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Enzymatic mediated polymerization of functional aniline derivatives in nonaqueous media

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Abstract

Horseradish peroxidase (HRP) catalyzed H_2O_2-dependent oxidation and polymerization of *p*-aminophenylmethycarbinol (*p*APMC), but not *p*-aminoacetophenone (*p*AAP) or other aniline derivative with elecron-withdrawing character. The effect of the H_2O_2 feed ratio on the polymerization was examined. Chemical structure was determined by IR and ^1H, ^{13}C NMR spectroscopic analysis. The p(*p*AMPC) formed primarily through a covalent bond between the benzilic carbons and the amino group of another *p*APMC molecule. Thermal stability of p(*p*APMC) was analyzed by DSC and TGA.

Introduction

It is well known that aromatic substrates having substituents with an unshared pair of electrons like OH and NH_2 can be polymerized via free radical oxidative coupling of rings catalyzed by horseradish peroxidase (HRP)[1,2]. The molecular weight of such polymers depends on several factors such as: the type of solvent, solvent/buffer ratio, and H_2O_2 feed ratio. According to Akkara[3] a high yield polymerization can be obtained by using a mixture of 80% dioxane and 20% buffer HEPES with pH between 6.5 and 7.5. The mechanism of enzyme-catalyzed polymerization can be explained as follows[4,5]. An electron acceptor (peroxide) oxidizes the native enzyme, which in turn accepts an aromatic substrate possesing an oxidizable substituent at its active center. The resulting free radical is released leaving the enzyme in a second oxidation state capable to oxidize another aromatic substrate, releasing a second free radical and returning the enzyme to its native state. The free radicals spontaneously combine to form dimers, trimers, oligomers, etc. The cohesive strengh of the growing chain becomes stronger, while the solvating power of the solvent remains constant which causes oligomer or polymer precipitation. Current interest to synthesize polymers by this technique is due to the fact that these materials possess unique electrical and optical properties resulting from their long conjugated π-bonds[1,3,4]

In the present work, *p*AAP and *p*APMC, aniline substrates with functional groups in the *para* position were oxidized by HRP, expecting to prepare new functional polymers. Peroxidase catalysed oxidation of such monomers has not been investigated before.

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Experimental

Reagents: The starting materials were used as received from Aldrich Chemical Company, Milwaukee, WI. HRP (Type II, 150-200 units/mg solid) was purchased from Sigma Chemical Company, St. Louis, MO.

Characterization: Infrared spectra were obtained using a FTIR Nicolet Magna 550 spectrophotometer on KBr discs. ^{1}H and ^{13}C-NMR spectra were recorded on a Varian Gemini 200-MHz instrument using $CDCl_3$ as solvent. The DSC and TGA were performed on a DuPONT 2000 and DuPONT 910-S model instrument. Molecular weights were determinated in a SEC (Waters 150-C) with Ultra Styragel column, nominal porosity of 500 Å. Molecular weight averages were calculated using a calibration curve constructed with polystyrene standards.

Chemical synthesis of *p*APMC[6]: 13.51 g (0.10 moles) of *p*AAP were dissolved in 200 ml of warm ethanol. Once the solution was cool, 3.78 g (0.10 moles) of sodium borohydride were added in three portions. The mixture was stirred at room temperature for 60 min. Later, 250 ml of water were added and the mixture stirred for another 15 min. The organic layer was extracted with diethyl ether and dried with sodium carbonate, obtaining 12.33 g (90 % yield) of brown crystals. Anal. Calcd. for $C_8H_{11}N$: C, 70.02; H, 8.08; N, 10.21. Found: C, 69.98; H, 8.78; N, 10.46.

Enzymatic polymerization: The monomer (350 mM) was dissolved in dioxane, and the HRP (0.5 mg/ml solubilized in HEPES buffer solution) was added slowly to the solvent with gentle stirring, giving a total mixture of dioxane/HEPES buffer of (80/20). Then, 350mM of hydrogen peroxide were added. The enzymatic polymerization was carried out at room temperature over a period of 26 hours.

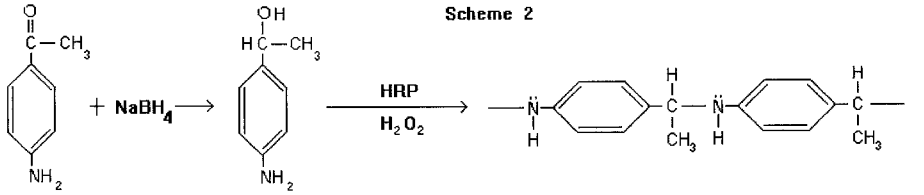
Results And Discussion

p-Aminoacetophenone does not polymerize using HRP catalyst, most likely because the negative inductive effect of the acetyl group[7], Scheme 1. In order to corroborate this assumption, *p*-nitroaniline was also subjected to polymerization and was found not to polymerize. We suggest that aniline monomers containing electron-withdrawing groups can not polymerize by enzymatic catalysis due to: (i) the nitrogen unshared electron pair is less available because is stabilized by the resonance hybrid **1a**, and (ii) when the conjugated Bronsted acid is formed, the resonance stabilization afforded by **1b** is no longer available because the previously unshared pair is now being shared by the proton[7].

1a **Scheme 1** **1b**

On the other hand, *p*APMC was readily polymerized most likely because the positive inductive effect of the $HC(OH)CH_3$ group[7], Scheme 2.

Scheme 2



The polymerization started when hydrogen peroxide was added and a typical color change occurred from yellow to deep brown. The addition of hydrogen peroxide was accomplished in two ways; in one step (mode I) or gradually (mode II) at a rate of about 20 mM every 3 hours, for the first 12 h, and 13.5 mM every hour until reaching the desired ratio of H_2O_2. The product was isolated by centrifugation (14 000 r.p.m) at 4 °C for 10 min and washed with water to remove residual buffer and enzyme. The product was washed several times with diethyl ether to remove residual monomer and low molecular weight fractions, the rest of the product was soluble in $CHCl_3$. Blanks were frequently run without enzyme and no color change was observed. Results of polymerizations are shown in Table 1. As can be observed from this table, yields are similar for both cases, and the average molecular weights do not differ markedly. However, for mode I there is a higher content of the low molecular weight fraction than that seen for mode II. By gradually adding the H_2O_2 , more uniform molecular weight could be obtained. A small fraction of insoluble product was obtained during precipitation which is indicative of a high molecular weight product, presumably with a crosslinked structure.

TABLE 1. Enzymatic polymerization of *p*-aminophenylmethylcarbinol

MODE I	Yield (%)	Mw_{min}	Mw_{max}	Mw	Mn	Mw/Mn
Adding of H_2O_2 in one step						
Reaction total product	83	274	3,238	1,034	887	1.15
After washed with diethyl ether						
Only Soluble in $CHCl_3$	21	274	14,460	1,492	893	1.67
Soluble in ether	62	274	2,424	855	707	1.20
MODE II						
Gradual adding of H_2O_2						
Reaction total product	84	274	13,888	1,372	778	1.76
Insoluble	3.72					
After washed with diethyl ether						
Only soluble in $CHCl_3$	44	274	18,636	1,914	992	1.92
Soluble in ether	40	274	5,177	887	662	1.33

The polymer structure was determined by IR and NMR spectroscopy. The spectra of the products from the mode I were similar to those for mode II, therefore only the products from mode I were analyzed.

Figure 1 shows the IR spectra for: a) *p*APMC monomer, b) p(*p*APMC) product soluble in $CHCl_3$, and c) p(*p*APMC) product soluble in ether. The *p*APMC monomer [Fig.1 (a)] shows the OH stretching (ν) at 3347 cm^{-1} and the C-O stretching at 1081 cm^{-1}, two characteristic peaks for the primary amine at 3300 and 3450 cm^{-1} are overlapped with the OH stretching; however, the N-H bending vibration (σ) appears at 1617 cm^{-1}. The spectrum for p(*p*APMC) soluble in $CHCl_3$ as well as the spectrum for p(*p*APMC) soluble in ether [Fig. 1 (b), (c)] show similar signals: a broad peak for the secondary amine at 3410 cm^{-1}, the peak at 1617 cm^{-1} due to N-H bond of secondary amine and the peak at 1519 cm^{-1}, due to C-N stretching (σ). The difference between the polymer and that of the monomer spectra is in the reduction of the signals corresponding to the OH stretching and the C-O stretching as well as the relative intensity of the signals between 780 and 1519 cm^{-1}.

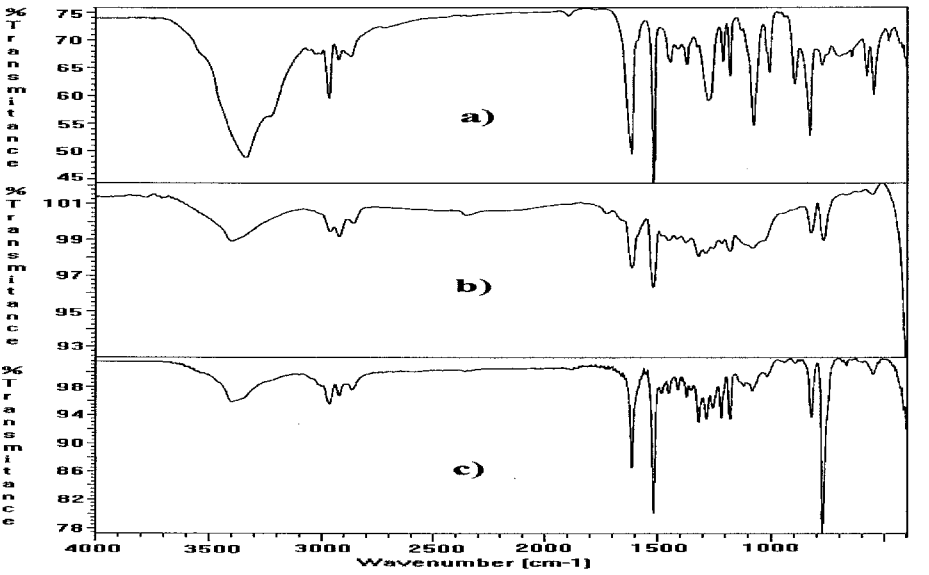


Figure 1. IR spectra for: a) *p*APMC monomer, b) p(*p*APMC) product soluble just in $CHCl_3$, and c) p(*p*APMC) product soluble in diethyl ether.

Figure 2 shows the ^1H NMR spectra for: a) *p*APMC monomer, b) p(*p*APMC) soluble in $CHCl_3$, and c) p(*p*APMC) soluble in $CHCl_3$ after heat treatment and deuteration. The spectrum of *p*APMC shows one doublet at δ 1.46 ppm corresponding to the methyl protons, a broad doublet for OH at 1.77 ppm, the amine protons gave a broad signal at 3.65 ppm, the proton in the secondary carbon was identified by a quadruplet at 4.78 ppm. The presence of two symmetrical doublet aromatic proton at 6.66 and 7.16 ppm indicates the substitution at *para* position. In the spectra corresponding to the polymers, it is interesting to note that most of the signals are those of the monomer, just broader, indicating a higher molecular weight. However, the integration of the amine group corresponds to only one proton and the most interesting aspect is that the OH signals

dissapeared. Further, after D_2O exchange, the amine proton signal disapeared. Finally, the heat treatment develops signals of vinyl groups at 4.96 and 5.46 ppm, maybe because traces of residual monomer were dehydrated giving *p*-aminostyrene[6]. This signal did not increase with longer heat exposition time.

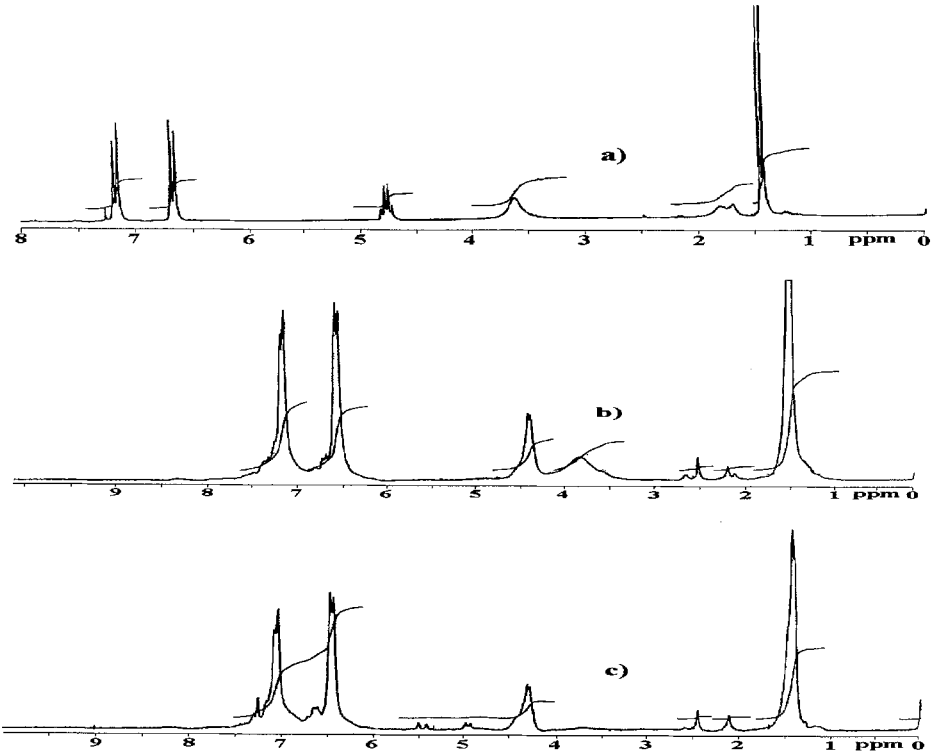


Figure 2. ^{1}H NMR spectra for; a) *pAPMC* monomer, b) p(*p*APMC) soluble in $CHCl_3$, and c) p(*p*APMC) soluble in $CHCl_3$ after heat treatment at 100 °C and D_2O exchange.

Figure 3 Shows the ^{13}C NMR spectra for: a) *p*APMC monomer, b) p(*p*APMC) soluble in $CHCl_3$, and c) off-resonance decoupled ^{13}C of p(*p*APMC) soluble in $CHCl_3$. The *p*APMC monomer spectrum shows the peak for methyl carbon at δ 24.84 ppm, the peak at 70.13 was assigned to the secondary carbon. In downfield absortions the two large peaks at 126.68 and 115.30 ppm represent the two pairs of equivalent aromatic ring carbon atoms. The peaks at δ 145.80 and 136.01 correspond to the quaternary ring carbon atoms. The polymer spectra peaks [Fig.3 (b)] also correspond to those of the monomer; however, a small upfield displacement is observed for the carbon atoms nearby the secondary carbon. These assignments were confirmed by the off-resonance decopled spectrum [Fig. 3 (c)]: quartet for methylene, doublet for secondary carbon, two symmetrical doublets for ring carbon, and singlets for the quaternary carbon.

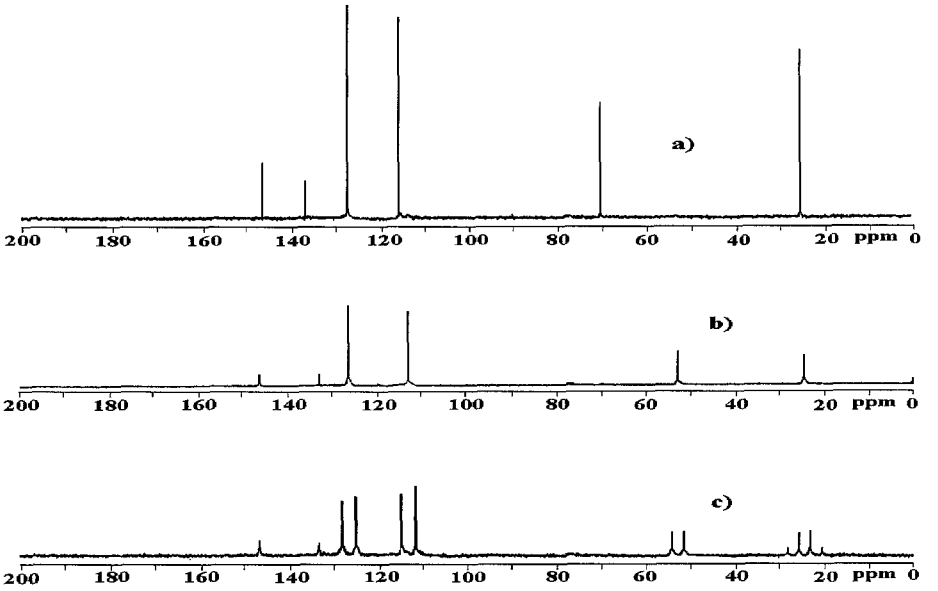


Figure 3. ^{13}C NMR spectra for; a) *pAPMC* monomer, b) p(*p*APMC) soluble in $CHCl_3$, and c) off-resonance decopled ^{13}C of p(*p*APMC) soluble in $CHCl_3$.

Thermal properties of p(*p*APMC) soluble in $CHCl_3$ were determined by TGA and DSC. The thermograms are illustrated in Figures 4 and 5, respectively. The TGA of p(*p*APMC) indicates that about 80 % of the polymer was lost either by water evaporation or polymer degradation on heating the sample to 600°C under nitrogen atmosphere. The weight lost from the polymer sample around 100 °C is in good agreement with the assumption of dehydration and the formation of vinyl groups already discussed.

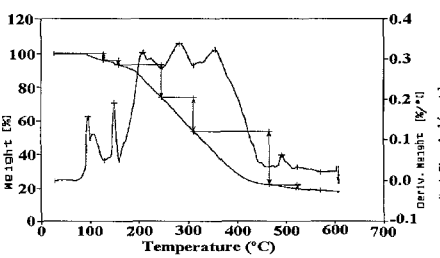


Figure 4. Thermogravimetric analysis p(*p*PMC) soluble in $CHCl_3$.

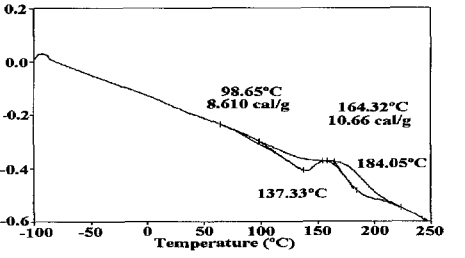


Figure 5. Differential scanning calorimetry of p(*p*APMC) soluble in $CHCl_3$

In general, the thermal analysis indicates that the polymer undergoes a series of weight losses at a temperatures higher than 100 °C. The DSC analysis of p(*p*APMC) (Fig. 5) shows an endothermic heat flow (8.610 cal/g) at about 98.65°C. The thermogram also

indicates a poorly defined melting point for the polymer, which was confirmed by observations from melting point determinations in a fusiometer.
The absence of hydroxy or ether signals in the IR spectra combined with the symmetrical ring signals detected in NMR spectra indicate that the polymer formed by this enzyme catalyzed polymerization is carried out through a condensation reaction with water elimination. According to this result, the polymer has the structure presented in Scheme 2. Further studies are on the way to determine physical properties.

Conclusions

Based on the results presented in this investigation we can conclude the following: First, anilines with functional groups substituted in *para* position with electro-withrawing character do not polymerize enzymatically; this statement can be extend to a wide range of monomers. Second, anilines with functional groups substituted in *para* position with electro-donating groups readily polymerize enzymatically. Third, the addition rate of H_2O_2 is an important parameter to obtain higher molecular weights. Fourth, this process may be used to synthesize novel polyfunctional materials.

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