Feature Article

Enzyme-mediated polymerization reactions: peroxidase-catalyzed polyphenol synthesis

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Peroxidase-catalyzed phenol polymerization in the presence of hydrogen peroxide has been extensively studied. The process offers many advantages over the conventional chemical process to produce phenol-based resins. The enzymatic process avoids the use of formaldehyde and results in minimal formation of by-products due to high specificity of the enzyme toward the reactants. A number of phenolic derivatives and aromatic amines can be polymerized in different reaction systems, such as bulk solvents, reversed micelles and Langmuir trough. The polymers produced exhibit morphology reflective of the templating effect of the medium used to prepare the materials. Polymer molecular weight and polydispersity can be controlled by suitable design of the reaction medium. Polymer characteristics such as structure, thermal and temporal stability, functionalization and processability are reviewed. Conductivity, nonlinear optical and related functional properties are discussed with regard to potential applications.

1. Introduction

The annual production of phenolic resins in the US for the year 1995 is projected to be about 3.2 billion pounds [1], which represents over 50% of the world production. The production and sales of the phenolic resins have been on the rise consistently over the past few years in line with the widespread utility of these materials. Phenolic resins are primarily used as bonding and adhesive materials [2]. The resins are also used in certain advanced fields such as electronics and aerospace applications.

Phenol-formaldehyde resins are commercially synthesized by acid- or base-catalyzed chemical reactions [2, 3]. Phenol or phenol derivatives are polymerized with an aldehyde, most commonly formaldehyde, to obtain a branched polymer. The reaction is exothermic and the phenolic resins produced are generally thermosetting in nature. Novolacs, one type of phenol-formaldehyde resin used commonly in photoresists, are produced in the presence of acid catalysts such as sulfuric acid and oxalic acid with a formaldehyde/phenol molar ratio of 0.5 to 0.8. Divalent metals are used as neutral catalysts to produce novolacs with high content of ortho-linked resins, while alkali catalysts such as sodium hydroxide and calcium hydroxide catalyze the synthesis of resoles, another type of phenol-formaldehyde resin, with formaldehyde/phenol molar ratios between 1.2 and 3.0. In these different types of phenolic resins, substitution at para or ortho on the aromatic ring is dictated by the type of catalyst and pH of the reaction medium. Molecular weights ranging from 500 to 5000 are possible [2, 3].

Commercial preparation of phenolic resins is carried out in a batch process in carbon steel or occasionally glass-lined reactors [3]. Temperatures during the reaction are as high as 100 °C at atmospheric pressures. Water is circulated in jackets to remove the heat generated due to the exothermic reaction. Excess water is removed from the product by heating to about 140 °C under vacuum. Monomer conversions run as high as 95% in the chemical process to produce phenolic resins. However, the reactions involve extremes of pH, toxic starting materials and excessive heat inputs to be regulated, resulting in high operational costs. Most importantly, toxicology studies on formaldehyde have prompted the Occupational Safety and Health Administration (OSHA) to regulate its use in the US industry. While phenol is designated as a questionable carcinogen (class III), formaldehyde is a confirmed carcinogen (class I) [4], and it is recommended that the use of formaldehyde in industry be phased out. Therefore, alternative technologies are desirable to produce phenolic resins without the use of formaldehyde. There are chemical and biological catalysts that can catalyze oxidative coupling of phenol and its derivatives in the absence of formaldehyde. Inorganic catalysts are known to catalyze the coupling of certain phenolic monomers in the presence of oxygen to form aromatic ethers [5]. A typical catalyst is composed of a copper halide and aliphatic amine(s). Phenol and a number of ortho-substituted phenols can be polymerized to form a variety of high melting poly(1,4-phenylene oxide)s. Since the monomers are linked by ether linkages, there is no conjugation along the polymer backbone, which makes the polymers unsuitable for electronic/photonics applications.

Polymer production by biological catalysts has long been known and forms the basis for macromolecules in the biosphere, including cellulose, chitin, lignin and rubber among many others [6–11]. These biochemical reactions can be carried out at mild conditions with minimal by-product formation due to the high specificity of the biocatalysts. For example, Biopol, a commercially available polyhydroxyalkanoate, is produced on a large scale from the bacterium *Alcaligenes eutrophus* [9]. Oligo and polysaccharides have been synthesized from activated sugars in the presence of cellulase or amylase [10, 11]. Lipase-catalyzed ring-opening polymerization of ε-caprolactone and other macrolides, and polycondensation of hydroxy fatty acids with secondary hydroxyl groups have been demonstrated [7, 12, 13]. In addition, combined chemical and enzymatic (or chemoenzymatic) reactions have also been used to produce optically active poly(acrylamide)s [14].

The biosynthesis of lignin can also be mimicked in vitro to synthesize lignin-like products. These synthetic reactions are catalyzed by laccases (with molecular oxygen as electron acceptor), peroxidases (with hydrogen peroxide as electron acceptor) and tyrosinases (with molecular oxygen as electron acceptor) [15]. Of these phenoloxidases, peroxidase extracted from the roots of horseradish is by far the most studied enzyme for its structural and functional properties because of its availability in pure form, catalytic effi-

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ciency, wide substrate specificity, and low cost. There are a few types of peroxidases, such as soybean peroxidase and chloroperoxidase, that also catalyze polymerization of phenol and phenol derivatives, but we will limit our discussion in this paper to horseradish peroxidase (HRP) catalyzed reactions. The important feature of these biocatalytic reactions is that formaldehyde is not necessary to polymerize phenol, its derivatives or aromatic amines. The reactions can be carried out at ambient conditions of temperature and pressure, and no heavy metal catalysts are needed for the reaction. There is minimal formation of byproducts due to the high specificity of the enzyme toward the reactants. The reaction rates, although strongly dependent on the type of the monomer, are relatively fast with HRP. In addition, the molecular weight and polydispersity of the polymers can be tightly controlled, as can the polymer composition based on the monomer types used in the reaction [16, 23]. Polymer composition can also be controlled by varying the stoichiometry of different monomers prior to their copolymerization [21]. In some cases, the biocatalyst is recyclable, which is an important factor in economic analysis of the process. The polymers produced by enzymatic process lack methylene bridges that are present in the phenol-formaldehyde resins, and the effect of this change in structure on the functional properties is under study [16].

Phenols and aromatic amines have been known to be oxidatively coupled using hydrogen peroxide in the presence of HRP [17, 18]. *p*-Cresol was oxidized in aqueous systems to produce, among other products, dimers and trimers [17]. A number of other phenols and aromatic amines were also oxidized and the products were identified and characterized [18]. However, limited solubility of the starting materials and the final products in aqueous reaction systems restricted the formation of higher molecular weight oligomers or polymers. Nevertheless, studies continued on the oxidative coupling of phenols and aromatic amines in aqueous media by laccases, peroxidases and tyrosinases from soil microorganisms [15].

With the advent of nonaqueous enzymology [19], limitations on monomer and polymer solubility were overcome [20]. A wide range of phenolic polymers have been prepared in mixtures of water and water-miscible organic solvents such as 1,4-dioxane, dimethylformamide (DMF), ethanol and tetrahydrofuran [20–26]. It is now possible not only to produce higher molecular weight oligomers and phenolic polymers, but also to control the polymer molecular weight and polydispersity by proper design of the reaction medium [23, 27]. Phenol polymerization has been carried out in other types of reaction media as well. For example, poly(*p*-ethylphenol) has been polymerized by HRP and laccase at the oil–water interface of water-in-oil microemulsions (reversed micelles) [28]. Likewise, a monolayer of *p*-tetradecyloxyphenol has been prepared at the air–water interface of a Langmuir trough and polymerized by HRP in the presence of hydrogen peroxide. Both the reversed micelles and the Langmuir trough act as three- and two-dimensional templates, respectively, for the organization of the monomer prior to polymerization [29, 30]. The effect of this templating on control of polymer morphology and macromolecular architecture will be discussed later. A biphasic system comprising water and water-immiscible organic solvent, such as chloroform or hexane, is also suitable for the polymerization reactions [16]. The enzyme remains soluble in the aqueous phase while the hydrophobic monomer remains largely in the organic phase. A dynamic macroemulsion can be created by vigorous stirring of the two phases. Upon addition of hydrogen peroxide the reaction takes place at the oil–water interface. Since no surfactant is involved in creating the emulsion, the product is free from contamination.

Enzymatic polymerization of phenols and aromatic amines in mixtures of water-miscible solvents and water was first reported by Klibanov and co-workers [20] and Pokora and Cyrus [31]. Aniline, phenol and a number of phenol derivatives were polymerized by HRP in mixtures of 1,4-dioxane and acetate buffer at different compositions [20]. Highest polymer yields were reported in 85% dioxane with molecular weights in excess of 25 000. Enzymatically prepared phenolic polymers exhibited nine orders of magnitude lower electrical resistivity than phenol-formaldehyde resins produced by chemical methods [20]. Phenolic polymers were enzymatically prepared in ethanol/water mixtures and evaluated as recording materials [31]. A number of patents were subsequently issued on biocatalytic processes to produce phenolic polymers for photoresists and to purify commercially available phenolic resins by removing phenol monomers [31–35].

Enzyme kinetics were analyzed in HRP-catalyzed oxidation reactions of *p*-cresol in mixtures of dioxane or methanol with water [36]. It was observed that the catalytic turnover number (k_{cat}) and the Michaelis constant (K_m) increased in the solvent mixture as compared to the aqueous system. The effect of organic solvents on the structure and function of HRP was analyzed using circular dichroism and electron paramagnetic resonance techniques [37]. The active-site structure of the enzyme is retained although it is exposed to organic media. It was emphasized that the solvent–substrate interactions should be considered in kinetic analysis [37]. HRP was also used to modify lignin by copolymerization with *p*-cresol or by oxidation in the presence of veratryl alcohol in nonaqueous media [38, 39]. However, whether lignin by itself can be degraded in the presence of HRP or milk lactoperoxidase is still unclear [40, 41]. Lignin degradation in the presence of lignin peroxidase or manganese peroxidase was reported [42, 43]. High molecular weight polymers from unsubstituted phenol were prepared in bulk solvents using HRP [26]. In addition to bulk solvents, phenol polymerization was carried out with *p*-alkylphenols in water-in-oil microemulsions [23, 28, 44, 45]. Rapid reaction rates and near-complete conversions were reported in these microemulsions. The surfactant-induced organization of the polymer chains into spherical particles was observed at various compositions of the reaction mixture [46]. Iron oxide particles were encapsulated into these polymer spheres, and they were found to be superparamagnetic [47].

The influence of molecular weight and polydispersity of phenolic polymers on their applications for electronics, photoresists and polymer batteries is a continuing area of focus [16, 23, 27]. Options for functionalization of the polymers to enhance optical properties and processability continue [23]. We will review in the following sections our recent work on HRP-catalyzed polymerization of phenols and aromatic amines in different reaction systems.

2. Discussion

HRP (EC 1.11.1.7) is a 44 kDa hemoprotein with 21% carbohydrate content [48]. The active site of HRP is very similar to those of other plant and fungal peroxidases [49,

50]. The enzyme maintains its activity over wide range of pH, temperature, and salt concentrations, which make HRP a useful enzyme in materials synthesis. Phenol polymerization in the presence of hydrogen peroxide occurs by the following generally accepted mechanism [51, 52].

$$\text{HRP} + H_2O_2 \longrightarrow \text{Compound I}$$
$$\text{Compound I} + AH_2 \longrightarrow \text{Compound II} + AH\cdot$$
$$\text{Compound II} + AH_2 \longrightarrow \text{HRP} + AH\cdot$$
$$AH\cdot + AH\cdot \longrightarrow AH\text{-}AH$$

Two electrons are extracted from the heme group by hydrogen peroxide to form compound I. The enzyme regains these electrons in two single-electron steps, producing two free radicals in the process. The free radicals may then combine to form a dimer. The formation of compound I is facilitated by His 170, Arg 38 and His 42 in the active site of HRP. The formation of the intermediate compounds and kinetic mechanism of HRP are well understood [50, 53], and there is experimental data available in the literature on the kinetics of oxidation of phenolic monomers in nonaqueous systems [23, 25, 36]. In this paper we will discuss the synthesis of a few phenolic polymers in different systems, along with a review of the characterization and potential applications of some of the polymers.

3. Synthesis

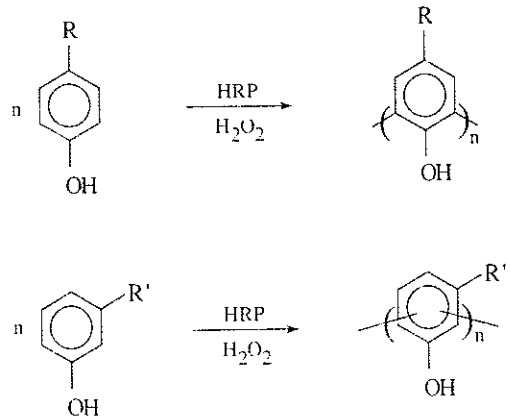
HRP-catalyzed polyphenol synthesis in the presence of hydrogen peroxide follows a step-wise free radical polymerization with a 1:1 stoichiometry between phenol and peroxide [28]. The enzyme kinetics are strongly influenced by the nature of the monomer and the solvent [37]. For example, phenol is one of the least reactive monomers. Likewise, ortho substituted phenols such as *o*-cresol and *o*-methoxyphenol are less reactive than the corresponding para and meta substituted phenols [25]. The solvent and substrate hydrophobicities play a crucial role in partitioning the substrate into the enzyme active site and thereby affecting the enzyme-substrate dissociation constant, K_m. Although the presence of hydrogen peroxide is required to initiate the reaction, it inhibits the enzyme at sufficiently high concentrations [28, 54]. It is therefore necessary to maintain a low molar ratio of peroxide to enzyme in the reaction mixture by adding hydrogen peroxide in small aliquots. Controlled addition of the peroxide is especially important with less reactive monomers, which therefore consume the peroxide at a slower rate [16, 28, 31]. The reaction is exothermic. An estimated 40 kcal of heat is released under adiabatic conditions per mole of *p*-ethylphenol oxidized in AOT/isooctane reversed micelles at 100% monomer conversion [44].

The discussion of the enzymatic synthesis of phenolic polymers is divided into (a) reactions in bulk solvent and (b) interfacial synthesis. Interfacial synthesis includes reactions carried out at (i) the oil-water interface of reversed micelles, (ii) oil-water interface in a biphasic system and (iii) the air-water interface of a Langmuir trough.

3.1. Reactions in bulk solvents

Enzyme-mediated phenol polymerization is often carried out in mixtures of water and water-miscible organic solvents such as DMF and 1,4-dioxane. Klibanov's group was the first to show that HRP is active in a number of organic solvents, and that phenol polymerization is feasible in water/1,4-dioxane mixtures [20, 55]. Subsequently, a number of phenolic monomers and aromatic amines have been polymerized and characterized [21, 24, 31]. Detailed characterization of polymer products from synthesis in water/dioxane mixtures has been reported by Akkara et al. [21]. The structures of some of the homo- and co-polymers generated from phenols and aromatic amines were characterized with the help of a variety of spectroscopic and thermal techniques. Kobayashi and co-workers [24] have also polymerized phenol and reported relatively high molecular weights for DMF-soluble fractions of the polymer. One objective in synthesizing phenolic polymers has been to evaluate their nonlinear optical properties. Due to the extensive backbone conjugation determined from the structural characterization of the materials [21], these polymers have potential application as nonlinear optical materials [56]. Applications in photoresists for electron-beam lithography are also possible [57, 58]. With the latter application in mind, we developed methods to control the molecular weights of various phenolic polymers by suitable solvent design to obtain low molecular weight materials with narrow polydispersity [16, 23].

To generate low molecular weight polyphenols with narrow polydispersity, three phenolic monomers viz., *p*-ethylphenol, *p*-cresol and *m*-cresol were used (Fig. 1). The solvent systems (Table 1) were selected on the basis of the range of solubility parameters covered by their mixtures in water [59]. These solubility parameters span approximately the same ranges covered by supercritical fluids through the manipulation of pressure [60]. The important difference is that we can achieve the same variation in solubility parameters at ambient conditions of pressure and temperature. The polymerization of *p*-ethylphenol was initially carried out in DMF/water mixtures. In general, the enzyme was active up to 85% DMF, and was able to produce oligomers up to the size of hexamers (Table 2). The highest molecular weight observed with poly(*p*-ethylphenol) in any reaction medium was about 2500 to 3600, a degree of polymerization (DP) of 20-30 [23]. Higher molecular weight polymer is difficult to produce with *p*-ethylphenol since there is little opportunity for branching as the para position on the



$R = CH_3$ or C_2H_5, $R' = CH_3$

Fig. 1. Reaction scheme for phenol polymerization.

Table 1. Ranges of solubility parameters and dielectric constants covered by the solvent systems used for polymerization reactions.

Solvent system	Solubility parameter [$MPa^{1/2}$]	Dielectric constant
Isooctane/chloroform	14–19	2– 5
DMF/water	25–48	37–78
1,4-Dioxane/water	20–48	30–78
Ethanol/water	26–48	24–78

monomer is blocked by the ethyl group. The monomer units in the oligomers formed are linked primarily via ortho–ortho couplings, as confirmed by NMR studies [23]. As a result, the chain growth is only from the two free ends, and the polymer is likely to be coiled. This is in contrast to polymers synthesized from phenol or *m*-cresol, which exhibit high molecular weights since both the para and ortho positions are available for reaction and branching [16, 24].

Polymerization of *m*- and *p*-cresols was carried out in ethanol-HEPES (*N*-[2-hydroxyethyl]piperazine-*N'*[2-ethanesulfonic acid]) buffer systems to study the control of molecular weight. *m*-Cresol was studied in greater detail since a much broader molecular weight range can be characterized than with *p*-cresol. Ethanol is a solvent of choice in view of its environmental compatibility and ease of regeneration. In addition, the enzyme is active in ethanol/buffer mixtures at levels up to at least 60% ethanol [25, 27, 32]. Enzyme activity was studied as a function of ethanol content, and it was found that 20–40% ethanol was optimal for the conversion of about 50% monomer into polymer (20 000 molecular weight, DP of 200) [27]. At an enzyme concentration of 2 μM, the conversion was essentially complete in about 10 min. Although there was no significant improvement in the monomer conversion when the reaction was continued for 24 h, the molecular weight of poly(*m*-cresol) increased by about 75% (Table 3). Ethanol appears to be a useful solvent when higher molecular weight polymer is desired. Replacing HEPES buffer with deionized water resulted in no noticeable change in reaction rates or in polymer properties.

Although high molecular weight polymers were produced in ethanol with *m*-cresol due to branching, in some applications, such as photoresists, oligomers are desirable [61]. Hence, *m*-cresol was polymerized in 100% buffer/water, and as a result, the polymer molecular weight decreased to 2500. During the reaction the polymer molecular weight gradually increased without significant improvement in monomer conversion. We therefore attempted to isolate the polymer as the polymerization process continued. This was achieved by carrying out the reaction in the presence of 0.5 to 1 (w/v)% KCl in the medium. While the salt, up to around 3%, had no effect on the enzyme activity, it caused precipitation of the polymer as it formed. The polymer precipitate was isolated by filtration and the filtrate was returned to the reaction vessel. The enzyme in the filtrate was no longer active, therefore, enzyme and peroxide were added in pulses such that fresh enzyme was always available after each filtration to assure continued polymer formation. However, the rate of polymerization dropped as the reaction continued after each filtration in spite of the presence of significant amounts of the residual monomer, fresh enzyme, and hydrogen peroxide. The polymer yield was about 40% after completely adding all the enzyme and hydrogen peroxide to a final concentration of 2 μM enzyme and 30% stoichiometric excess of the peroxide. The polymer molecular weight was about 1400. Further polymerization was still possible in the remaining reaction mixture if fresh enzyme and peroxide were added. Therefore, the polymerization process could be continued, in theory, until all monomer was consumed. When the polymer was not removed by filtration and the reaction was run with the identical additions of enzyme and hydrogen peroxide, the final polymer molecular weight was over 2000. The polydispersity was

Table 2. Effect of DMF content on monomer conversion and the molecular weight of poly(*p*-ethylphenol) in DMF/water systems.

Synthesis medium	Monomer conversion	Polymer yield	M_w	M_w/M_n	Comments
100/0 DMF/water	0%	0%	–	–	no reaction
85/15 DMF/water	20%	10%	281	1.23	dimers soluble in 85% DMF
60/40 DMF/water	80%	75%	612	1.20	oligomers soluble in 60% DMF
40/60 DMF/water	80%	80%	675	1.05	oligomers soluble in 40% DMF
20/80 DMF/water	75%	75%	658	1.02	oligomers soluble in 20% DMF
0/100 DMF/water	50%	35%	400	1.90	oligomers soluble in 100% DMF

Table 3. Effect of ethanol content on monomer conversion and the molecular weight of poly(*m*-cresol) in ethanol/water systems. Polydispersity in all cases was > 2.5.

Solvent composition	Monomer conversion	Polymer M_w	Comments
100% buffer	40%	2 200	polymer removed once at the end; 60% conversion possible when HRP & H_2O_2 added in pulses
100% buffer with 1% KCl	40%	1 400	polymer removed as it formed; 60% conversion possible when HRP & H_2O_2 added in pulses
80/20 water/EtOH	90%	22 000	M_w of 6 000 to 22 000 possible at intermediate stages of reaction
60/40 water/EtOH	47%	10 000	Lower molecular weights possible at intermediate stages of reaction
40/60 water/EtOH	11%	3 000	90% conversion & 24 000 M_w at 5 × enzyme concentration
20/80 water/EtOH	3%	–	20% conversion & 2000 M_w at 5 × enzyme concentration
100% EtOH	0%	–	Insoluble enzyme

about 2.5 in both cases, whether the polymer was removed intermittently or not.

It is thus possible to control the polymer molecular weight by manipulating the reaction and/or process conditions. In the case of poly(*m*-cresol), we have been able to obtain control of molecular weight from 1400 to 25 000, with a polydispersity of about 2.5, by reaction medium choice. Enzyme activity is a function of the reaction medium composition which influences monomer conversion. However, the polymer molecular weight is also strongly influence by the polymer solubility and the length of time it is in contact with the enzyme and reacting species in the reaction mixture, even after precipitation.

Water-immiscible solvents, such as hexane and benzene, are not suitable for enzyme-mediated phenol polymerizations due to the poor dispersion of the enzyme powder. In addition, hydrogen peroxide is also immiscible with these solvents and tends to be heterogeneous in distribution. This can inhibit enzyme activity. Although small amounts of polymer form, the overall process is very inefficient. To overcome these practical limitations, HRP can be covalently modified with polyethylene glycol to create an organic solvent soluble enzyme for homogeneous catalysis [66].

3.2. Interfacial reactions

Three interfacial systems, reversed micelles, the Langmuir trough and a biphasic system, have been studied. Our initial interest in these systems had been to study the feasibility of phenol polymerization in highly ordered structures [28, 29, 44, 63] in the anticipation that, by enhancing control of macromolecular architecture, the functional attributes for the polymer can be optimized for specific applications.

3.2.1. Reversed micelles

Reversed micelles are nanometer-sized water droplets suspended in hydrophobic solvents such as isooctane with the help of a surfactant. The most commonly studied reversed micellar system is a ternary system composed of isooctane/water/sodium dioctylsulfosuccinate (an anionic surfactant commercially known as AOT) [44]. Since a number of hydrophiles, including enzymes, can be encapsulated in the reversed micelles, a variety of enzyme-catalyzed reactions can be carried out in this pseudo-homogeneous phase. In addition to excellent dispersion of the enzyme, reversed micelles provide enormous interfacial area, providing an ideal medium for interfacial reactions. Although HRP activity was studied in reversed micelles [64, 65], reports on phenol polymerization are rather recent [28, 44, 46].

Substituted phenols such as *p*-ethylphenol have poor solubility in water and partition to the oil–water interface when added to a reversed micellar solution (Fig. 2). The monomer acts as a cosurfactant based on the dramatic increase in droplet size on adding sufficient *p*-ethylphenol [28]. When hydrogen peroxide is introduced under these conditions, and with encapsulated HRP in the micelles, the reaction proceeds instantaneously. Under appropriate conditions, the reaction rates are so rapid that the monomer is completely converted into polymer within the first few seconds. On losing solubility in isooctane, the polymer drops out of solution and can be easily retrieved. While some enzyme is trapped in the precipitating polymer and is taken out of the reversed micelles, the enzyme remaining in the micelles is still active and fresh monomer and hydrogen peroxide can be added to continue the polymerization. Subsequent to the removal of the polymer, very little surfactant was lost, and the essentially monomer-free solution could be recycled as a fresh reaction medium [44]. Thus a process can be developed for the continuous polymerization of phenol and its derivatives using reversed micelle templates.

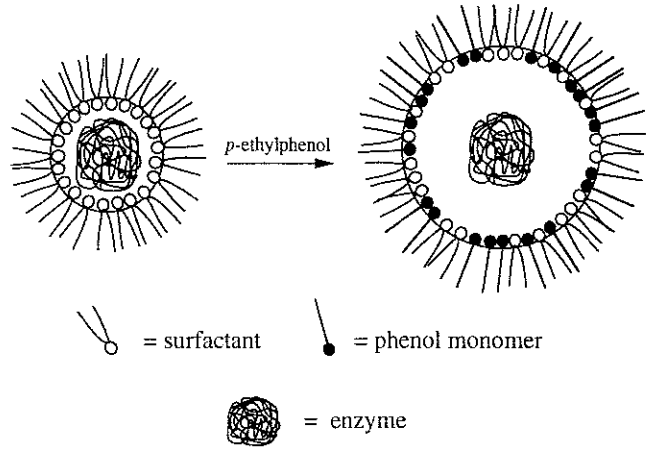


Fig. 2. Schematic of reversed micelle before and after the addition of *p*-ethylphenol.

The poly(*p*-ethylphenol) precipitated from reversed micelles exhibits interesting morphology. For example, with 0.15 M each of AOT and *p*-ethylphenol, and at appropriate water to surfactant molar ratio (which dictates the micellar size), the polymer powder is composed of about 1 μm diameter spheres [44]. A systematic study of the polymer morphology revealed that the polymer spheres always formed with AOT to monomer molar ratios greater than 3 [46]. Further electron microscopic studies revealed that the spheres are hollow, and it is possible to trap enzymes and inorganic particles in them. Accordingly, ferrite paticles were dispersed in the polymer spheres and their superparamagnetic properties characterized [47].

The polymer synthesized with reversed micelles exhibited narrower molecular weight distribution than that

Table 4. Monomer conversion and polymer molecular weight and dispersity in different reaction media.

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

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synthesized in bulk solvents (Table 4) [23]. Molecular weight control of poly(*p*-ethylphenol) by solvent design was also demonstrated in reversed micelles. Mixtures of chloroform and isooctane were used to prepare polymers with molecular weights from 1000 to 3600 [23]. However, poor polymer yield at a higher chloroform content was observed and was due to the formation of an unstable microemulsion and subsequent phase separation. Phenol polymerization was also carried out in reversed micelles of cationic and non-ionic surfactants [16]. The polymers prepared in these systems exhibited similar molecular weights to those of the polymers prepared in AOT/isooctane micelles.

3.2.2. Langmuir trough

The Langmuir trough provides an environment in which orient and organize amphiphilic molecules in a two-dimensional monolayer at the air–water interface. Phenols, derivatized with long alkyl chains, were enzymatically polymerized at this interface (Fig. 3) [29, 63]. A solution of monomer in a volatile solvent such as chloroform is deposited at the air–water interface (with HRP in the subphase) between stationary and mobile barriers on the trough. After the solvent has been allowed to evaporate, the monomers are compressed from gaseous to solid phase by moving the mobile barrier. Introduction of hydrogen peroxide into the subphase initiates the reaction in the monolayer. A reaction is observed within minutes, with the exact time depending on monomer and catalyst. The polymer film can be transferred to suitable substrates for analysis. Attempts to form monolayers with *p*-alkylphenols up to a carbon number 9 were not successful. However, *p*-tetradecyloxyphenol, synthesized from hydroquinone [63], formed a monolayer and could be polymerized only when premixed with unmodified phenol prior to spreading on the subphase. The reaction were carried out at phenol to *p*-tetradecyloxyphenol molar ratios of 1:1 to 500:1. The reaction rates were faster at higher concentrations of phenol. Similar observations were made in the polymerization of *p*-hexadecylaniline in the presence of the comonomer aniline. Polymer structures were studied with FTIR-ATR spectroscopy and mechanistic molecular modeling [29, 63]. An idealized polymer structure had ortho–meta linkages between the monomer units. It should be noted that the alkoxy group on the phenyl ring is also an ortho–para directing group, as is the hydroxy group, and hence linkage via meta positions on the ring is equally possible, unlike with the *p*-alkylphenols. Para–para linkages were not present in the polymer because of the para-substituted phenolic monomers used, thus enhancing control over the polymer structures generated.

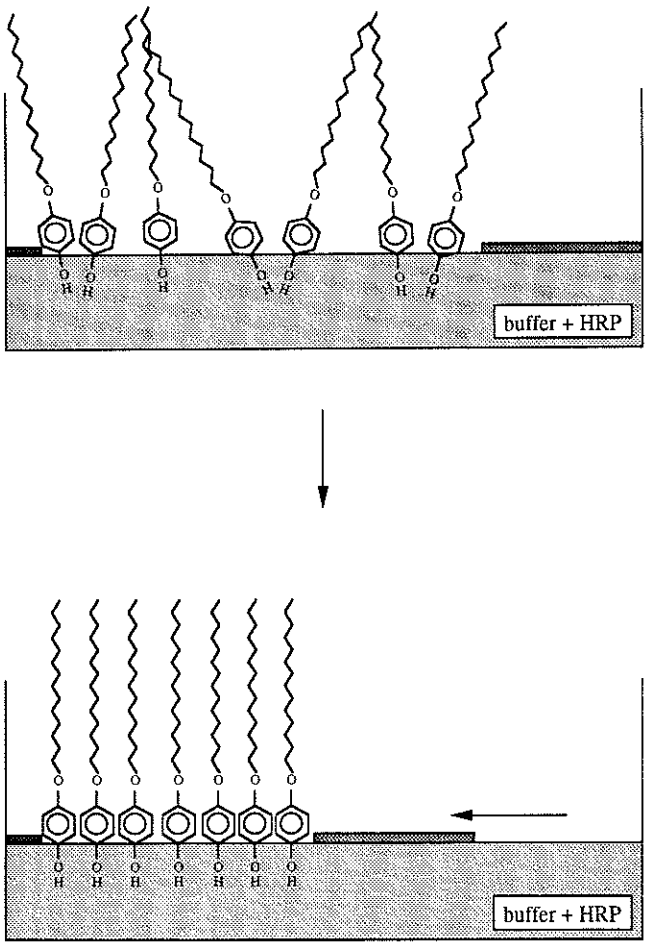

Fig. 3. Schematic of monolayer preparation with *p*-tetradecyloxyphenol on a Langmuir trough.

3.3. Biphasic system

A biphasic system consists of two mutually immiscible phases such as water–isooctane and water–chloroform. For the enzymatic polymerization reactions, equal volumes of HEPES buffer and isooctane or chloroform were used in a reaction vessel equipped with a paddle stirrer positioned at the interface. A baffle was used to break vortex formation. The aqueous phase contained the enzyme and the organic phase contained the monomer. A number of monomers used in this study are largely soluble in the organic phase. A dynamic macroemulsion formed when the two phases were stirred at a constant paddle stirring rate. The phases were stirred for about 15 min to allow equilibrium to be reached before hydrogen peroxide was slowly introduced into the reaction vessel to initiate the polymerization. The reaction was continued for about an hour before the agitation was terminated. As there was no clean separation of the phases, the polymer was recovered by evaporating the phases under a stream of air while maintaining a solution temperature of not more than 50 °C. The recovered polymer was washed with isooctane and water to remove any unreacted monomer and enzyme, respectively.

The biphasic system resulted in near complete conversion of *p*-cresol, *p*-ethylphenol, *p*-isopropylphenol and *p*-butylphenol to their respective polymers. The molecular weight of all polymers was in the range 1500 to 3500 (Fig. 4) with monomers containing longer alkyl chains yielding higher molecular weight. The volume fraction, Φ, of water in the emulsion was observed to effect monomer conversion in the case of *p*-ethylphenol (Table 5). As Φ decreased the monomer conversion decreased. The increased local concentration of hydrogen peroxide in water droplets containing the enzyme may have an inhibitory effect on the enzyme at smaller values of Φ. However, the monomer conversion was high at $\Phi > 0.2$. Thus biphasic systems are another useful reaction medium for interfacial reactions. One immediate advantage of the biphasic system is that there is no need for surfactants, resulting in minimal contamination of the product.

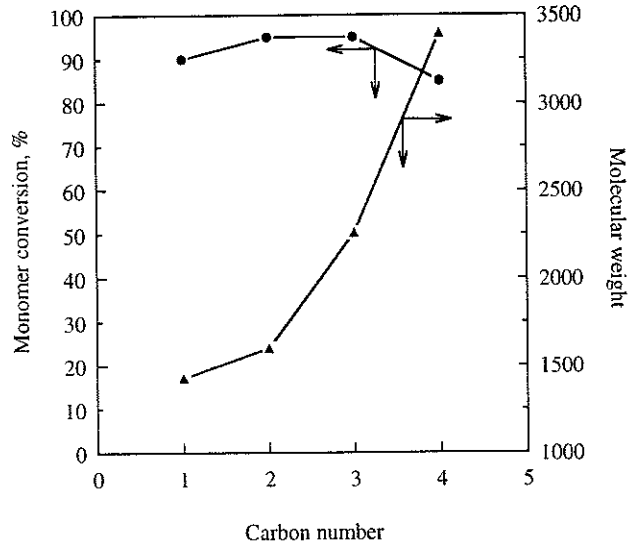

Fig. 4. Monomer conversion and polymer molecular weight in isooctane/water biphasic system as a function of carbon number in *p*-alkylphenol.

Table 5. Effect of water volume fraction, Φ, on the monomer conversion in water/isooctane biphasic system.

Φ	Monomer conversion [%]	Comments
0.00	0	Enzyme poorly dispersed
0.02	50	Polymer easily recovered
0.05	62	Polymer easily recovered
0.20	82	Polymer distributed in two phases as an emulsion
0.33	90	Polymer distributed in two phases as an emulsion
0.50	80	Polymer distributed in two phases as an emulsion
0.67	90	Polymer distributed in two phases as an emulsion

4. Polymer properties

Phenolic polymers synthesized by enzyme catalysis in the presence of hydrogen peroxide were analyzed using optical, thermal, spectroscopic and chromatographic methods to gain insight into their molecular weight, dispersity, structure, thermal stability and processability. In general, the phenolic polymers are thermally stable up to at least 300 °C. Figure 5 shows the differential scanning calorimetry (DSC) and thermogravimetry analysis (TGA) thermograms for poly(*p*-ethylphenol) synthesized in AOT/isooctane reversed micelles. The polymer exhibits a large exotherm centered at about 113 °C in the DSC thermogram. The exotherm may be due to cross-linking of the polymer and/or heat of crystallization. Based on gel permeation chromatography (GPC) analysis, the polymer molecular weight tripled on heating to 150 °C. Poly(*p*-phenylphenol), synthesized by HRP-catalyzed reactions in bulk solvents, was heated to over 600 °C under nitrogen atmosphere to increase the carbon to hydrogen ratio by ring fusion. About 20% of the starting material was recovered as shiny crystals after the heating, and its properties are under analysis for battery electrodes [65]. Most of the phenolic

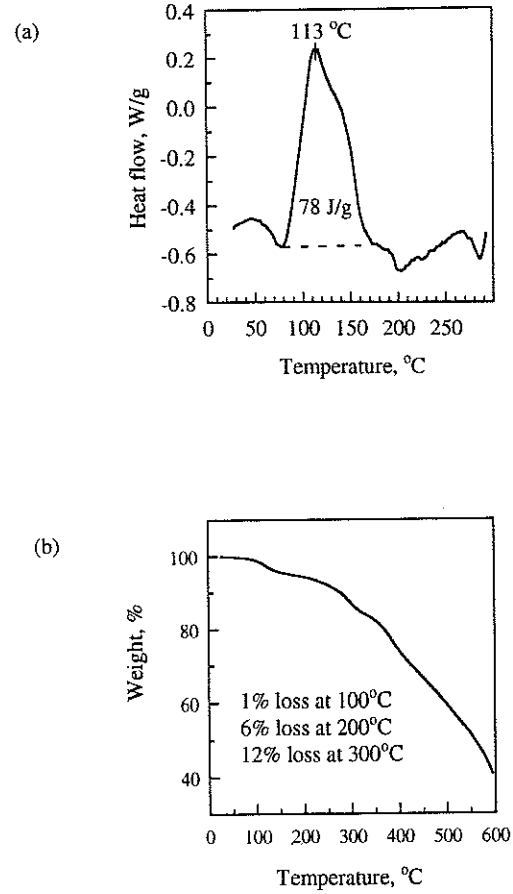

Fig. 5. Thermal characteristics of poly(*p*-ethylphenol) prepared in AOT/isooctane reversed micelles. (a) DSC and (b) TGA thermograms.

polymers are soluble in chloroform, methanol or acetone, thus making it possible to solvent-cast thin films of the polymers. Some of the polymers are also soluble in alkaline solutions (pH > 11) [16], an important requirement for their application as photoresists.

Polymer morphology was studied using electron and atomic force microscopy. In general, the poly(*p*-ethylphenol) synthesized in bulk solvents exhibited irregular shapes as inferred from scanning electron microscopy (SEM) studies (Fig. 6) [44]. On the other hand, polymerization in reversed micelles created polymer spheres (Fig. 7) [44, 46]. Although the phenomenon of polymer aggregation into spheres is not fully understood, the surfactant appears to play a key role in this templating effect. Atomic force microscopy of the polymer film generated on a Langmuir trough indicated that the polymer film exhibited vertical homogeneity as expected from the two-dimensional network at the air-water interface [62]. The polymer film average thickness, measured by ellipsometry, was about 28 Å per layer measured from five deposited layers.

As noted earlier, poly(*p*-ethylphenol) synthesized with reversed micelles exhibited a molecular weight of about 2500 (DP of about 20). As inferred from the ^{13}C-NMR spectra shown in Figs. 8a and 8b, the monomer units appeared to be largely linked via ortho-ortho coupling on the rings. Samples for ^{13}C-NMR were prepared in deuterated acetone. In addition, a comparison of FTIR spectra (Fig. 9) for the monomer and the polymer (cast on KBr windows from solutions of chloroform) indicates that the

Fig. 6. Scanning electron micrograph of poly(*p*-ethylphenol) synthesized in the 85/15 1,4-dioxane/water system.

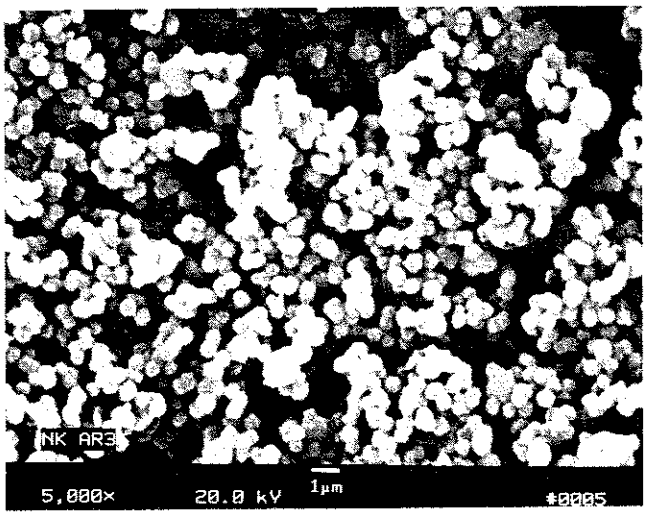

Fig. 7. Scanning electron micrograph of poly(*p*-ethylphenol) synthesized in AOT/isooctane reversed micelles.

polymer structure lacked ether linkages. The presence of the broad peak due to OH stretch at 3300 cm^{-1} in the IR spectrum for poly(*p*-ethylphenol) also shows that the hydroxyl groups in the polymer structure are largely intact. Thus it is possible to modify these hydroxyls to functionalize the polymer for specific applications [23]. Esterification was carried out with acid chlorides to improve processability and to enhance UV absorption of the polymer [23]. It is also possible to esterify the hydroxyl groups with biotinic acid for binding proteins to the polymer backbone for applications in chromatography. Included in Fig. 9 is an IR spectrum for poly(*p*-ethylphenol) derivatized with palmitoyl chloride. Near complete esterification of the hydroxyls can be readily achieved.

Polymer molecular weight is a strong function of solubility in solvent and the monomer structure. For example, molecular weights over 50 000 are possible in reactions with phenol and HRP in the presence of hydrogen peroxide [67]. This is due to the availability of ortho and para positions on the rings for linkages, resulting in highly branched products. If the para position is blocked, as in the case of *p*-cresol and *p*-ethylphenol, the polymer is forced to grow largely via ortho linkages, and maximum molecular weights drop to about 3000. On the other hand, poly(*p*-methoxyphenol) prepared in dioxane/water mixture exhibited a molecular weight of over 50 000, in concurrence with its structure in which all the hydroxyls are involved in ether linkages, resulting in branching [68]. *m*-Cresol, which allows linkage at ortho and para positions, can also be polymerized to molecular weights of about 25 000. Molecular association or aggregation also plays an important role in the determination of molecular weights [23]. DMF or mixtures of DMF and methanol are usually used as GPC solvents to measure molecular weights and dispersities of phenolic polymers [20, 21, 24, 28]. Methanol is added to DMF to break the molecular aggregation in phenolic polymers which otherwise erroneously show high apparent molecular weights. However, even with 20% methanol in DMF/methanol mixtures, the polymer aggregates are not completely disrupted [23]. Inorganic salts such as lithium bromide at about 0.5 (w/v)% in DMF are necessary to completely dissociate the polymer aggregates and provide accurate estimations of molecular weights [23]. It is also possible to avoid molecular aggregation by proper choice of GPC solvent. For example, use of tetrahydrofuran in place of LiBr/DMF provided accurate results for poly(*p*-ethylphenol) synthesized in reversed micelles [69].

The polymers of phenols and aromatic amines synthesized by enzyme catalyzed reactions in various systems have been analyzed for functional properties relative to electronic and optics applications. Polyphenols synthesized on a Langmuir trough exhibited enhanced conductivity in comparison to the polymers synthesized in bulk solvents [62]. The use of polyphenols and polyaromatic amines for their third-order nonlinear behavior in devices such as all-optical modulators and directional couplers is a potential application [70]. Table 6 summarizes the third-order susceptibility ($\chi^{(3)}$) values for some of the enzymatically polymerized products measured by degenerate four wave mixing with frequency-doubled Nd:YAG laser with 17 ps pulses at 532 nm [56]. In general, the values for the polyaromatic amines are superior to the phenolic polymers.

In enzyme-mediated phenol polymerization in bulk solvents and reversed micelles, the reaction time is of the order of minutes to hours with good polymer yields. It is

Table 6. Third order susceptibility ($\chi^{(3)}$) values for various polymers prepared in different reaction systems.

Reaction medium and polymer	$\chi^{(3)} \times 10^{-10}$, esu
Bulk organic solvent (enzymatic)	
Poly(*p*-ethylphenol)	0.68
Poly(*p*-dodecylphenol)	0.54
Poly(*p*-phenylphenol)	1.00
Polyaniline	75.0
Polybenzidine	150.0
Reversed micelles (enzymatic)	
Poly(*p*-ethylphenol)	3.70
Poly(*p*-butylphenol)	4.80
Poly(*p*-octylphenol)	6.00
Polybenzidine	15.0
Langmuir trough (enzymatic)	
Copoly(tetradecyloxyphenol-phenol)	8.80
Chemical synthesis (for comparison)	
Polyaniline	8.00

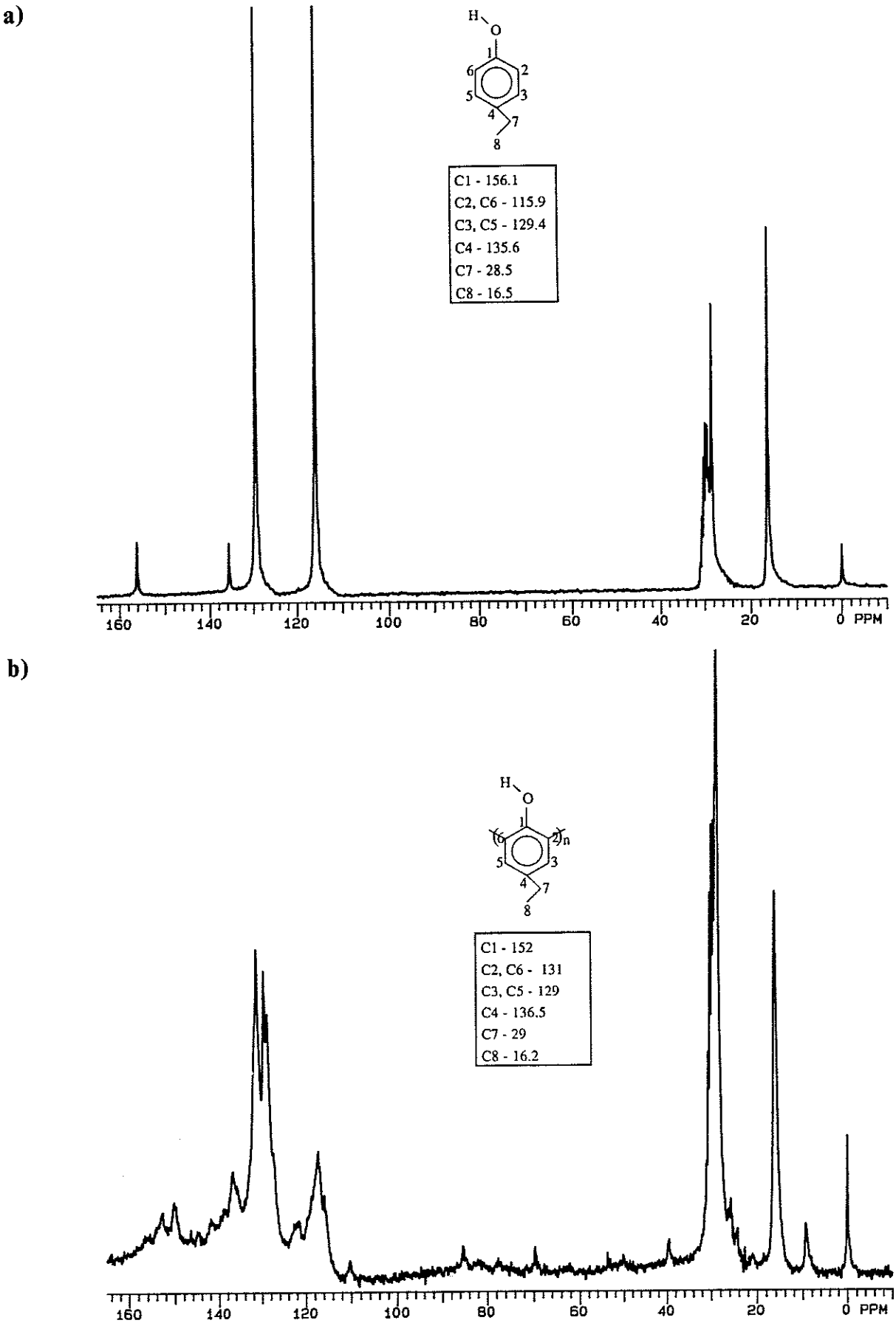

Fig. 8. (a) ^{13}C NMR spectrum of the monomer, *p*-ethylphenol, (b) ^{13}C NMR spectrum of poly(*p*-ethylphenol) prepared in AOT/isooctane reversed micelles.

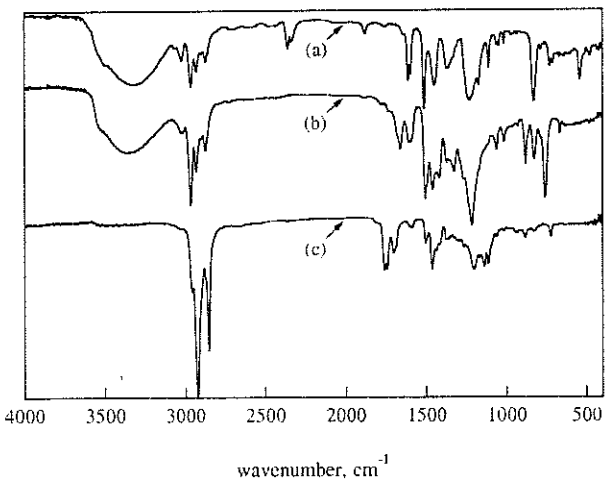

Fig. 9. FTIR spectra of (a) *p*-ethylphenol, (b) poly(*p*-ethylphenol) and (c) poly(*p*-ethylphenol) modified with palmitoyl chloride.

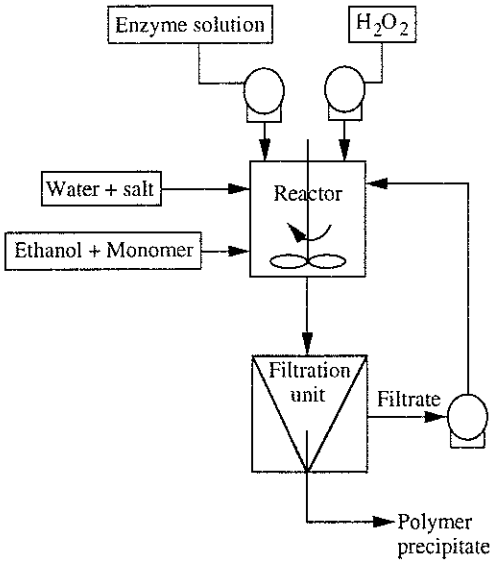

Fig. 10. A process flow sheet for production of phenolic polymers in ethanol/water mixtures.

possible to conceptualize a process scheme to produce these polymers in a batch or continuous mode for commercial-scale needs. Figure 10 illustrates a bulk polymerization process which incorporates recycling of the reaction medium in the case of ethanol/water mixtures. The medium is recycled to enhance monomer conversion as discussed earlier and to minimize the use of solvents and maximize reuse of enzyme.

5. Conclusions

In light of the health hazards posed by formaldehyde, enzymatic approaches for the synthesis of phenolic and aromatic amine polymers is a viable alternative. Inorganic catalysts are also useful in polymerizing phenols in the absence of formaldehyde, but the resultant polymers are poly(phenylene oxide)s, which are electrically inactive. While the economics of the enzymatic process still need to be evaluated, it should be a competitive process in view of small amounts of the biocatalyst required.

We have briefly reviewed the current status of the work reported in the literature with more detailed accounts of our own research. Polymerization is possible in aqueous media, mixtures of water and water-miscible organic solvents, reversed micelles, Langmuir trough and biphasic systems. Polymer morphology is strongly dependent on the reaction system. Polymer synthesized in bulk solvents, in the absence of any additives, is largely irregular in morphology as inferred by SEM. On the other hand, reversed micelles and the Langmuir trough provide three- and two-dimensional templating effects, respectively, to create hollow spheres and two-dimensional thin films. The spherical morphology of the polymer prepared in reversed micelles allows incorporation of a number of additives in the polymer structure. Methods to control polymer molecular weight and polydispersity have been successful. Polymers can also be modified at unreacted hydroxyl groups to improve processability, UV absorption, and other characteristics. Nonlinear optical properties of different polymers synthesized enzymatically in different reaction systems indicate that the third-order susceptibility values are higher for polymers with the ordered structure. Scaled-up production of phenolic polymers can be carried out both in reversed micelles and in bulk solvents.

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