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# Enzymatic Mediated Synthesis of Conjugated Polymers at the Langmuir Trough Air–Water Interface

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An approach for the enzyme-mediated in-situ synthesis of polymers in two dimensions on a Langmuir trough surface is reported. Horseradish peroxidase-mediated polymerization of monomer mixtures of unsubstituted and long alkyl chain substituted phenols and aromatic amines at the air–water interface was demonstrated. The use of the Langmuir trough to organize and orient the reactants results in improved control of monomer reactivity and orientation in the resulting polymer compared to the polymer synthesized in bulk solvent. In turn, this enhanced control results in improved processability because of reduced chain branching. Good thermal stability and significant electronic and optical properties of the ordered polymer were demonstrated. This interfacial polymerization procedure creates opportunities in the development of improved materials for applications in biology, medicine, and microelectronics.

## Introduction

Recently we reported on the synthesis of high molecular weight polymers using substituted phenols with horseradish peroxidase (HRP)-mediated polymerization in dioxane.<sup>1</sup> Poor solubility and high branching of these polymers make it difficult to process these polymers. An approach was sought to overcome the processing limitations while the optical and electronic properties were maintained or enhanced. We report here on enzyme-mediated polymerization at an air–water interface, which accomplishes this goal.

Enzyme-mediated polymerization on the Langmuir trough is also desirable because of the ability to order the monomer at the air–water interface.<sup>2</sup> We have successfully applied this approach for the first time to polymerize monomer mixtures of unsubstituted and alkyl chain substituted phenols and aromatic amines on the Langmuir trough surface by HRP. The resulting polymeric product has a degree of order not seen in the bulk-synthesized material. This rational design of monomers, and their assembly at the air–water interface, to form a polymerizable template is used to produce ordered conjugated macromolecules with interesting electronic and optical properties. These polymers for example, could be useful in the design of semiconducting polymers with tunable band gaps that may be processed in the form of large-area thin films.<sup>3</sup>

The Langmuir–Blodgett (LB) technique is used to assemble surfactant molecules at an air–water interface.<sup>4,5</sup> Polymerization of the assembly into ultrathin films can be catalyzed by UV irradiation, in the case of diacetylene monomers through 1,4-additions of stacked

adjacent monomer units,<sup>6–8</sup> 1,2-additions for 2,4-heptadecadynoic acid,<sup>9</sup> or chemical oxidation with ferric chloride for pyrrole monomers.<sup>10,11</sup> Polymerization of *o*-alkylanilines, on the Langmuir trough, has been recently carried out in the presence of ammonium peroxydisulfate.<sup>12</sup>

## Experimental Section

A Lauda film balance, equipped with a constant temperature bath (Langmuir Filmwaage, Model D, Lauda-Brinkman, Westbury, NY) was used. The surfactant 4-(tetradecyloxy)phenol (C14PP) was synthesized by *O*-alkylation of hydroquinone with 1-bromotetradecane. The 4-hexadecylaniline (C16PA) (Aldrich Chemical Co., Milwaukee, WI) was used as received. Monomers, 1–2 mg/mL, were solubilized in chloroform and spread at the air–water interface. The subphase contained 2 L of 0.85 mM *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES) buffer, pH 7.5, with 12–25 mg of enzyme per liter. HRP (EC 1.11.1.7) Type II, 150–200 units/mg solid was from Sigma Chemical Co. (St. Louis, MO). The surfactant was compressed with a 1.0 Å<sup>2</sup> mol<sup>−1</sup> min<sup>−1</sup> rate to a surface pressure of 15 mN/m prior to polymerization. Once the monomer at the air–water interface was assembled and compressed, hydrogen peroxide (0.30 mL of 3.0% in water) was injected into the aqueous subphase containing HRP to initiate the polymerization process.

The reaction progress could be assessed visually by a significant decrease of the interfacial area within minutes. Reactions were conducted at constant pressure and were terminated after 12 h. The subphase containing HRP was slowly pumped out and replaced with fresh MilliQ water. Free radical polymerization inhibitors, like benzoquinone, were found to inhibit the reaction. Furthermore no reaction was observed in the presence of HRP or H<sub>2</sub>O<sub>2</sub> alone. The polymer monolayers were transferred onto appropriate slides using the Langmuir–Blodgett technique. Polymerization of substituted and unsubstituted phenols (ratio 1:10) catalyzed by laccase enzyme (benzenediol:oxygen oxidoreductase, EC 1.10.3.2; 120–150 units/mg solid; Sigma Chemical Co.) was also studied. Polymerization in the monolayer was initiated by the injection of 2 mL of a laccase solution (10 mg/mL) into the buffered (pH 7.5) subphase (no hydrogen peroxide was required for this polymerization). The film was

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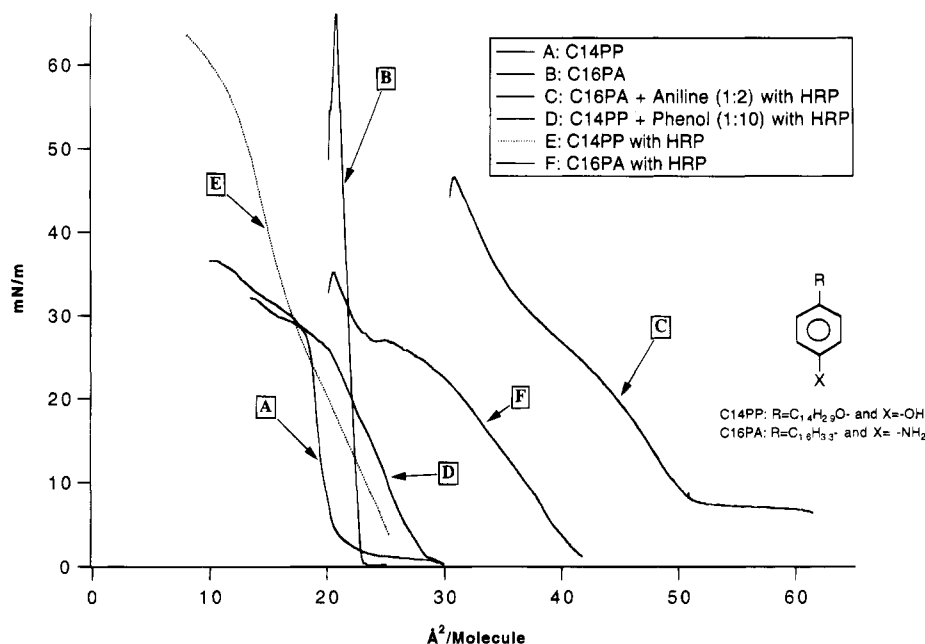
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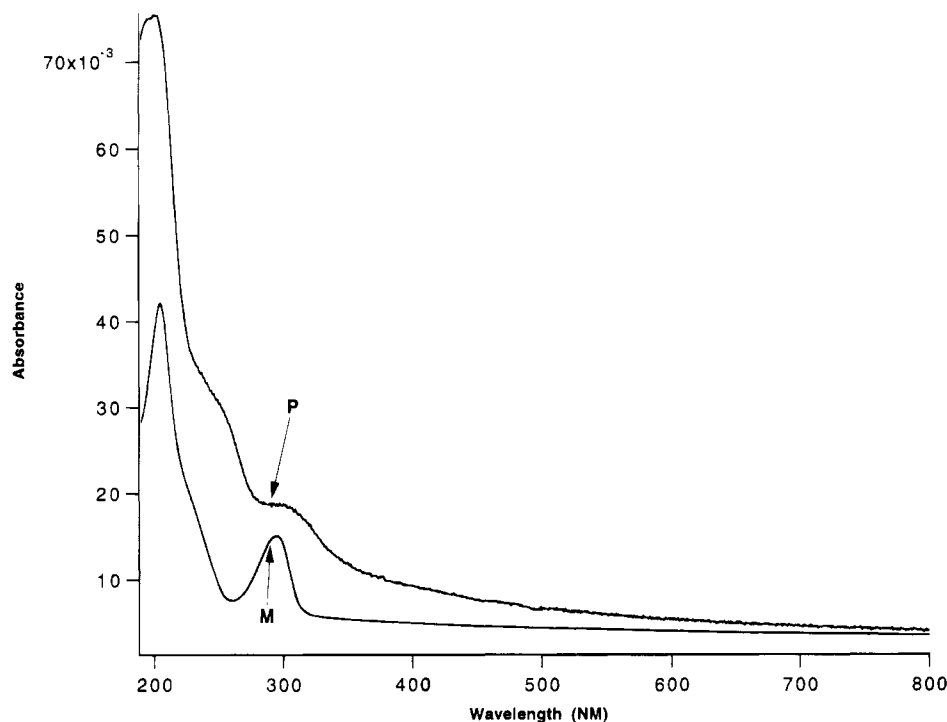
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**Figure 1.** Isotherms of listed monomers on MilliQ water (A, B), monomer mixtures, and derivatized monomer alone, prior to polymerization, in the presence of HRP (C-F) at 20 °C.



**Figure 2.** UV-vis spectra of 10 layers of the C16PA/aniline (ratio 1:2) system polymer/monomer: (A) 10 layers of the polymer, (B) 10 layers of the monomer. Similar results were found for the C14PP/phenol (ratio 1:10) mixture.

compressed with a  $1.0 \text{ Å}^2 \text{ mol}^{-1} \text{ min}^{-1}$  rate to surface pressure of 15 mN/m prior to polymerization.

The molecular weight of the polymer formed by the C14PP/phenol mixture (ratio 1:10) was established as 300 000 g/mol by GPC measurements. Spectral characterization of 10 layers on quartz slides was assessed with a Perkin-Elmer Lambda-9 UV-vis-near IR spectrophotometer (Norwalk, CT). The transfer ratio ranged between 90% and 100%, and the transfer was of type Y. Spectral characterization of monolayers on ZnSe slides for the attenuated total reflection (ATR) mode and in the transmission mode was assessed with a Perkin-Elmer 1760 FTIR-FT Raman spectrophotometer. The transfer ratio ranged between 90% and 100%, and the transfer was of type Y. FTIR spectra of 10 layers were collected in transmission at 45° incidence with s and p polarization. The dipole orientation of the alkyl chain was

calculated according to established procedures.<sup>13</sup> The film was deposited on a silicon wafer with a transfer ratio of 100%, for the Y type film. Atomic force microscopy (AFM) measurements were performed using a Digital Instrument Inc. nanoscope AFM (Santa Barbara, CA). Ellipsometry (43603-200E, Rudolph Research, Flanders, NJ) was used to measure the thickness of the deposited films. One and five monolayers of C14PP/phenol polymer (reactive index  $n \approx 1.50$ ) deposited on fused silica (refractive index  $n = 1.457$ ) substrates were used for the ellipsometric measurements at  $\lambda = 0.6328 \mu\text{m}$ , where absorption was negligible (imaginary part of refractive index  $k = 0$ ). The readings were averaged over four different regions of the film. Third-order nonlinear optical susceptibility was determined in solution by

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degenerate four-wave mixing with a frequency doubled Nd:YAG laser with 17 ps pulses at 532 nm.<sup>14</sup>

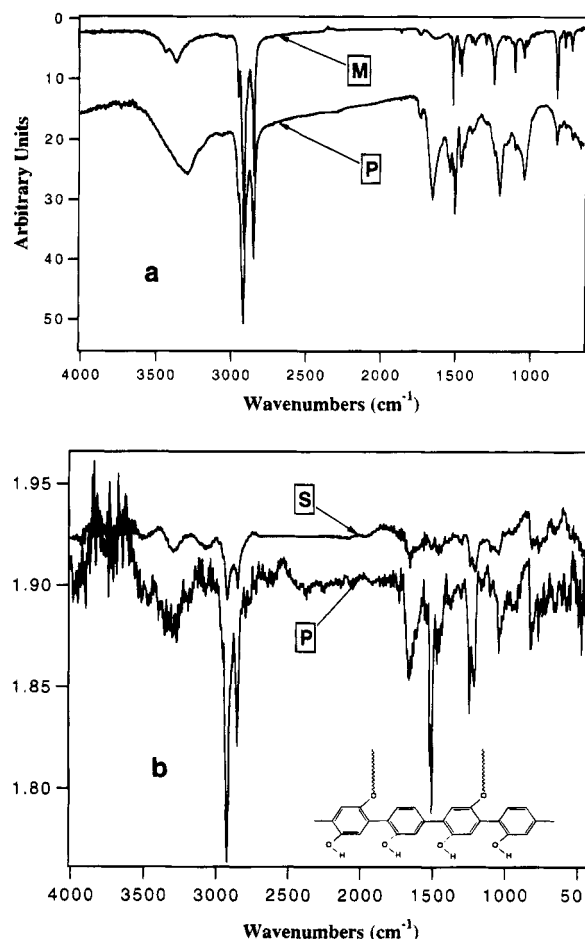
## Results and Discussion

Pressure–area isotherms with surfactant monomers C14PP and C16PA, and mixtures of these monomers with aniline and phenol, are shown in Figure 1. An approximately 22– to 25 Å<sup>2</sup> area per monomer was observed for the pure monomer systems. The C16PA formed highly incompressible, solid analogous films which reached relatively high compression values of around 70 mN/m and rapidly collapsed upon further compression. Underivatized phenol or aniline was added to the trough in ratios ranging from 1:1 to 500:1 to facilitate the polymerization reaction. The substituted phenols and substituted anilines were not reactive when run alone and within the time frame of the experiment (12 h). The function of the underivatized aniline and phenol is to form a reactant array, with the derivatized monomer, in a manner such that the coupling reaction may proceed. Isotherms with HRP in the subphase are shown in Figure 1. The decreased slopes and increased areas per molecule are indicative of enzyme and phenol penetration into the monolayer. Alternatively, the reaction can proceed without the addition of hydrogen peroxide in the case of the injected enzyme laccase.

Polymer multilayers of the mixes C14PP/phenol (ratio 1:10) and C16PA/aniline (ratio 1:2) show a broad absorption in the visible region (Figure 2), indicative of backbone conjugation. This pattern was absent in controls containing only monomer and those run without hydrogen peroxide or enzyme. The collapsed films were highly extensible and elastomeric. Surface characterization was assessed by FTIR–ATR and transmission spectroscopy. The ATR spectra obtained from 10 monolayers of the C14PP/phenol monomer and polymer are shown in Figure 3a. The FTIR–ATR spectrum of the polymer shows a dramatic difference in the 1600–1700 and 1190–1290 cm<sup>−1</sup> regions from the monomer spectrum. In the former region the presence of backbone conjugation in resonance with the phenolic part of the polymer is clearly seen.<sup>15</sup> An upper limit in the ratio of C14PP to phenol in the polymer backbone is also established as 1:2 from the OH stretching intensities (region 3300 cm<sup>−1</sup>).

Spectroscopy in the transmission mode for s and p polarization of C14PP/phenol indicates a near normal alignment of the alkyl chains on the surface from a 10 monolayer film using s and p polarized beams (Figure 3b). The tilt angles calculated from these spectra for the O–H, C–OH, and C=C groups are consistent with the structure proposed in the inset of Figure 3b.<sup>15</sup> Specifically, the band at 1510 cm<sup>−1</sup>, assigned to the aromatic C=C, shows a high relative intensity for the p polarization compared with the s polarization spectrum, indicating that the transition moment for the benzene ring vibration is oriented nearly perpendicular to the substrate surface. The verticality of the alkyl chain and the phenyl ring corroborate the structure in Figure 3b.<sup>16</sup>

Mechanistic molecular modeling using optimized geometries allowed normal arrangement for the alkyl chains only for the regiospecific isotactic polymer model in Figure 3b from many possible choices including the ortho–ortho structure. We have used the PM3 hamiltonian in MOPAC 5.0 to optimize the geometries of the monomers in the



**Figure 3.** FTIR spectra of monolayers: (a) FTIR–ATR spectra of 10 layers (M) of the monomer and (P) of the polymer and (b) FTIR transmission spectra of 10 layers (S) collected at 45° incidence with s polarization and (P) collected with p polarization.

polymer unit cell. The valence-effective hamiltonian method was used for the electronic structure calculations on the resulting polymers. The structure shown in Figure 3b also had the calculated minimum energy conformation. It is further seen that the OH groups in this structure have the proposed arrangement associated with the water subphase.

Optical ellipsometry was used to measure the monomolecular thickness and quality of the films formed. We obtained a monolayer film thickness of  $28.2 \pm 5$  Å. An average monolayer thickness of  $27.8 \pm 5$  Å was obtained from a five layer C14PP/phenol polymeric film.<sup>17</sup> These results are in reasonable agreement with the end atom to end atom length of 24.8 Å, obtained by modeling calculation. Vertical homogeneity results were confirmed by AFM measurements, where the micrograph showed a regular flat surface (Figure 4).

Upon doping with iodine the conductivity for the bulk polymer was measured to be  $1 \times 10^{-5}$  S/cm.<sup>18</sup> However the monolayer upon doping with iodine exhibited a conductivity of  $1.07 \times 10^{-2}$  S/cm. The conductivity was stable for more than 4 weeks under ambient condition. There was also significant enhancement of the third-order optical nonlinearity ( $\chi^3$ ) upon polymerization, as expected for a conjugated backbone, reaching values of  $1 \times 10^{-9}$  esu at 532 nm. An order of magnitude increase in the

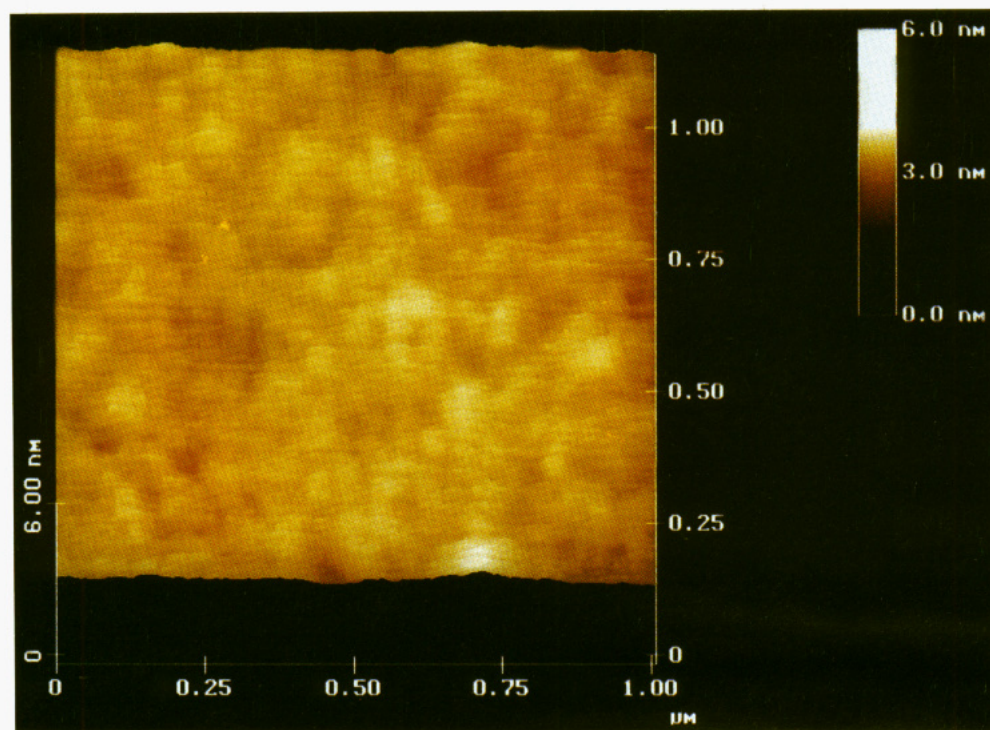
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**Figure 4.** AFM of one layer of the polymeric film formed by the C14PP/phenol mixture.

third-order nonlinear optical properties was also observed over bulk polymerized samples.

### Conclusions

This approach of polymer synthesis in two dimensions is based on an enzyme-mediated radical polymerization process.<sup>19</sup> Specific monomers, such as pyrrole, were not reactive at the air–water interface in the presence of HRP, evidence for a more complex mechanism of reaction rather than just the simple free radical generation by the enzyme. This seems to confirm past research<sup>20,21</sup> on HRP that established stereospecific binding of phenol derivatives to the enzyme before undergoing reaction. It is important

to note that the gentle nature of the enzymatic activity and the control of stereochemistry, by using the enzyme, may present significant processing and synthetic advantages. In our present work, a wide range of monomers were found to react under these conditions which provide a diversity of potential polymer products for systematic studies of the effect of monomer substituents on mechanical, thermal, electronic, and optical properties.<sup>22</sup> In addition, preordering and orienting of monomers prior to polymerization improves processability and optical/electronic properties.

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