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Characterization of phenolic polymers synthesized by enzyme-mediated reactions in bulk solvents and at oil–water interfaces

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Abstract

Phenolic polymers are synthesized from horseradish peroxidase-catalyzed reactions in the presence of hydrogen peroxide. The reactions are carried out in homogeneous mixtures of water and polar solvents such as DMF as well as at the oil–water interface of AOT/isooctane reversed micelles and isooctane–water biphasic systems. Polymer characteristics are analyzed using thermal, spectroscopic and chromatographic techniques. Polymer molecular weight dependence on the reaction medium composition is demonstrated for poly(*p*-ethylphenol) and poly(*m*-cresol). It is shown that thermal curing of polymers leads to cross-linking, and as a result, there is a significant increase in molecular weight. Cross-linking appears to be directly to the aromatic rings, and not via ether links, as inferred from infrared spectra. A process scheme for the production, under controlled conditions, of phenolic polymers in ethanol–water mixtures is presented. The process allows enhanced monomer conversions and ability to tailor polymer molecular weight. Possible applications for phenolic polymers are discussed.

Keywords: Phenolic polymers; Synthesis; Enzymes; Bulk solvents; Oil–water interfaces

1. Introduction

Peroxidase-catalyzed oxidative coupling of phenols is one of the most studied enzymatic polymerization reactions in nonaqueous systems [1–8]. A number of phenolic monomers and aromatic amines have been polymerized in different reaction media, including organic solvents and microemulsions [1,3,5]. Polymer structure and properties have been studied and near complete monomer conversions, quantitative polymer yields and high molecular weights have been reported [5]. Enzymatic methods to produce polyphenols are a potential alternative to chemical processes that are currently used in the industry. Polyphenols are commercially produced as novolacs and resoles by condensing phenol with formaldehyde at different molar ratios depending on the type of polymer desired [9]. Formaldehyde is a confirmed carcinogen, and its large-scale use in the industry presents a health risk. Also, its presence in the finished product is unavoidable and undesirable. Inorganic catalysts such as copper halides are known to catalyze phenol polymerization in the presence of molecular oxygen [10]. As in the case of enzymatic catalysis, the inorganic catalysis does not require the use of formaldehyde, and the resultant poly(phenylene oxides) exhibit good thermal and temporal stability.

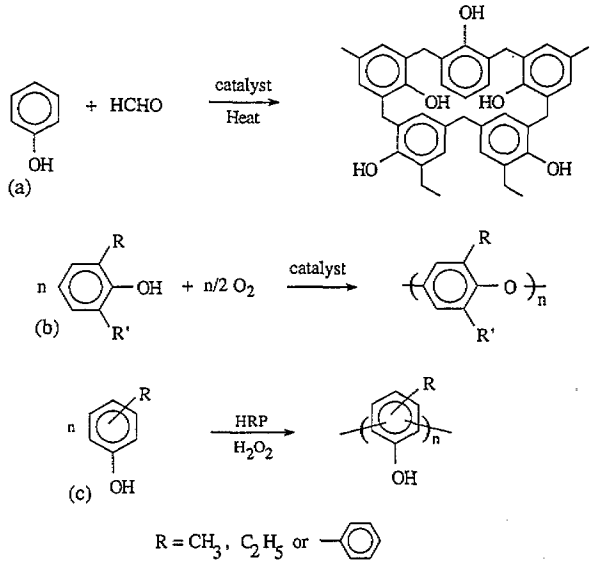

Fig. 1. Schematic of phenol polymerization by different methods.

There is a significant fundamental difference in the structure of polyphenols synthesized by the above methods. The polymer produced by enzymatic methods has a continuous conjugated backbone, whereas in the polyphenols produced by acid/base and inorganic catalysts, the conjugation is broken by methylene bridges and ether links, respectively (Fig. 1). However, chemical synthesis of conjugated poly-

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Table 1
Poly(*p*-ethylphenol) molecular weight and polydispersity in different reaction media

Synthesis medium	M_w (p.d.)[a]	Comments
AOT/isooctane reversed micelles	2500 (1.4)	100% monomer conversion to polymer
85/15 dioxane/water	3400 (>2.0)	Good monomer conversion; poor polymer yield
Isooctane/buffer biphasic system	1700 (>2.0)	Good monomer conversion; fair polymer yield
85/15 dioxane/water	3000 (>2.0)	Good monomer conversion; fair polymer yield

[a]Molecular weights were determined with 1% LiBr in DMF as GPC solvent; p.d. = polydispersity.

mers has long been known. For example, the synthesis of conjugated polymers such as polydiacetylene and polyaniline by chemical processes is well documented. The fully conjugated backbone generated in the enzymatic polymerization processes provides important opportunities for the phenolic polymers in optics and electronic applications. Third-order nonlinear optical materials, photolithography materials and rechargeable battery components are just some of the potential areas of utility under active study. In addition, enzymatically synthesized polyphenols may also find applications for which novolacs and resoles are currently being used. Polyphenols for photolithography should preferably be of low molecular weight, soluble in alkaline solution and filterable at high concentrations. On the other hand, the hydrogen-to-carbon ratio in the polymer should be low for battery applications. We address in this paper polyphenol molecular weight issues that are pertinent to a number of applications, including photolithography.

2. Materials and methods

All phenolic monomers and solvents were purchased from Aldrich Chemicals Company (Milwaukee, WI). Horseradish peroxidase (HRP) (Type II) was purchased from Sigma Chemicals Company (St Louis, MO). All chemicals were of highest purity available and were used as received.

Preparation of reaction mixtures and samples for thermal, spectroscopic and chromatographic analyses have been described previously [6]. Thermal analyses were carried out on Du Pont thermal analyzers (TA Instruments, Inc., New Castle, DE). The samples were heated at a rate of 10 °C min^{-1} under a nitrogen atmosphere for all thermal analyses. Molecular weights were determined on a Waters LC Module I instrument (Millipore, Milford, MA) with an on-line gel permeation chromatography (GPC) column (GBR mixed-bed linear column with a molecular weight range of 100 to over 20 million; Jordi Associates, Inc., Bellingham, MA). 1% LiBr/dimethylformamide (DMF) at a flow rate of 1 ml min^{-1} was used as mobile phase. Infrared spectra were recorded on a Perkin-Elmer 1760 FTIR-FT Raman spectrophotometer (Norwalk, CT) at 4 cm^{-1} resolution. Samples were cast as thin films on a KBr window from chloroform solutions.

3. Results and discussion

In our earlier work, we observed that *p*-cresol and *p*-ethylphenol are readily polymerized by horseradish peroxidase (HRP) in the presence of hydrogen peroxide [5,6]. The reactions are carried out in homogeneous mixtures of water and a polar solvent such as DMF, at the oil–water interface of dioctylsodiumsulfosuccinate (known as AOT)–isooctane–chloroform reversed micelles or in isooctane–water biphasic systems. However, the highest degree of polymerization (DP) is *ca* 30. Molecular weights larger than 3500 have not been possible for poly(*p*-ethylphenol), perhaps due to the lack of extensive cross-linking in the polymer structure [6]. However, as noted in Table 1, the molecular weight of poly(*p*-ethylphenol) can be controlled in the range of 1700–3500 by varying the solvent composition and by using different reaction media. Synthesis of materials with molecular weights lower than 1700 is also possible. In addition, good control of polydispersity has been demonstrated by carrying out the reactions in reversed micelles [6]. Likewise, the ability to control the molecular weight of poly(*m*-cresol) is studied in ethanol–water mixtures. It is possible to tailor the polymer molecular weight between 1400 and 24 000 by varying the reaction medium composition from 100% buffer to 100% ethanol (Table 2). These results appear to be related to a combination of solvent–polymer interactions and solvent–enzyme interactions. Studies are currently underway to quantify these interactions to predict polymer molecular weight at a given solvent composition.

Large scale reactions are carried out to synthesize poly(*p*-ethylphenol) in reversed micelles and poly(*m*-cresol) in ethanol–water mixtures. While 100% conversion of *p*-ethylphenol to poly(*p*-ethylphenol) is possible, only 40% conversion of *m*-cresol to poly(*m*-cresol) is obtained. However, *m*-cresol conversion can be enhanced to greater than 60% by controlled addition of hydrogen peroxide and HRP (Table 2). Further, ability to control the polymer molecular weight is possible by intermittent separation of the polymer from the reaction mixture and by adjusting the residence time of the reactants in the reactor (Fig. 2). Polymer at any stage can be separated from the reaction mixture in the filtration unit. The filtrate, with some unreacted monomer, can be recycled to the reactor for higher conversion. Using this process, poly(*m*-cresol) at a molecular weight of 1400 is synthesized at 60% monomer conversion (Table 2). A similar process

Table 2
Effect of ethanol content on monomer conversion and the molecular weight of poly(*m*-cresol) in ethanol/water system; polydispersity in all cases was >2.5

Solvent composition	Monomer conversion	Polymer M_w	Comments
100% buffer	40%	2200	Polymer removed once at the end; 60% conversion possible when HRP and H_2O_2 added in pulses
100% buffer with 1% KCl	40%	1400	Polymer removed as it formed; 60% conversion possible when HRP and H_2O_2 added in pulses
80/20 water/EtOH	90%	22000	M_w of 6000 to 22 000 possible at intermediate stages of reaction
60/40 water/EtOH	47%	10000	Lower molecular weights possible at intermediate stages of reaction
40/60 water/EtOH	11%	3000	90% conversion and 24 000 M_w at 5× enzyme concentration
20/80 water/EtOH	3%	–	20% conversion and 2000 M_w at 5× enzyme concentration
100% EtOH	0%	–	Inactive enzyme

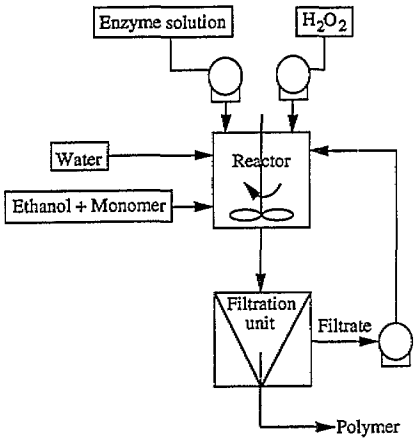

Fig. 2. A process flow sheet for the production of phenolic polymers in ethanol–water mixtures.

scheme can be designed for polymer synthesis in reversed micelles.

Structural characteristics of the polymers are analyzed using thermal and spectroscopic techniques. Earlier results from ^{13}C-NMR studies on poly(*p*-ethylphenol) synthesized in reversed micelles suggest that the repeat units in the polymer are primarily linked through the aromatic rings [6]. A comparison of FTIR spectra for *p*-ethylphenol and its polymer indicates that a significant fraction of unreacted hydroxyl groups are retained in the polymer as manifested by the presence of a strong peak for O–H stretch at *ca* 3400 cm^{-1} in the polymer spectrum (Fig. 3). In addition, both FTIR and ^{13}C-NMR spectra provide no evidence for the presence of ether linkages in the polymer structure. In light of the results from the spectroscopic studies, the polymer, therefore, is not expected to be significantly cross-linked. However, thermal curing of the polymer has been found to result in cross-linking [6]. Poly(*p*-ethylphenol) samples are heated to 200 °C for different periods of time under controlled conditions, and its effect on polymer molecular weight is studied. GPC results indicate that the polymer molecular weight increases up to 25-fold after 4 h of curing (Fig. 4(b)). Higher molecular weight fractions in the cured sample are not soluble in the GPC solvent (i.e., 1% LiBr/DMF solution). Solubility of

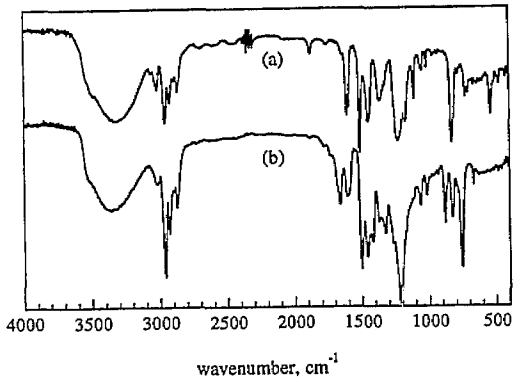

Fig. 3. FTIR spectra of (a) *p*-ethylphenol and (b) poly(*p*-ethylphenol).

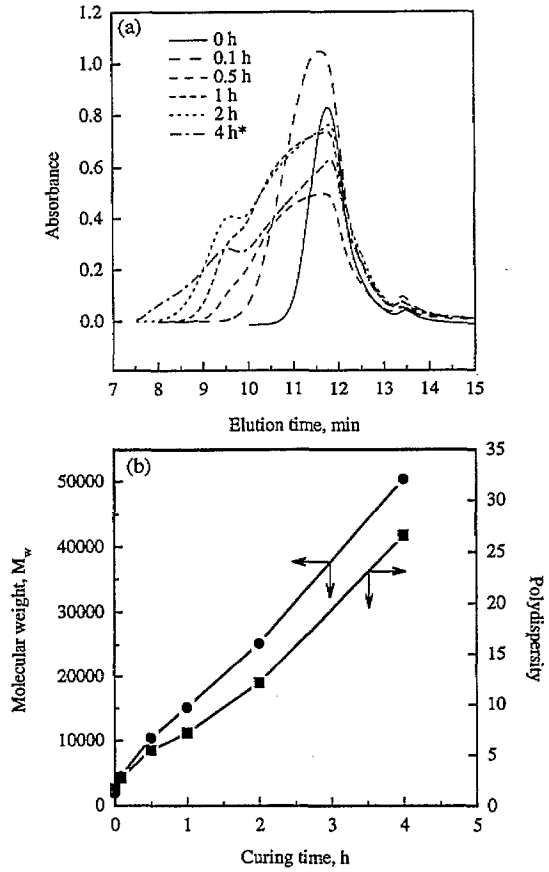

Fig. 4. GPC profiles of poly(*p*-ethylphenol) cured at 200 °C (* = sample partially soluble in GPC solvent). Molecular weight and polydispersity of poly(*p*-ethylphenol) as a function of curing time at 200 °C.

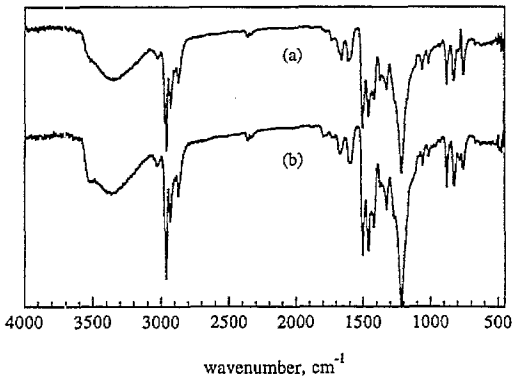

Fig. 5. FTIR spectra of poly(*p*-ethylphenol) (a) before and (b) after curing.

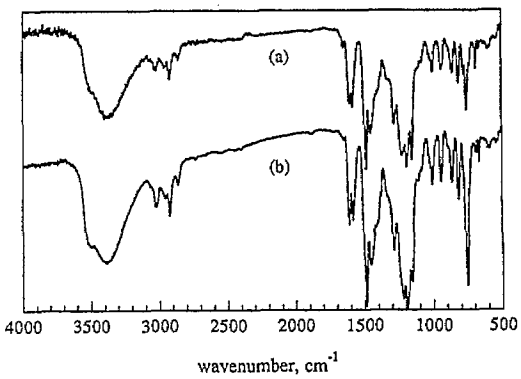

Fig. 6. FTIR spectra of poly(*m*-cresol) (a) before and (b) after curing.

the cured samples in the GPC solvent is found to decrease with increase in curing time, and after 8 h of curing at 200 °C the polymer is found to be completely insoluble in GPC solvent. It is apparent from the GPC profiles that the polymer becomes significantly more polydisperse on curing (Fig. 4(a)). Interestingly, the cross-linking due to curing appears to be on the aromatic ring. FTIR studies carried out on a sample cured for 2 h reveal that a significant fraction of the hydroxyl groups is intact (Fig. 5). In addition, an examination of the O–H stretch region (3100–3700 cm^{-1}) suggests the presence of two populations of OH groups. This is more prominent in the cured polymer than in uncured polymer. The O–H stretch of hydroxyl groups that are involved in hydrogen bonding is characterized by a broad peak centered at *ca* 3400 cm^{-1}. A weak shoulder at *ca* 3600 cm^{-1} in the uncured polymer characterizes the presence of some hydroxyls that are weakly hydrogen bonded. The latter population is seen to increase as the polymer is cured, as manifested by the increase in 3600 cm^{-1} to 3400 cm^{-1} peak height ratio. If cross-linking were to occur on phenyl rings due to curing, some of the hydroxyls would be rearranged in the cured sample leading to the breakage of some of the hydrogen bonds. As a result, there may be a shift in population balance of hydroxyls seen in Fig. 5. Similar observations are made with poly(*m*-cresol) whose molecular weight is found to increase only marginally by about 25% on curing (Fig. 6). The increase in relative intensity of the band at *ca* 750 cm^{-1} in the cured sample reflects on the multiple substitutions on aromatic rings.

While thermal cross-linking in poly(*p*-ethylphenol) synthesized in reversed micelles is evident from the GPC analysis, it is not clear if the exotherm at about 120 °C in the differential scanning calorimetry (DSC) thermogram for the polymer is due to cross-linking or heat of crystallization or both (Fig. 7). The exotherm is absent in the second heating, and the glass transition temperature, T_g, is apparent at *ca* 80 °C. There is no further change in thermal profile in subsequent cycles of heating. As a comparison, a DSC thermogram is shown in Fig. 7 for a sample of poly(*m*-cresol) synthesized in ethanol–water system. The flat profiles for the sample during first and second cycles of heating indicate thermal stability of the polymer.

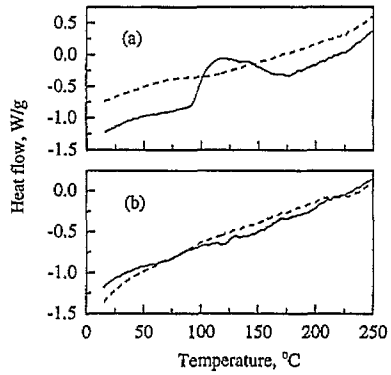

Fig. 7. DSC thermograms of (a) poly(*p*-ethylphenol) (b) poly(*m*-cresol). Solid line for first heat and dashed line for second heat.

Thermogravimetric analysis (TGA) of the samples show that poly(*p*-ethylphenol) is thermally stable even without curing. There is a weight loss of *ca* 5%, presumably due to the loss of moisture, when the sample is heated to 200 °C and an additional 5% when heated to 300 °C (Fig. 8). While the cured polymer exhibits no weight loss up to 250 °C and 5% loss at 300 °C, there is no significant improvement in stability considering the 5% loss during the curing. The availability of the hydroxyl groups for chemical derivatization and the enhanced thermal stability on curing may indicate utility for the polymer in high temperature applications.

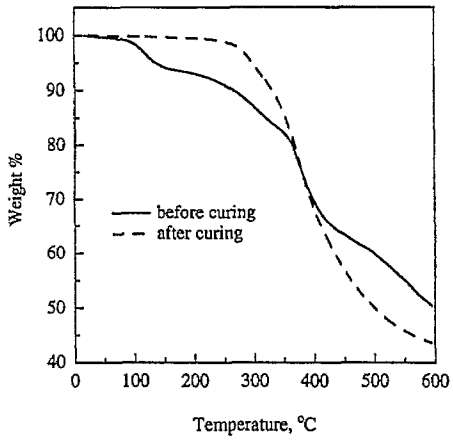

Fig. 8. TGA profiles of poly(*p*-ethylphenol) before and after curing.

4. Conclusions

HRP-catalyzed polyphenol synthesis is carried out in solvent–water mixtures and AOT–isooctane reversed micelles. Dependence of polymer molecular weight and polydispersity on solvent composition is demonstrated. Thermal analysis of polyphenols shows that the polymers are stable up to at least 300 °C. Thermally cured samples of poly(*p*-ethylphenol) show significant cross-linking in their structure. However, the hydroxyl groups in the cured polymer are intact, as confirmed by FTIR spectroscopy. Thus functionalization is still possible on cured polymers for applications requiring thermal stability. Poly(*m*-cresol), on the other hand, exhibits only a marginal increase in molecular weight on curing.

Acknowledgements

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