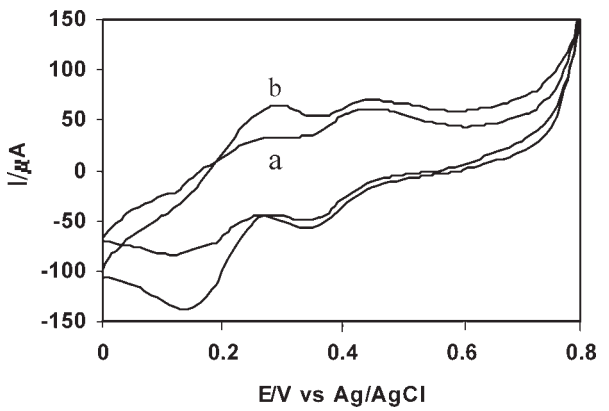

Figure 9 Cyclic voltammograms of chemically synthesized (a) POA/SPS and (b) POP/SPS complexes obtained at 100 mV/s.

the presence of bulky substituents can induce some nonplanar conformations and result in higher oxidation potentials. However, for the second anodic peaks, contribution of the electron-donating of alkoxy groups become more important and leads to the negative shift for the second oxidation peak. For poly(*m*-alkoxyanilines) obtained by enzymatic and chemical oxidation, we did not get convenient cyclic voltammograms that confirm other results.

CONCLUSIONS

Methoxy and ethoxy mono-substituted polyaniline have been synthesized in ambient condition at pH 4 by enzymatic polymerization in the presence of a template. The electroactive complexes were characterized by spectroscopy. The presence of alkoxy substituents affects the polymerization reactions. It has been observed that the size and position of the substituent have strong effect on the polymerization yields. Similar results were obtained for every pair of ortho- and meta-alkoxy substituted polyanilines. We compared the enzymatic oxidation of alkoxyanilines with chemical oxidation. The results of enzymatic polymerization are different from chemical polymerization. These differences are probably due to different interaction of enzyme and chemical oxidants with monomers. The FTIR spectroscopic investigations are consistent with the UV-vis spectroscopy and cyclic voltammetry results.

References

1. Kobayashi, S.; Uyama, H.; Kimura, S. Chem Rev 2001, 101, 3793.
2. Shan, J.; Han, L.; Bai, F.; Cao, S. Polym Adv Technol 2003, 14, 330.
3. Gross, R. A.; Kumar, A.; Kalra, B. Chem Rev 2001, 101, 2097.
4. Akkara, J. A.; Senecal, K. J.; Kapalan, D. L. J Polym Sci Part A: Polym Chem 1991, 29, 1561.
5. Dordick, J. S.; Marletta, M. A.; Klibanov, A. M. Biotechnol Bioeng 1987, 30, 31.
6. Alva, K. S.; Kumar, J.; Marx, K. A.; Tripathy, S. K. Macromolecules 1997, 30, 4024.
7. Liu, W.; Kumar, J.; Tripathy, S. K.; Senecal, K. J.; Samuelson, L. J Am Chem Soc 1999, 121, 71.
8. Liu, W.; Kumar, J.; Tripathy, S. K. Langmuir 2002, 18, 9696.
9. Ikeda, R.; Uyama, H.; Kobayashi, S. Macromolecules 1996, 29, 3053.
10. Samuelson, L. A.; Alva, K. S.; Anagnostopoulos, A.; Kumar, J.; Tripathy, S. K. Macromolecules 1998, 31, 4376.
11. Karamyshev, A. V.; Shleev, S. V.; Koroleva, O. V.; Yaropolov, A. I.; Sakharov, I. Y. Enzyme Mirob Technol 2003, 33, 556.
12. Sakharov, I. Y.; Ouporov, I. V.; Vorobiev, A. K.; Roig, M. G.; Pletjushkina, O. Y. Synth Met 2004, 142, 127.
13. Ma, Y.; Zhang, J.; Zhang, G.; He, H. J Am Chem Soc 2004, 126, 7097.
14. Nabid, M. R.; Entezami, A. A. Eur Polym Mater 2003, 39, 1169.
15. Nabid, M. R.; Entezami, A. A. J Appl Polym Sci 2004, 94, 254.
16. Amano, K.; Ishikawa, H.; Kobayashi, A.; Satoh, M.; Hasegawa, E. Synth Met 1994, 62, 229.
17. Wei, Y.; Focke, W. W.; Wnek, G. N.; Ray, A.; MacDiarmid, A. G. J Phys Chem 1989, 93, 495.
18. Kang, E. T.; Neoh, K. G.; Tan, K. L. Eur Polym Mater 1994, 30, 529.
19. Mattoso, L. H.; Manohar, S. K.; MacDiarmid, A. G.; Epstein, A. J. J Polym Sci Part A: Polym Chem 1995, 33, 1227.
20. Koval'chuk, E. P.; Stratan, N. V.; Reshetnyak, O. V.; Blazejowski, J.; Whittingham, M. S. Solid State Ionics 2001, 141/142, 217.
21. Nabid, M. R.; Entezami, A. A. Iran Polym J 2003, 12, 401.
22. Nabid, M. R.; Entezami, A. A. Polym Adv Technol 2005, 16, 305.
23. Laska, J. Synth Met 2002, 129, 229.
24. Cataldo, F.; Maltese, P. Eur Polym Mater 2002, 38, 1791.
25. Li, X. G.; Huang, M.-R.; Jin, Y.; Yang, Y.-L. Polymer 2001, 42, 3427.
26. Widera, J.; Patys, B.; Bukowska, J.; Jackowska, K. Synth Met 1998, 94, 265.
27. Yuan, G.-L.; Kuramoto, N. Macromolecules 2002, 35, 9773.