Enzymatic Polymerization of Phenolic Biomonomers Derived from Cashew Nut Shell Liquid

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ABSTRACT

Enzymatic polymerization of alkyl phenols derived from a renewable resource, viz., cashew nut shell, has been carried out in a dioxane-water mixture. The reaction and reaction products have been characterized by a number of spectroscopic, microscopic, and thermometric methods. These monomers have also been copolymerized with various substituted phenols. The homopolymers are soluble in organic solvents

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while the copolymers are crosslinked, with negligible solubility. Enzymatic polymerization in a dioxane–water solvent mixture results in spherical particles in the case of homopolymerization while structures without distinctive morphologies are obtained in the case of copolymerization. The effects of dioxane–water composition and solution pH on the polymerization reaction have also been explored.

INTRODUCTION

The use of enzymes in polymer synthesis has attracted the attention of many researchers in recent years. Enzymes are becoming increasingly popular in organic synthesis [1–8]. Horseradish peroxidase (HRP) has been used for the enzymatic synthesis of polyphenols and polyanilines [1–5]. In the presence of hydrogen peroxide, peroxidases catalyze the oxidation of electron-rich systems such as phenols and aromatic amines that eventually give rise to high molecular weight polymers through oxidative free-radical coupling. The molecular weight and properties of these biochemically formed polymers can be manipulated by controlling the experimental conditions, unlike the nonenzymatic reactions.

In recent years the synthesis of polymers from renewable resources has attracted the attention of many research workers for a number of reasons [9, 10]. Growing environmental concern in the expanded use of petrochemicals is one reason. Cashew nut shell liquid (CNSL) (from *Anacardium occidentale, L.*) is one of the renewable resources rich in phenolic compounds [10]. CNSL contains four major components: anacardic acid, cardanol, cardol, and 6-methyl cardol, whose structures are furnished in Fig. 1. Although a variety of R groups is possible, all

Anacardic acid

Cardanol
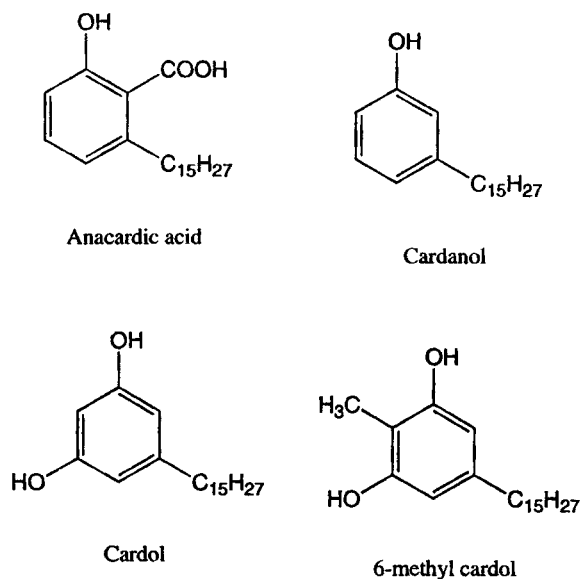
Cardol

6-methyl cardol

FIG. 1.   Structures of the components of cashew nut shell liquid.

have the meta substitution of a 15-carbon-long hydrocarbon chain, with varying degrees of unsaturation with an average of two double bonds. Tyman and Jacobs [11] showed that the relative proportions of unsaturated components of anacardic acid are monoene (38.7%), diene (36.3%), and triene (45.1%). Cardanol upon hydrogenation in the presence of Pd/C gives hydrogenated cardanol where the C_{15} hydrocarbon chain is saturated. Hence, cardanol is a potential renewable monomeric source for the synthesis of a variety of specialty polymers. In the present program, both cardanol and hydrogenated cardanol have been used for HRP-catalyzed polymerization in the presence of hydrogen peroxide in a dioxane–water mixture.

EXPERIMENTAL PROCEDURES

Horseradish peroxidase (EC 1.1.11.7, 200 units/mg, RZ = 2.0) was obtained from Sigma Chemicals Company, St. Louis, MO. All other chemicals were purchased from Aldrich Chemicals Company, Milwaukee, WI, and used as obtained.

Cashew nut shell liquid was obtained from Kerala Cashew Development Corporation, Quilon, India. Cardanol was obtained by double vacuum distillation of CNSL at 3–4 mgHg, and the fraction distilled at 230–240°C was collected. Hydrogenated cardanol was obtained by catalytic hydrogenation of cardanol. Hydrogen was passed over Pd/C catalyst at 25 psi at 60°C for a period of 6–8 hours to saturate the side chains of the cardanol. The reaction mixture was extracted with ether, which was later evaporated off to obtain 3-pentadecyl phenol in the form of a pale yellow solid (yield > 95%).

Under atmospheric conditions 5 mM of cardanol and 300 units of HRP were dissolved in 17 mL of 1,4-dioxane and 3 mL of phosphate buffer (pH 7.0), and 0.25 mL of hydrogen peroxide (30%) was added to the reaction mixture every 15 minutes for 20 times with vigorous stirring at room temperature. After 24 hours the resulting polymer was isolated by evaporation of the solvent at reduced pressure followed by washing of the residue with ice-cold methanol and water followed by drying in vacuum.

Spectrophotometric experiments were carried out using a Perkin-Elmer Lambda-9 UV-Vis-near IR spectrophotometer. Vibration spectra were recorded using a Perkin-Elmer FT-IR spectrometer 1760 with a room-temperature TGS detector. The pellets of the sample with KBr were used for FT-IR studies. TGA experiments were carried out using a TGA 2950 (TA Instruments, Inc.) thermobalance. The temperature was increased at a speed of 20°C/min under nitrogen atmosphere. SEM experiments were performed using an Amray 1400 Scanning Electron Microscope/Electron Probe Microanalyzer. Gel permeation chromatographic (GPC) analysis was carried out on a GBR mixed bed linear column (Jordi Associates, Inc., Bellingham, MA). A UV detector at 270 nm was used to follow the elution. The eluent was DMF containing 1% lithium bromide. The flow rate was kept at 1 mL/min. NMR spectra were recorded using a Bruker 250MHz NMR spectrometer.

RESULTS AND DISCUSSION

Hydrogenation of liquid cardanol was followed by ^{13}C NMR. Figure 2 gives a selected region of the ^{13}C-NMR spectra of the cardanol and hydrogenated cardanol in $CDCl_3$. It can be observed from the spectra that there is complete hydrogenation
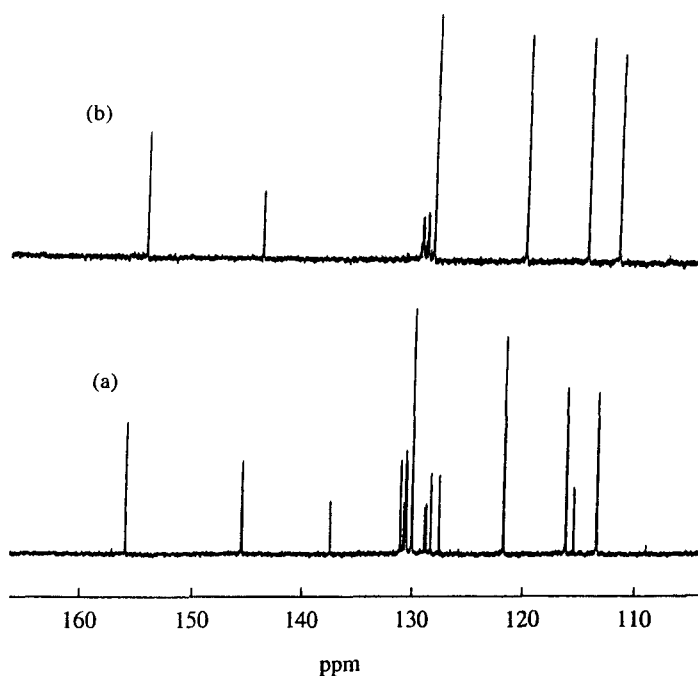
FIG. 2. ^{13}C-NMR spectra of (a) cardanol and (b) hydrogenated cardanol in chloroform-d.

of the cardanol. The resonance peaks arising from the unsaturated hydrocarbons disappear upon hydrogenation.

The enzymatic polymerization of cardanol and hydrogenated cardanol (3-pentadecyl phenol) was carried out using horseradish peroxidase (HRP) in a mixture of dioxane and buffer (85:15) for 24 hours at room temperature. The system becomes turbid almost instantaneously upon the addition of hydrogen peroxide; this is a visual indication of reaction progress. From the spectroscopic observation of the polymerization reaction at 250 nm it was determined that the reaction attains equilibrium in about 2 minutes. On further addition of hydrogen peroxide, similar changes in absorbance were observed.

The dioxane–water composition for the polymerization was optimized through a set of experiments as summarized in Table 1. Upon increasing the dioxane concentration from 20 to 95 (v/v with buffer), the percentage yield of the polymer increased from very low to 72% at 85% dioxane. On further increasing the dioxane concentration, the yield was lowered. At low dioxane concentrations the enzymatically formed polymers precipitate out of the solution as oligomers. Mostly dimers or trimers were formed when the reaction was carried out at low dioxane content.

The polymerization of cardanol was carried out in 85% dioxane–15% water while varying the pH from 6 to 7.5. The objective here was to optimize the pH condition for optimum yield. It was observed that by increasing pH from 6 to 7, the percentage of yield increased, and with a further increase of the pH it decreased. The enzyme activity was maximum at pH 7.0.

TABLE 1. Effect of Solvent/Buffer Ratios on the
Enzyme Activity in the Polymerization of Cardanol

Synthesis medium: dioxane–buffer[a]	Polymer yield (percentage)	
	Cardanol	Hydrogenated cardanol
20:80	Negligible	Negligible
40:60	10	12
60:40	18	25
70:30	35	35
80:20	52	55
85:15	75 (M_n = 2200)	85
95:5	37	35

[a]Phosphate buffer, pH 7.0.

Cardanol and hydrogenated cardanol (3-pentadecyl phenol) were copolymerized with phenol, p-ethyl phenol and p-phenyl phenol, the results of which are furnished in Table 2. The numbers in parentheses in the first column indicate the mass ratio of the cardanol (unsaturated and hydrogenated) and the comonomer. Copolymerization in 85% dioxane-15% buffer resulted in a rapid change of color of the solution to brown or dark brown as soon as hydrogen peroxide was added; thus indicating the formation of the polymer. The reactions were completed within 20-30 minutes. The yields of the copolymers were above 90%. The polymers are insoluble in common solvents, which limited the determination of the molecular mass of these polymers.

The morphology of the polyphenols of cardanol and hydrogenated cardanol in the presence and absence of the comonomer has been studied. Figure 3 shows scanning electron micrographs of polymers of (a) hydrogenated cardanol and of (b) hydrogenated cardanol with phenol copolymer (in 1:5 ratio), polymerized in a diox-

TABLE 2. Effect of Substitution on Phenols
on the Copolymerization of Hydrogenated
Cardanol (3-pentadecyl phenol). The Reaction
Was Carried Out in 85:15 Dioxane–Buffer
Mixture at pH 7.0

Phenol	Percentage yield
Phenol (3:1)	95
p-Ethyl phenol (3:1)	90
p-Phenyl phenol (2:1)	98
Cardanol–phenol (1:2)[a]	95

[a]Cardanol was used instead of hydrogenated cardanol.
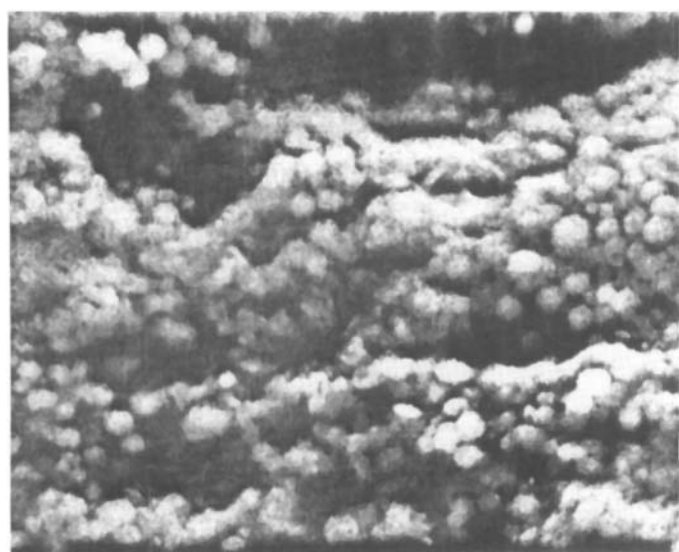
FIG. 3.   Scanning electron micrographs of polymers of (a) cardanol and (b) cardanol with phenol.

ane–water mixture. It can be observed from the figure that the homopolymer shows spherical particle structures with a radius of the order of 0.5 μm while the copolymer does not show any ordered morphology. It was recently reported that short-chain derivative of phenols form spherical particles upon polymerization in the presence of surfactants [12]. We speculate that the long hydrocarbon chain of the cardanol and hydrogenated cardanol organizes the monomers along the water–dioxane interface in the dioxane–water microemulsion, creating a micellar environment. The positions of these monomers are fixed in the spherical form during polymerization, forcing chain curvature along the micellar curvature. However, the addition of phenols or short-chain derivatives of phenols lowers the effect of hydrocarbon chain in organizing the monomers, resulting in featureless structures. Similar structures have also been observed in the case of polymer of cardanol and cardanol–phenol copolymer.

The spectroscopic characterization of the polymers was carried out using FT-IR and NMR spectroscopic techniques. The NMR of the copolymers could not be taken due to the insolubility of the polymers. The homopolymer of cardanol showed characteristic changes in the aromatic region of the ^{1}H spectrum. The aliphatic region of the spectrum resembled the monomer spectrum. New peaks appeared in the aromatic region due to the polymerization, and they were down shifted compared to the monomer peaks. The monomer shows aromatic peaks at 6.7, 6.8, and 7.2 ppm. The polymeric peaks appear at 6.9, 7.6, 7.8, and 8.1 ppm. The appearance of more than one peak due to polymerization can be attributed to the complex nature of the polymer structure. The FT-IR spectra of monomers and polymers were compared. Figure 4 shows the FT-IR spectra of hydrogenated cardanol and its polymer. It can be observed from the figure that the linewidths of the peaks in the polymer have broadened as compared to those of the monomer. The peak at 950 cm^{-1}, present only in the polymer spectrum, corresponds to the phenyl-phenyl C—C bond. Similar observations were made in the case of copolymers.
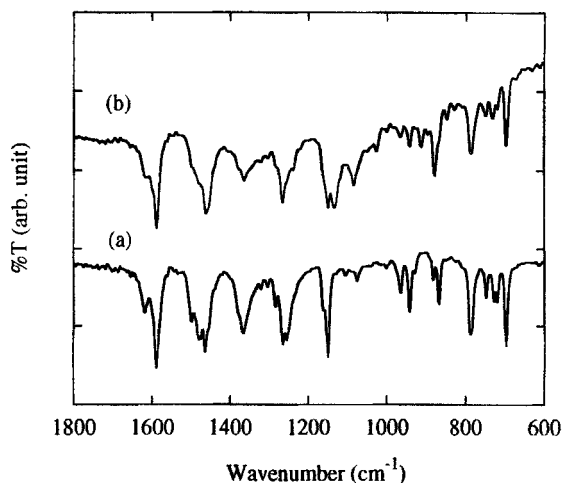

FIG. 4.   FT-IR spectra of (a) hydrogenated cardanol and (b) polymer of hydrogenated cardanol.
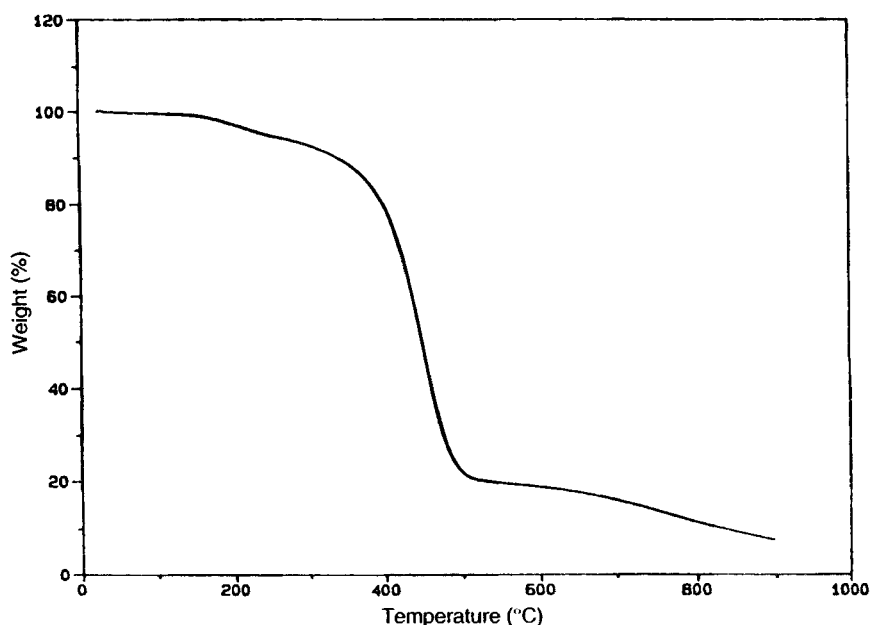
FIG. 5.   Thermogram of copolymer of hydrogenated cardanol with p-phenyl phenol.

The TG thermogram of the copolymer of hydrogenated cardanol with p-phenyl phenol is shown in Fig. 5. The characteristic thermal data of the copolymers prepared from cardanol and hydrogenated cardanol with substituted phenols are provided in Table 3 with the weight loss at different temperatures. A 2 to 10% weight loss occurs for all the copolymers when they are heated up to 200°C. This may be attributed to moisture retained in the sample. About 50–70% weight loss occurs in the 450–500°C region in the case of all the copolymers, and 80% weight loss occurs at 800°C. In the case of the copolymer prepared from hydrogenated cardanol and p-phenyl phenol, 20% weight loss occurs at 400°C and thereafter degradation is rapid. Kobayashi et al. reported the TG analysis of the polyphenol prepared using HRP–hydrogen peroxide catalyst [13]. By comparing the thermal

TABLE 3.   Thermal Decomposition of the Copolymers Measured by Thermogravimetric Analysis

Polymer system[a]	Percentage weight loss at various temperatures						
	200°C	300°C	400°C	500°C	600°C	700°C	800°C
Card + phenol	8	17	35	55	62	68	75
CardH + phenol	12	23	38	48	–	–	–
CardH + p-ethyl phenol	5	12	28	58	67	76	81
CardH + p-phenyl phenol	3	8	22	78	81	85	88

[a]Card = cardanol. CardH = hydrogenated cardanol.

data of the polyphenol with cardanol–phenol or hydrogenated cardanol–phenol copolymer, it is evident that the homopolymer has greater thermal stability than the copolymers. Under nitrogen, a 10% weight loss was observed at 387°C and a 57% weight loss occurred at 1000°C. The difference in thermal stability is due to the structural differences of these two types of compounds. In the case of cardanol or hydrogenated cardanol, there is a long hydrocarbon chain at the meta position. The degradation of the long hydrocarbon chain occurs at a lower temperature. Similar observations were reported earlier by Nayak et al. while studying the degradation pattern of cardanol–formaldehyde and phenol–formaldehyde resins [14].

Further, comparing the thermal stability of the three copolymers prepared from hydrogenated cardanol with phenol, *p*-ethyl phenol and *p*-phenyl phenol, it is observed that the copolymer from *p*-phenyl phenol is more stable up to 400°C (~20% weight loss) than the other two copolymers. The enhancement in stability is due to the rigidity of the copolymer because of the presence of a phenyl group at the para position. On the other hand, above 400°C the degradation of this copolymer becomes rapid because breakage of the carbon—carbon bond of the phenyl group at the para position might take place along with the degradation of the long hydrocarbon chain at the meta position.

CONCLUSIONS

Polymers of monomers derived from cashew nut shell liquid have been enzymatically synthesized. The copolymers with derivatives of phenols are insoluble in common solvents, while the homopolymers are low molecular weight oligomers. The polymer yield was dependent on the nature of the comonomer. The interfacial polymerization reaction was observed to be very fast, which required continuous stirring to achieve increased polymer chain length. The monomers organize in the micellar form, with the polymer chain growing in micellar curvature. SEM studies indicate that the homopolymer forms a spherical particle while the copolymers with phenols show featureless structures. The surfactant properties of the monomer aid in the formation of these spherical particles. The thermal properties of the copolymers are dependent on the cosubstrates. The copolymers degrade at lower temperatures compared to the homopolymeric polyphenols.

ACKNOWLEDGMENTS

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