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NOVEL ENZYMATIC POLYETHYLENE OXIDE-POLYPHENOL SYSTEM FOR IONIC CONDUCTIVITY

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ABSTRACT

Phenol formaldehyde resins are of interest for different industrial and electronic applications. However, the toxic nature of formaldehyde and the extreme reaction conditions for the synthesis of these polymers have severely limited their use in today's markets. We present here a novel, biocatalytic approach where the enzyme horseradish peroxidase is used to polymerize phenol in the presence of a template such as polyethylene oxide. Here the template assists as a surfactant that can both emulsify the phenol and polyphenol chains during polymerization and provide water/solvent solubility of the final polyphenol/template complex. The reactants and the reaction conditions of this method are mild and result in high molecular weight, electrically and optically active, water-solvent soluble complexes of polyphenol and the template used. High molecular weight water-soluble polyphenol/polyethylene oxide complexes were formed.

Key Words: Polyethylene; Polyphenol; Horseradish peroxidase; Ionic conductivity; Batteries

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INTRODUCTION

Phenolic compounds and aromatic amines have wide-ranging industrial applications, particularly as bonding agents for coatings, laminates, particleboards.[1] Phenol-formaldehyde polymers constitute a major segment of the commercial phenolic resin market.[2] However, the manufacture of these resins is of great environmental concern because of the toxic nature of the starting materials, such as formaldehyde, and the harsh temperature, pressure and pH conditions involved in the production of these materials.[1,3] Furthermore, the reaction is complicated to control and unfavorable side products are normally produced.[1]

An alternative synthetic approach has involved the use of enzyme based catalyst systems.[4–6] It was reported that horseradish peroxidase (HRP) induced oxidative polymerization of phenol and aniline derivatives produced conjugated polyaromatics with novel properties. The benign, gentle and specific reactivity of the enzyme is extremely desirable for a more environmentally compatible synthesis. Efforts were focused to improve the control of molecular weight, mechanical properties, and processability of these polyphenols.[7] Polymerization in organic solvents using several methods was successful in increasing the molecular weight and improving the mechanical properties of the polyphenols.[4,7] However, most of these polymers were not easily processable and the synthesis still involved the use of toxic solvents. Another approach involved enzymatic polymerization of phenol at the air-water interface of a Langmuir trough.[8] Although this approach improved processability, large-scale production using this technique is costly.

A new enzymatic route for the polymerization of phenol that minimizes parasitic reactions and results in water soluble, high molecular weight, thermally stable and processable polyphenol has been reported recently. [9] This procedure was originally used in our laboratories for the polymerization of aniline.[10] It comprises polymerization of phenol, using the enzyme HRP, in the presence of a polyelectrolyte such as polystyrene sulfonate (SPS). This method generates a favorable local environment[11] for the formation of high molecular weight polymer complex composed of both polyphenol and the polyelectrolyte. This complex is water-soluble and thermally stable. In this paper, we present the polymerization of phenolic compounds using this new biological approach in the presence of polyethylene oxide (PEO). The synthesis of these PEO/polyphenol complexes and their spectroscopic, thermal, electrical, and optical properties are reported.

EXPERIMENTAL

Horseradish peroxidase (HRP, EC 1.11.1.7) was obtained from Sigma Chemical Co. (St. Louis, MO) as a salt free powder. The specific activity was 240 purpurogallin units/mg solid. Phenol, polyethylene oxide (PEO) (Mw

1.0 M 10,000, 400 g/mol), hydrogen peroxide (30% solution), phosphate buffer, and all solvents (reagent grade or better) were purchased from Aldrich (Milwaukee, WI) and used as received. A typical enzymatic polymerization was carried out in 10 mL of aqueous phosphate buffer (10 mM, pH 7.0). Phenol (71.3 mM), lithium iodide trihydrate (37.4 mM, Strem Chemicals, MA) and equimolar concentrations of PEO and hydrogen peroxide (no more than 20 mM aliquots added every 5 min) were then added to the buffered solution. The HRP concentration was 0.1–0.15 mg/mL. Finally, hydrogen peroxide was introduced to commence the reaction.

The reaction was carried out for 30 min at room temperature and the final products were dialyzed using Centricon concentrators (10,000 cut off, Amicon Inc., Beverly, MA). The samples were then dried under vacuum at 60°C and used for further analysis. The yield was typically 95% or higher. Control samples, using denatured enzyme, were prepared using a similar procedure. The enzyme was denatured by leaving it in buffered water at 100°C for 30 min. The denatured HRP was tested using purpurogallin, and was found to be inactive. In all control experiments, polymer formation was not observed. Spectral characterization of the water-soluble polymers and controls was performed with a Perkin-Elmer Lambda-9-UV-Vis-Near IR spectra-photometer (Norwalk, CT). FTIR spectra, of the samples deposited on ZnSe, were measured on a Perkin Elmer FTIR 1720X. The ^{1}H-NMR spectrum was recorded on a Bruker ARX 500-MHz NMR spectrometer. Thermal gravimetric analysis (TGA) and Dielectric analysis (DEA) were conducted using a TA instrument 2950, and a DEA instrument 2970 (New Castle, DE) respectively. TGA was carried out under nitrogen and a heating rate of 10°C/min was used. Static light scattering (SLS) measurements were performed on the PEO-polyphenol polymer (after enzymatic polymerization) using a Brookhaven instrument (Model SG-7B Rigaku Denki, Japan). The PEO-Polyphenol was dissolved in a 50:50 DMSO/water solvent mixture. NaCl was added to a final concentration of 0.1 M and the solution was filtered several times using a 0.45 µm filter. A series of concentrations required for the measurements were prepared by diluting the stock solution, with filtered buffer added directly into the scattering cell. The data was analyzed using Zimm-plot software supplied by Brookhaven instrument (Version. 2.04).

The ionic conductivity of all polyphenols was measured using DEA. All samples were dried on the cell in a vacuum oven at 60°C for 48 h The hygroscopic nature of the template-polyphenol necessitates proper drying procedures to minimize secondary ionic conductivity.

RESULTS AND DISCUSSION

The UV-Vis spectra of the enzymatic polyphenol/PEO complex and of the control is reported in Fig. 1.

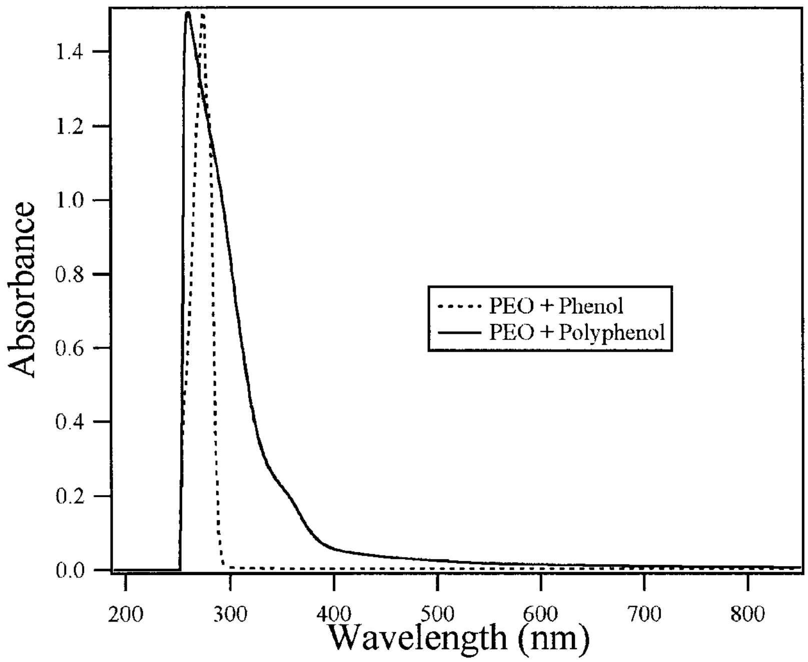

Figure 1. UV-Vis spectra of polyphenol polymerized on PEO and of control phenol/PEO.

In the region from 300 to 800 nm, a large broad absorption tail is observed for the PEO/polyphenol indicating an extended degree of conjugation in the PEO/polyphenol complex. This broad absorption was observed for phenol enzymatically polymerized on a Langmuir trough[8] and in polystyrene sulfonate(SPS)/polyphenol.[9] The PEO/polyphenol, did not show any gel formation as was previously observed with the (SPS)/polyphenol.[9] Figure 2 shows the ^{1}H NMR spectrum of the PEO/polyphenol. The broadening of the aromatic peaks in the region of 6–8 ppm indicates the presence of a polymeric compound.

Figure 3 shows the Fourier transformer infrared (FTIR) spectra of the polyphenol in the presence of PEO.

Three principal absorptions are observed in the spectra. The first is a significant broadening of frequency in the OH stretch region (3000–3500 cm^{-1}) of the polymer with respect to the monomer. This is explained by stronger hydrogen bonding in the polymer. The second absorption peak of interest is in the region 1550–1690 cm^{-1} that is assigned to conjugation and to the presence of a quinoid group in the main chain. The last feature is the presence of an absorbance peak in the 1250–1300 cm^{-1}region that is assigned to ether groups. The presence of ether groups seen here are from the PEO. Furthermore, for these enzymatic reactions the ether group formation has also been reported earlier.[12] We are unable to distinguish the two peaks.

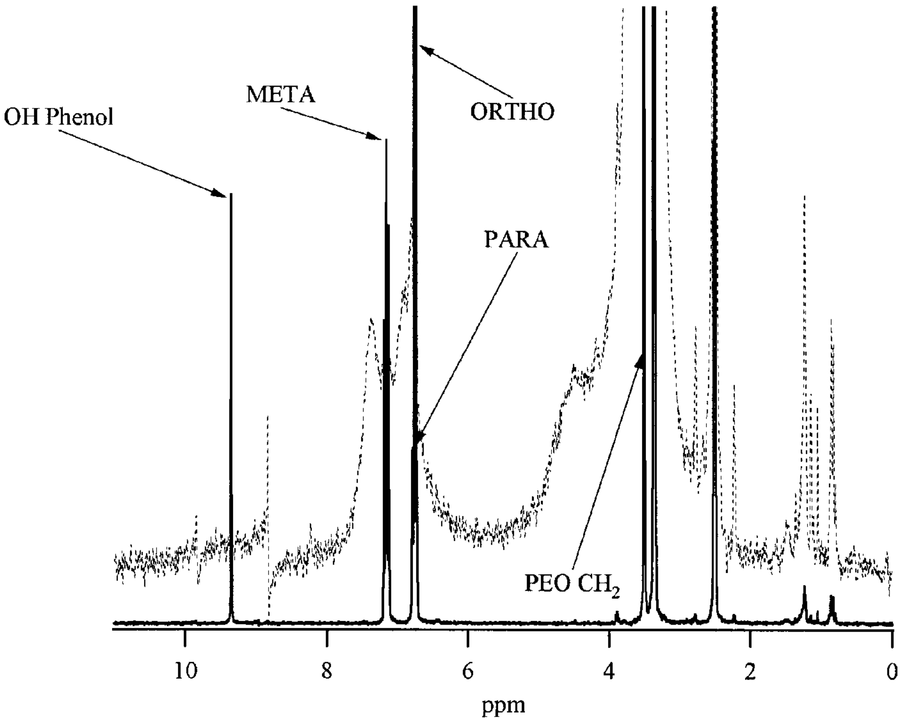

Figure 2. ^{1}H-NMR spectra of phenol/PEO (—)and of polyphenol/PEO (------).

Moreover, a peak at 3733 cm^{-1} is observed for the PEO/polyphenol system associated with a shift of 146 cm^{-1}for the ether group from the solitary PEO to the PEO/polyphenol system. This can be attributed to a reaction of the PEO with the polyphenol forming an ether group on the benzene ring, resulting in intermolecular H bonding between the phenolic OH and the PEO.

The PEO/polyphenol molecular weights were in the range of 1.1×10^6 g/mol as measured by SLS. The Flory-Huggin's interaction parameter, which is a measure of goodness of the solvent, was 0.45. This parameter value is below 0.5, which indicates that the solvent system used acts as a good solvent for the polymer complex.[13] However, we cannot separate the molecular weight of the polyphenol from that of the complex.

These results indicate that PEO has several important roles in the enzymatic reaction. It functions as a matrix to provide water solubility and processability to the final complex. Moreover, the polyelectrolyte, complexed to the polyphenol through hydrogen bonding and hydrophobic interactions, acts as an emulsifier to solubilize the polymer during the course of the reaction. This promotes the synthesis of high molecular weight polymer. The good thermal stability for these complexes supports that high molecular weight PEO/polyphenol complex has been formed.

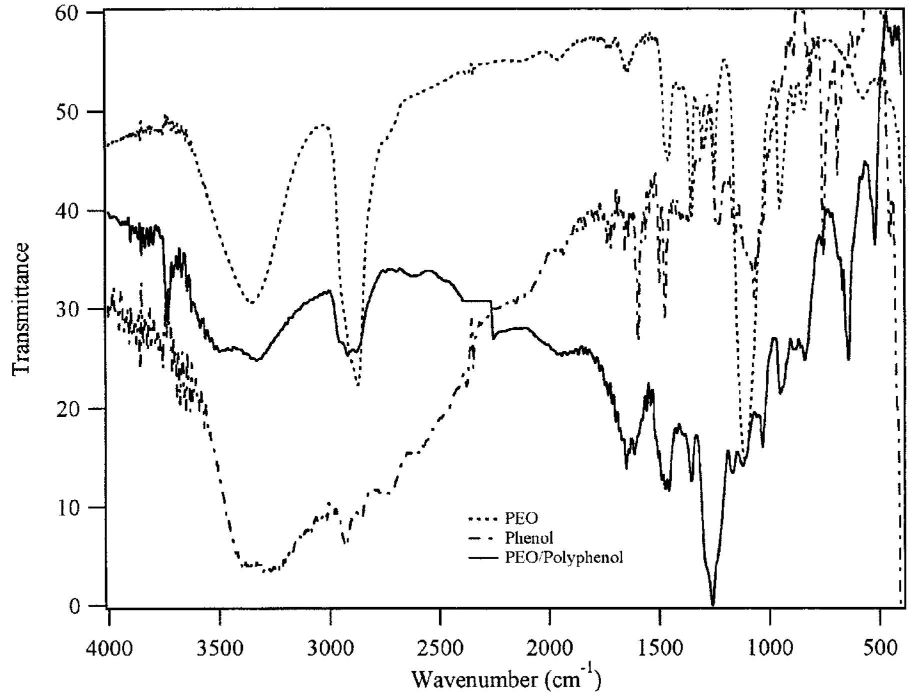

Figure 3. FTIR spectrum of phenol, PEO, and polyphenol/PEO complex.

The TGA in Fig. 4 shows the PEO/polyphenol thermogram before and after polymerization. A significant amount (85–90%) of complex remains after heating to 250°C. Three degradations are observed at 310°C, 370°C, and 520°C; and 31% of residue is left after 600°C. These results were similar to thermograms obtained from insoluble polyphenols synthesized from organic solvents.[4] The ionic conductivity of these complexes was found to be approximately 0.8×10^{-4} S/cm for all samples. These electrical and optical properties are encouraging and indicate that the template directed enzymatic polymerization might be used to obtain these electro active polymers.

CONCLUSION

A simple novel biological route for the enzymatic synthesis of water-soluble, high molecular weight, polyphenol is described. This approach is advantageous in that it is simple, biochemically mild and requires minimal separation and purification. Varying the polyelectrolyte and the introduction of different concentrations of enzyme initiator can optimize the final polymer properties. The polyelectrolyte-polymer complex also exhibited reasonable ionic conductivity. This process is generic in that numerous phenol functional comonomers and polyelectrolytes may be employed to

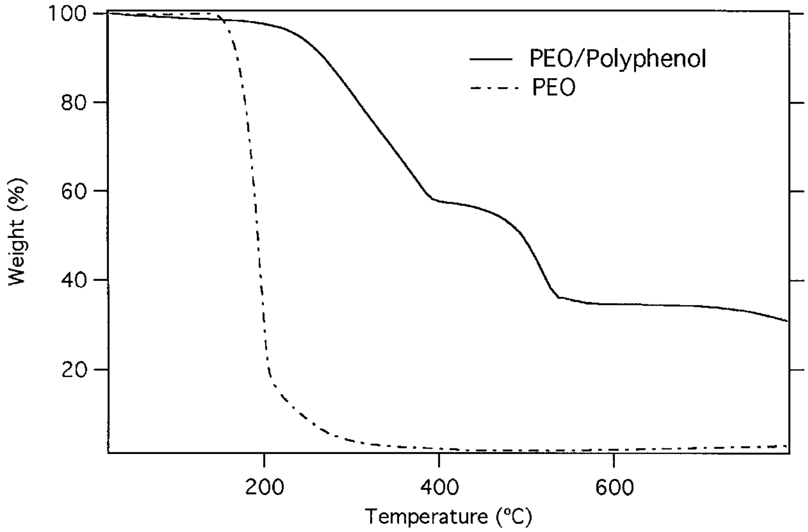

Figure 4. TGA of PEO/polyphenol and PEO (C) before enzymatic reaction.

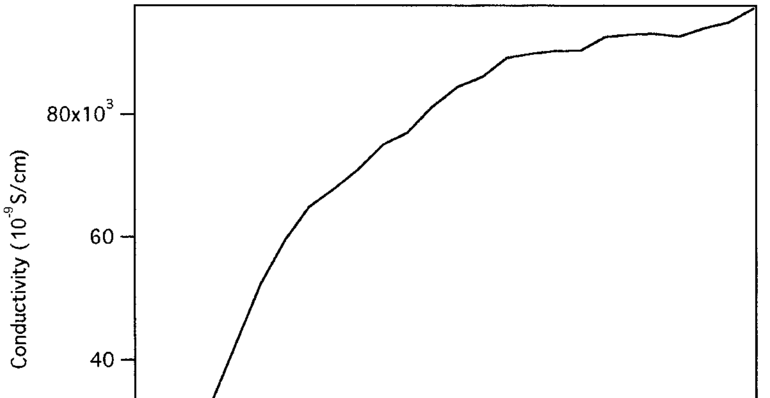

Figure 5. DEA of PEO/polyphenol in presence of LiI.

produce new phenolic resins. Furthermore, it is anticipated that suitable immobilization of the enzymes or the use of synthetic heme group based catalysts with this approach could potentially lead to a facile, cost effective and environmentally friendly route for large scale production of processable phenolic resins.

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REFERENCES

1. Brode, G.L.V. In *Kirk-Othmer Encyclopedia of Chemical Technology*; John Wiley & Sons: New York, NY, 1982; Vol. 17, 384.
2. White, D.M.; Cooper, G.D. In *Kirk-Othmer Encyclopedia of Chemical Technology;* John Wiley & Sons: New York, NY, 1982; Vol. 18, 594.
3. Clary, J.J.; Gibson, J.E.; Waritz, R.S. Formaldehyde Toxicology, In *Epidemiology, Mechanisms*; Marcel Dekker: New York, 1983; 1.
4. Akkara, J.A.; Senecal, K.J.; Kaplan, D.L. Synthesis and Characterization of Polymers Produced by Horseradish Peroxidase in Dioxane. J. Polym. Sci.: Part A: Polym. Chem. **1991**, *29*, 1561.
5. Dordick, J.S.; Marletta, M.A.; Klibanov, A.M. Polymerization of Phenols Catalyzed by Peroxidase in Nonaqueous Media. Biotechnol. Bioeng. **1987**, *30*, 31.
6. Kobayashi, S.; Ikeda, R.; Sugihara, J. Enzymic Oxidative Polymerization of 2,6-Dimethylphenol. Macromolecules **1996**, *29*, 8702 and ref. in the paper.
7. Ra, A.M.; John, V.J.; Gonzalez, R.D.; Akkara, J.A.; Kaplan, D.L. Catalytic and Interfacial Aspects of Enzymic Polymer Synthesis in Reversed Micellar Systems. Biotechnol. Bioeng. **1993**, *41*, 531.
8. Bruno, F.F.; Akkara, J.A.; Samuelson, L.A.; Kaplan, D.L.; Mandal, B.K.; Marx, K.A.; Kumar, J.; Tripathy, S.K. Enzymic Mediated Synthesis of Conjugated Polymers at the Langmuir Trough Air-Water Interface. Langmuir **1995**, *11*, 889.
9. Bruno, F.F.; Nagarajan, R.; Sidhartha, J.S.; Yang, K.; Kumar, J., Tripathy, S.; Samuelson, L. Enzymatic Template Synthesis of Polyphenol. Mater. Res. Soc. Symp. Proc. **2000**, *600*, 255.
10. Liu, W.; Kumar, J.; Tripathy, S.; Senecal, K.J.; Samuelson, L. Biologically Derived Water Soluble Conducting Polyaniline. J. Am. Chem. Soc. **1999**, *121* (1), 71.
11. Liu, W.; Cholli, A.L.; Nagarajan, R.; Kumar, J.; Tripathy, S.; Bruno, F.F.; Samuelson, L.A. The Role of Template in the Enzymatic Synthesis of Conductive Polyaniline. J. Am. Chem. Soc. **1999**, *121* (49), 11345.
12. Higashimura, H.; Fujisawa, K.; Moro-oka, Y.; Kubota, M.; Shiga, A.; Terahara, A.; Uyama, H.; Kobayashi, S. Highly Regioselective Oxidative Polymerization of 4-Phenoxyphenol to Poly(1,4-phenylene oxide) Catalyzed by Tyrosinase Model Complexes. J. Am. Chem. Soc. **1998,** *120*, 8529.
13. Sperling, L.H. *Physical Polymer Science*; John Wiley & Sons: New York, 1986; 394.