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# Enzyme Catalyzed 2-D Polymerization of Phenol and Aniline Derivatives on a Langmuir-Blodgett Trough

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## ABSTRACT

Previous studies have shown that polymeric materials may be synthesized from substituted phenols and aromatic amine compounds through a Horseradish Peroxidase (HRP) enzyme catalyzed reaction in the bulk. We have developed a novel approach for this enzyme mediated polymer synthesis which involves in-situ polymerization using the Langmuir-Blodget (LB) technique. Here, the LB technique is used to organize and manipulate the reactants during polymerization at the molecular level. This methodology provides direct control over the resulting structure, order and orientation of the final polymeric material. This enhanced control leads to improved processability, tensile strength, and functional properties for the polymers. This new synthetic approach and the resultant ordered polymer product can form the basis for intelligent material design.

## INTRODUCTION

Monolayers are generated by self assembly or Langmuir-Blodgett (L-B) techniques. Self-assembly is used to generate monolayers on metal support surfaces[1-3]. The L-B technique can provide unsupported monolayers and is used routinely to assemble surfactant molecules at an air-water interface. Polymerization of the surfactant monomer assembly may than be carried out in a variety of ways. For example polypyrrole monolayers may be prepared with the addition of a strong chemical oxidizer such as ferric chloride, in the subphase of a monomer monolayer[4]. The products are polyheterocycles with useful electronic and optical properties.

Recently we reported on the synthesis and characterization of polymers in monophasic organic solvents with peroxidase in dioxane[5]. A wide range of substituted phenols and anilines were reactive under these conditions and formed homo- and co-polymers. High molecular weight polymers (400K) were synthesized that were stable to high temperatures and exhibited melting points in the 215-250°C range. The substitution pattern for chain or network

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growth was dependent on the monomers and their ring substituents[5]. The poor solubility and high branching or 3-dimensional network configuration of these polymers prevented easy processing into films or fibers. An approach was sought to overcome the processing limitations while maintaining or enhancing the optical and electronic properties of the polymers. Ordered arrays of these conjugated macromolecules can form components in an intelligent material design. We report here a biocatalytic approach to polymerization at an air-water interface which accomplishes this goal.

## MATERIALS AND METHODS

Lauda film balance with trough and equipped with a constant temperature bath (Langmuir Filmwaage, Model D, Lauda-Brinkman, Westbury, NY) was used. The surfactant 4-tetradecyloxyphenol (C14PP) was chemically synthesized by O-alkylation of hydroquinone with 1-bromotetradecane. Monomers, in 1-2 mg/ml concentration, were solubilized in chloroform and spread at the air-water interface. The subphase contained two liters of 0.85 mM HEPES buffer, pH 7.5 with 12-25 mg of enzyme per liter. Horseradish Peroxidase (HRP) (EC 1.11.1.7) Type II, 150-200 units/mg solid was used (Sigma Chemical Co., St. Louis, MO). The surfactant was compressed at the rate of 1.0 $Å^2$ $mol^{-1}$ $min^{-1}$ to a surface pressure of 15 mN/m prior to deposition. Hydrogen peroxide (0.30 ml of 30% in water) was injected by syringe into the subphase. All reactions were carried out under ambient conditions.

Spectral characterization of monolayers on quartz slides was performed with a Perkin-Elmer Lambda-9 UV-Vis near IR spectro-photometer (Norwalk, CT). Spectral characterization of monolayers on ZnSe slides was performed with a Perkin-Elmer 1760 FTIR-FTRaman spectrophotometer (Norwalk, CT). For electrical measurement on the polymer we used an "Interdigitated Microsensor Electrodes" (IME). The IME was composed of fifty gold fingers, each 15μm wide, 4985 μm long, and with a space of 15 μm between each one. This sensor was covered with bulk C14PP + Phenol polymer and was then placed in a sealed chamber where nitrogen is alternatively flushed and vacuumed three times. At this point the resistance of the undoped polymer was recorded. Nitrogen is gradually added but this time dopant (iodine) is also introduced into the chamber and measurements of the decrease of the polymer's resistance is initiated. The measurement process is halted when saturation in conductivity results.

The polymer formation of a mixtures of substituted C14PP and phenol monomer (ratio 1:10 ) was initiated by the injection of 2 ml Laccase solution (10mg/ml) into the buffered (pH 7.5) subphase (no hydrogen peroxide was required for this polymerization). Laccase (Benzenediol:oxygen oxidoreductase EC 1.10.3.2),120-150 units/mg solid was used (Sigma Chemical Co., St. Louis, MO). The surfactant was compressed at the rate of 1.0 $Å^2$ $mol^{-1}$ $min^{-1}$ to a surface pressure of 15 mN/m prior to deposition. The system was left to react for 15 hours. Similar results to HRP catalyzed system were found.

## RESULTS AND DISCUSSION

Pressure-area isotherms with unreacted monomers 4-tetradecyloxyphenol (C14PP) and 4-hexadecylaniline (C16PA), and 1:10 mixtures of these monomers with aniline and phenol on pure water subphases are shown in Fig. 1. An area per molecule of approximately 21 to 23 Å$^2$ was observed for the pure alkyl derivatized phenol and aniline materials, indicative of a well defined monolayer, while a slight expansion in the area per molecule was observed when the underivatizied phenol and aniline monomer was mixed into the monolayer[6-8]. The C16PA formed beautiful isotherms, reached relatively high compression values of around 70 mN/m, and rapidly collapsed upon further compression. The expansion in area with the mixed monolayers was encouraging as it suggests that the small co-monomers are incorporating into the monolayer and it is believed that these monomers will enhance the polymerization reaction.

Underivatized phenol or aniline was added to the trough in ratios ranging from 1:1 to 500:1 to accelerate the reaction, since the substituted phenols and substituted aniline were observed to be significantly less reactive by themselves. Once the monomer at the air-water interface was spread on to the aqueous subphase containing HRP and compressed to 15 mN/m, hydrogen peroxide was injected into the subphase to initiate the polymerization process.

The progress of the reaction could be visually monitored by the formation of a slight change in appearance which occurs within the first few minutes. Reactions were terminated after 12 hours and the resultant two-dimensional polymer networks were transferred onto quartz slides for subsequent characterization. UV-Vis spectroscopic characterization (Fig.2) of the polymer in transferred multilayers show a broad absorption in the visible spectrum, indicative of conjugated polymeric backbone. This absorption pattern was absent in controls containing only the monomer mixtures. In addition controls prepared in the absence of either hydrogen peroxide or HRP enzyme gave no evidence of polymer formation. Collapsed films, skimmed from the subphase surface, were dark in appearance, and highly extensible.

Further spectroscopic characterization was carried out by FTIR-ATR. Ten monolayers of each of the polymer and the monomer were deposited on ZnSe slides. The resulting spectra are shown in Fig.3. The FTIR-ATR of the polymer shows appropriate changes in the region 1600-1700 cm$^{-1}$ and 3500-3300 cm$^{-1}$ as compared to that of the monomer. The broadened peak in the 1600-1700 range cm$^{-1}$ confirms a C-O bond attached to a delocalized conjugated backbone. The broadened intense peak at 3500 cm$^{-1}$ shows the changed nature of the hydrogen bonding of the OH groups due to polymerization.

Conductivity measurements, of iodine doped polymerized multilayers were carried out using Interdigitated Microsensor Electrodes (IME). The conductivities ranged from 1.07*10$^{-1}$ to 2.09*10$^{-4}$ S/cm. It was determined that the polymer formed with the mixture C14PP + Phenol (1:10) was the most stable as it was able to mantaine a conductivity of 2.00*10$^{-3}$ S/cm over a 3 day time period.

Fig.4 illustrates the postulated reaction scheme and polymer products for the C14PP/phenol and C16PA reactions, although more random distributions of monomers in the chain would be expected. Mechanistic studies indicate that the ortho positions in the derivatized lipids would be the preferred sites of oxidative coupling[9].

## CONCLUSIONS

The described biocatalytic approach to 2-dimensional polymer network synthesis is a free radical polymerization process. It has general applicability and a wide range of monomers will

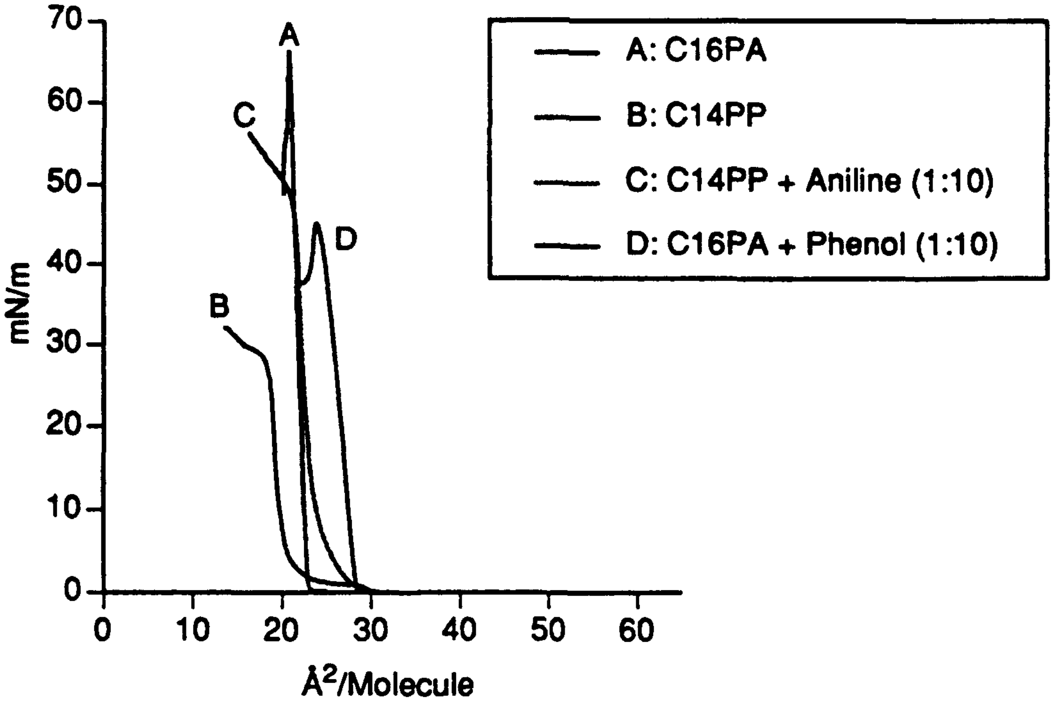

Figure 1. Isotherms of different monomers on MilliQ water at 20°C

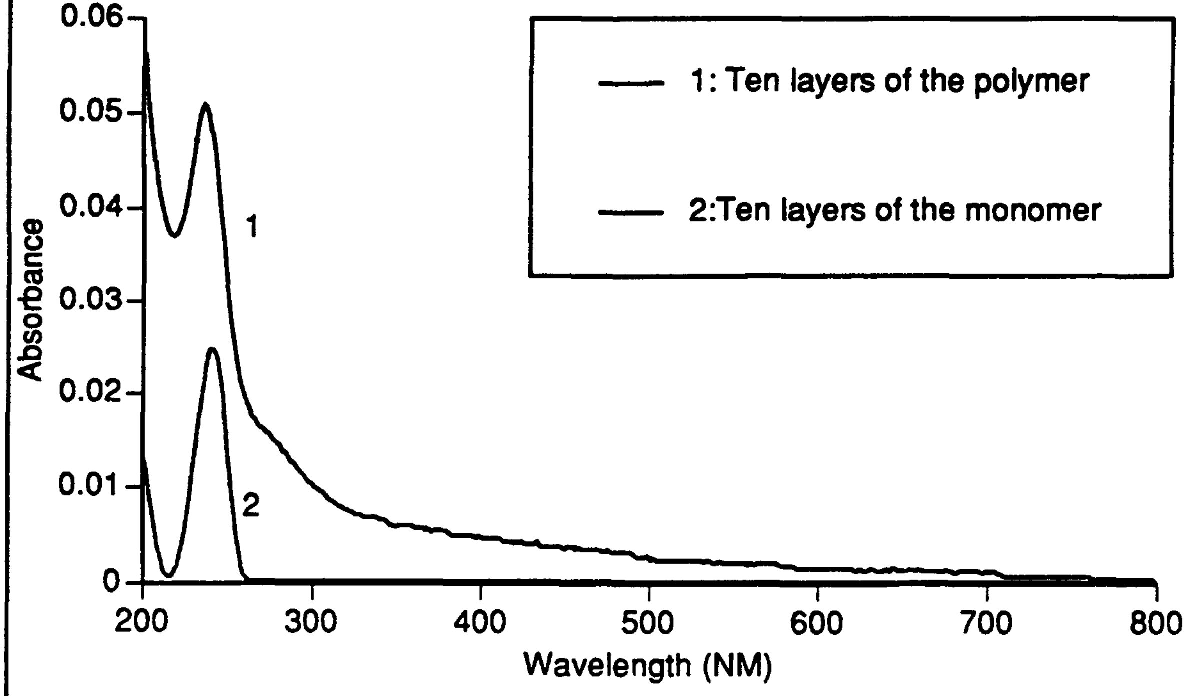

Figure 2. UV-Vis. Spectra of ten layers of polymer/monomer on quartz

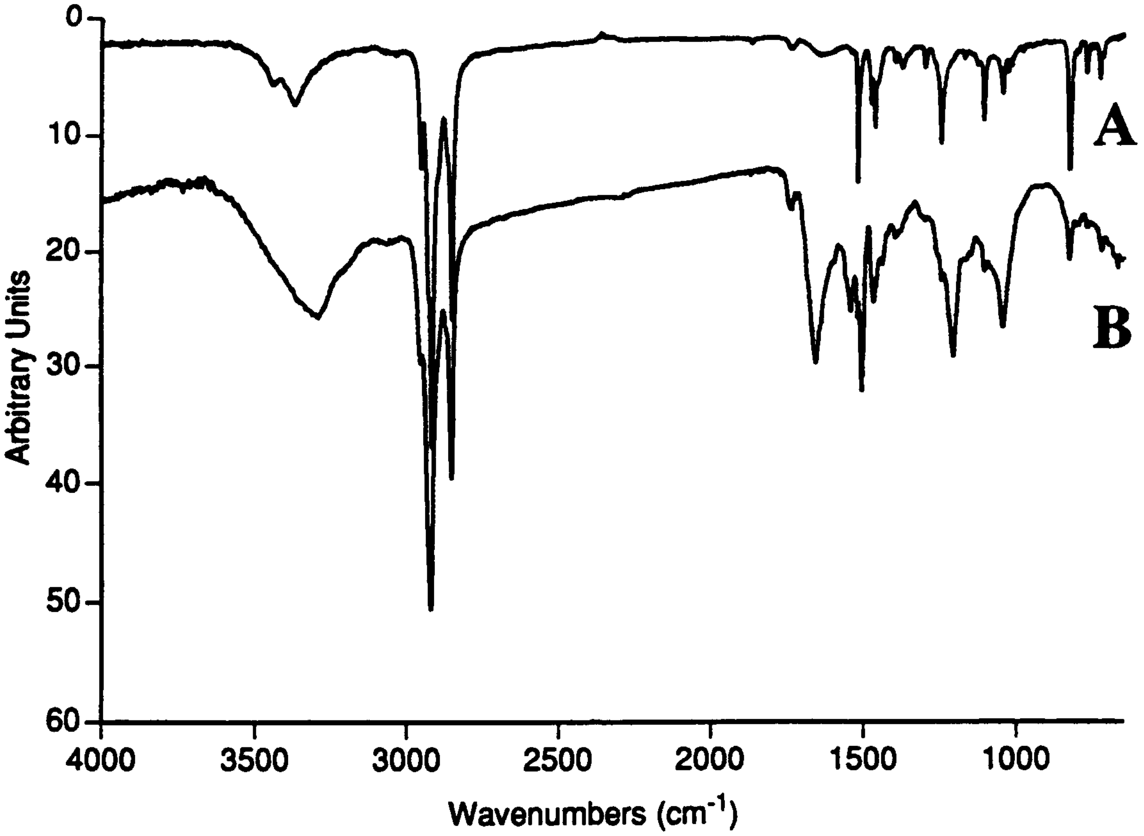

Figure 3. FTIR-ATR spectra of ten layers (A) of the monomer and (B) of the polymer

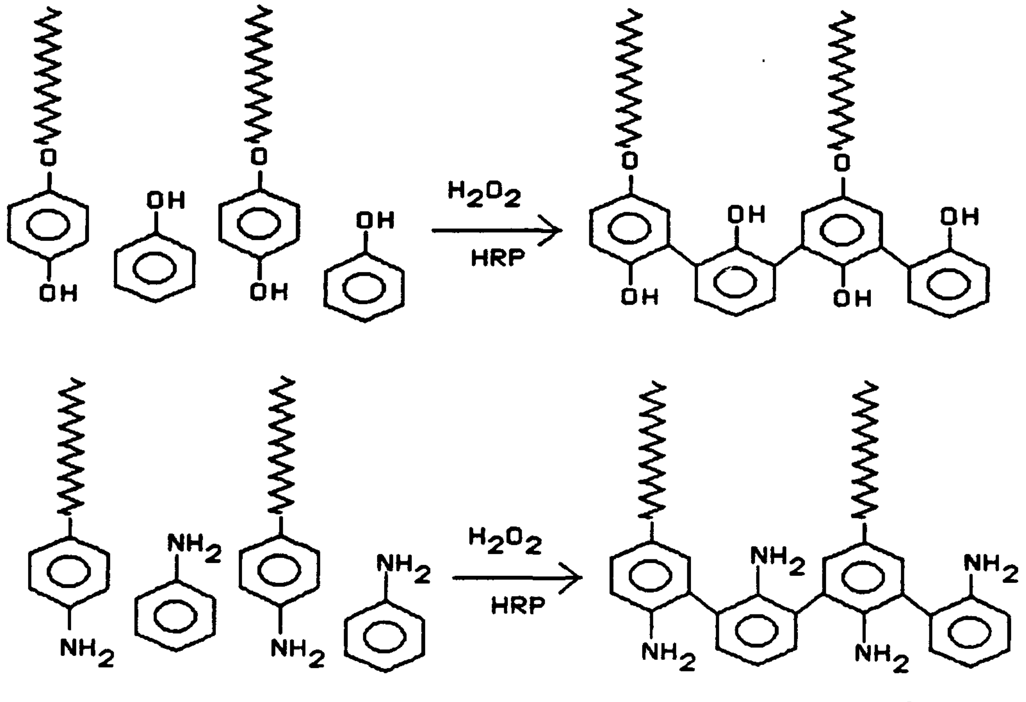

Figure 4. Proposed possible schematic arrangement of the polymerization at the air-water interface

react under these conditions to produce polymeric products. The process described represent a general methodology for the simultaneous assembly and polymerization of conductive and optically active polymers in a two-dimensional network. In addition, direct manipulation and preordering of the reactants prior to, and during polymerization substantially improves in optical and electronic properties. 2-D polymeric materials with appropriate electronic and optical signature for sensing and actuation may be designed.

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