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ORIGINAL ARTICLE

Horseradish peroxidase-catalyzed polymerization of *ortho*-imino-phenol: Synthesis, characterization, thermal stability and electrochemical properties

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KEYWORDS

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Imine functionality;
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Abstract Enzymatic polymerization of phenols has been investigated extensively over the last decades. However, involving imine functional group in the side chain of an oligophenol and its effect on polymerization is poorly understood. Therefore, the influence of the imine functionality in the side chain of oligophenol for enzymatic polymerization is explored in this work. *Ortho*-imine substituted phenol, (*E*)-2-((*p*-tolylimino)methyl)phenol (PTIMP), was enzymatically polymerized using horseradish peroxidase (HRP) enzyme in aqueous organic solvents and hydrogen peroxide (H_2O_2) as an oxidant. Different parameters (solvent system, pH and reaction temperature) on polymerization were investigated. EtOH/pH 6.0 buffer (50:50 vol.%) at 25 °C in 24 h under air was found to be the optimum polymerization condition with 65% of yield and M_n = 6100 g/mol (DP ≈ 29, PDI = 1.09). Polymerization of PTIMP in the presence of HRP enzyme catalyst leads to the formation of an oligophenol containing phenylene and oxyphenylene repeat units. The resulting oligophenol is soluble in most of the organic solvents. Characterization of oligo(PTIMP) was achieved by NMR, UV–Vis, CV, FT-IR spectroscopy and thermogravimetric analysis.

1. Introduction

Enzymes have gained increasing attention due to their unique properties including high stability, selectivity, catalytic activity, low solubility in water and low toxicity. Enzymes have been used in a wide range of fields including biochemistry, chemistry, pharmaceutical applications, medicine and industry (food, textile, and etc.) [1,2]. Enzymes are usually derived from renewable resources and known as green catalysts. For exam-

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ple, Horseradish peroxidase (HRP) enzyme is obtained from horseradish plant [3].

In the last few decades, enzymatic polymerizations of phenolic compounds have been extensively investigated by many polymer research groups since they require milder reaction conditions (physiological pH, ambient temperature and pressure) and facile methods [4,5]. Oxidative polymerizations of a variety of phenols catalyzed by peroxidase enzymes produced various polyphenols with excellent toughness and high thermal stabilities [6]. Dordick et al. reported the first study on the polymerization of phenolic compounds using HRP enzyme in aqueous organic solvents [7]. HRP is a widely used enzyme catalyst in enzymatic reactions and has received considerable attention due to its catalytic properties under a variety of conditions. HRP enzyme activated by hydrogen peroxide (H_2O_2) is known to oxidize a wide range of aromatic compounds including phenols, anilines, phenolic acids, amines, indoles, and sulfonates to form free radicals, which then undergo polymerization via Carbon–Carbon (C–C) and Carbon–Oxygen (C−O) couplings [8,9]. Polymerization occurs via couplings of phenolic monomers through *ortho*- and *para*-positions given in the formation of phenylene and oxyphenylene repeat units.

Phenol–formaldehyde resins are extensively used in industrial applications as surface coatings, laminates, adhesives, and friction materials due to their excellent toughness and thermal-resistant properties. In the literature, phenol–formaldehyde resins are also reported as good antioxidants for plastics and rubber. But, formaldehyde is known to be a toxic compound, and the health hazards of formaldehyde have become a public concern. Therefore, it is necessary to discover environmentally benign and green synthesis methods for the production of phenol–formaldehyde resins [4,10,11]. Enzymatic oxidative polymerization is considered as a benign method compared to the conventional oxidative polymerizations. Polymerization of phenol derivatives using peroxidase enzymes provided an alternative opportunity for the production of polyphenols (phenolic resins) with no need to use toxic formaldehyde comonomer in the process [12]. A variety of phenolic compounds have been subjected to the enzymatic polymerization using HRP enzyme [13]. Phenol derivatives having different functionalities can be chemoselectively polymerized using this method without involving side groups during polymerization [14,15]. Conducting polymers can also be efficiently produced by enzymatic polymerization [16,17]. Moreover, HRP is used to remove phenolic compounds (especially, chlorophenols labeled as ''priority pollutants'' by the Environmental Protection Agencies) from wastewater [18].

Polyazomethines have gained widespread interest due to their useful properties such as high thermal stability, electrical and magnetic properties, mechanical properties, semiconducting and crystalline properties, and etc [19]. We previously described enzyme-catalyzed polymerization of a phenolic compound containing *para*-imine functionality at RT under air, affording an oligophenol with high thermal stability and good electroactivity [20]. We have pursued the study of *ortho*-imine functionality in the side chain of oligophenols as a means of tailoring the physical properties and to develop new types of materials. For this purpose; a new type of imine functionalized phenol derivative, PTIMP, was synthesized with a condensation reaction of 2-hydroxybenzaldehyde and *p*-toluidine. Obtained PTIMP monomer was then enzymatically polymerized in the presence of HRP enzyme and H_2O_2 oxidizer under different reaction conditions. A wide pH range from 5.0 to 9.0 and reaction temperatures from 20 to 55 °C were examined. The optimum polymerization was achieved in EtOH/pH 6.0 phosphate buffer (50:50 vol.%) at 25 °C resulting in the highest molecular weight (M_n = 6100 g/mol, DP * 29, PDI 1.09) and yield (65%). A new type of useful and high performance phenolic oligomer, which is not easily obtained by conventional polymerization methods, has been prepared by enzymatic polymerization of *ortho*-imine functionalized phenol employing HRP enzyme catalyst. Enzymatic polymerization of PTIMP leads to the formation of an oligomer containing phenylene and oxyphenylene repeat units as reported in previous studies. Oligo(PTIMP) was characterized using ^1H and ^{13}C NMR, FT-IR, CV, UV–Vis, TGA and GPC analyses.

2. Experimental

2.1. Materials and reagents

2-Hydroxybenzaldehyde (salicylaldehyde, Merck, Catalog #800640), *p*-toluidine (Merck, Catalog #808315), phosphate buffers (pH = 5.0, 6.0, 7.0, 8.0 and 9.0), hydrogen peroxide (H_2O_2, Sigma–Aldrich, Catalog #18304), *N,N′*-dimethylformamide (*N,N′*-DMF, Merck, Catalog #110983), dimethylsulfoxide (DMSO, Merck, Catalog #802912), chloroform ($CHCl_3$, Merck, Catalog #102431), acetone (Merck, Catalog #100013), methanol (MeOH, Merck, Catalog #106007), ethanol (EtOH, Sigma–Aldrich, Catalog #34923), dichloromethane (CH_2Cl_2, Sigma–Aldrich, Catalog #24233) and 1,4-dioxane (Merck, Catalog #103115) were used without further purification. Horseradish peroxidase (HRP) enzyme was purchased from AppliChem (practical grade II, Catalog #A3800) and used as received.

2.2. Synthesis of (E)-2-((p-tolylimino)methyl)phenol (PTIMP)

(*E*)-2-((*p*-tolylimino)methyl)phenol (PTIMP) was synthesized according to the synthetic routes reported in the literature [21]. In a 50 mL flask, 10.0 mmol 2-hydroxybenzaldehyde and 10.0 mmol *p*-toluidine were dissolved in 20 mL of ethanol and a piece of molecular sieve was added to the flask. The solution was stirred and refluxed at 80 °C for 4 h. The product, (*E*)-2-((*p*-tolylimino)methyl)phenol (PTIMP), was obtained by removal of ethanol, and followed by flash column chromatography (SiO_2 column, elution with 9:1 hexane/acetone) (Fig. 1).

FT-IR (KBr) of PTIMP: 3027 cm^{-1} (aromatic, =CH), 2916 cm^{-1} (azomethine, —N=CH—), 1605 cm^{-1} (—CH=N—), 1504 cm^{-1} (—C=C—), 1283 cm^{-1} (=C—O). ^1H NMR (400 MHz, DMSO-d_6) δ: 13.2 (s, H1), 8.9 (s, H6), 7.6 (d, H5), 7.3 (t, H3), 7.2 (m, H4, H7, H7′, H8 and H8′), 6.9 (d, H2), 2.33 (s, H9). ^{13}C NMR (100 MHz, DMSO-d_6) δ:

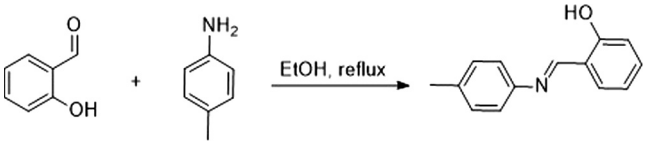

Figure 1 Preparation of the monomer.

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163 ppm (C1), 161 ppm (C7), 146 ppm (C8), 137 ppm (C11), 134 ppm (C3), 133 ppm (C5), 130 ppm (C10 and C10′), 122 ppm (C9 and C9′), 117 ppm (C4), 116 ppm (C6), 114 ppm (C2), 21 ppm (C12). UV–Vis of PTIMP in DMSO: 271, 291, 339 nm.

2.3. Enzymatic oxidative polymerization of PTIMP

0.211 g PTIMP and 10 mg HRP enzyme was dissolved in a mixture of 12.5 mL of an organic solvent and 12.5 mL of phosphate buffer at different pH conditions (5.0, 6.0, 7.0, 8.0 and 9.0). Then, 0.03 mL hydrogen peroxide was dropwise added to the mixture every 5 min for 10 times at desired reaction temperature. The mixture was stirred for an additional 6 h. After that, oligomer precipitates were vacuum filtrated and washed with water to remove unreacted monomer, enzyme and residual H_2O_2. The product was dried in an oven at 60 °C to give the target oligomer (Fig. 2) [22].

FT-IR (KBr) of oligo(PTIMP): 3035 cm^{-1} (aromatic, ═CH─), 2921 cm^{-1} (imine, ─N═CH─), 1580 cm^{-1} (─C═N─), 1479 cm^{-1} (─C═C─), 1280 cm^{-1} (C─O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.3 (s, H1), 8.9 (s, H6), 7.64 (d, H5), 7.32 (t, H3), 7.27 (m, H4, H7, H7′), 7.21 (m, H8 and H8′), 6.96 (t, H2), 2.31 (s, H9). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163 ppm (C1), 161 ppm (C7), 146 ppm (C8), 137 ppm (C11), 134 ppm (C3), 133 ppm (C5), 131 ppm (C10 and C10′), 121 ppm (C9 and C9′), 119 ppm (C4 and C6), 117 ppm (C2), 21 ppm (C12). UV–Vis of oligo(PTIMP) in DMSO: 271, 288, 302 nm.

2.4. Solubility results

Both the monomer (PTIMP) and oligo(PTIMP) were fully soluble in following organic solvents; 1,4-dioxane, *N,N′*-DMF, DMSO, acetone, chloroform, toluene and methanol. They were insoluble in water.

2.5. Characterization

Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded using a Bruker 400 FT-NMR Spectrometer at 25 °C using DMSO-d_6, and TMS was used as an internal standard. Number of scans is 32 with a 5 s relaxation delay for ^{1}H NMR. Chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS, 0.0 ppm). The FT-IR spectra were recorded on Spectrum BXII spectrometer using KBr disks in the region of 4000–400 cm^{-1} and 64 scans/sample. Ultraviolet–Visible (UV–Vis) spectroscopy experiments were carried out on Shimadzu 160A instrument using quartz cuvettes (path length: 1 cm, volume: 3 mL, DMSO solvent). Thermogravimetric analysis (TGA) was performed under nitrogen using a Perkin-Elmer pyrisdiamond 6.0 instrument. About 5–10 mg of sample was heated at 10 °C/min from RT to 1000 °C. Gel permeation chromatography (GPC) was performed at 40 °C using a Perkin-Elmer Series 200 instrument with an internal differential refractive index detector, and two TSK gel AM SEC Gel columns using a solution of 0.01 M LiCl in HPLC grade *N,N′*-dimethylformamide (*N,N′*-DMF) as the mobile phase at the flow rate of 1.0 mL/

Table 1 Enzymatic polymerization of PTIMP by HRP enzyme and H_2O_2 at 25 °C under air.

Entry*	Solvent	Buffer pH	Yield (%)	M_n (g/mol)	PDI
1	MeOH	5.0	35	1400	1.23
2	MeOH	6.0	42	1900	1.18
3	MeOH	7.0	58	2600	1.12
4	MeOH	8.0	51	2400	1.15
5	MeOH	9.0	39	1700	1.26
6	EtOH	5.0	46	4500	1.29
7	EtOH	6.0	65	6100	1.09
8	EtOH	7.0	52	5800	1.11
9	EtOH	8.0	39	5400	1.22
10	EtOH	9.0	25	4700	1.32
11	Acetone	5.0	24	1300	1.42
12	Acetone	6.0	33	1700	1.34
13	Acetone	7.0	35	2000	1.30
14	Acetone	8.0	28	1900	1.38
15	Acetone	9.0	21	1800	1.41
16	THF	5.0	34	2400	1.37
17	THF	6.0	49	2500	1.33
18	THF	7.0	50	2800	1.31
19	THF	8.0	44	2300	1.32
20	THF	9.0	30	2000	1.41
21	1,4-Dioxane	5.0	27	3300	1.30
22	1,4-Dioxane	6.0	38	3700	1.27
23	1,4-Dioxane	7.0	56	4500	1.22
24	1,4-Dioxane	8.0	52	4400	1.23
25	1,4-Dioxane	9.0	35	4100	1.22

* All polymerizations were carried out in a mixture of an organic solvent/phosphate buffer (1:1 vol.%) at 25 °C in 24 h under air. PDI, polydispersity index.

Table 2 Influence of reaction temperature on the polymerization of PTIMP at pH 6.0.

Entry*	T (°C)	Yield (%)	M_n (g/mol)	PDI
26	20	57	5500	1.13
27	25	65	6100	1.09
28	30	63	5900	1.10
29	35	61	5700	1.15
30	40	54	4900	1.22
31	45	45	4400	1.21
32	55	37	3000	1.34

* All polymerizations were carried out in EtOH/pH 6.0 buffer (1:1 vol.%) in 24 h under air. PDI, polydispersity index.

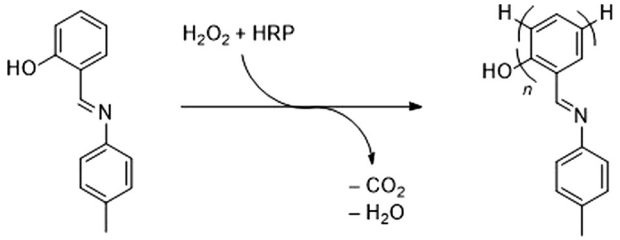

Figure 2 Chemoselective polymerization of PTIMP.

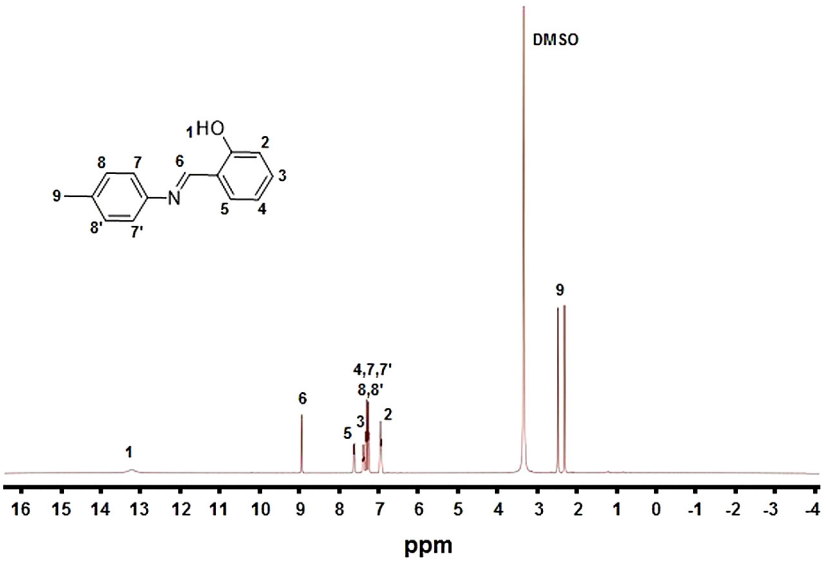

Figure 3 ^{1}H NMR spectrum of PTIMP.

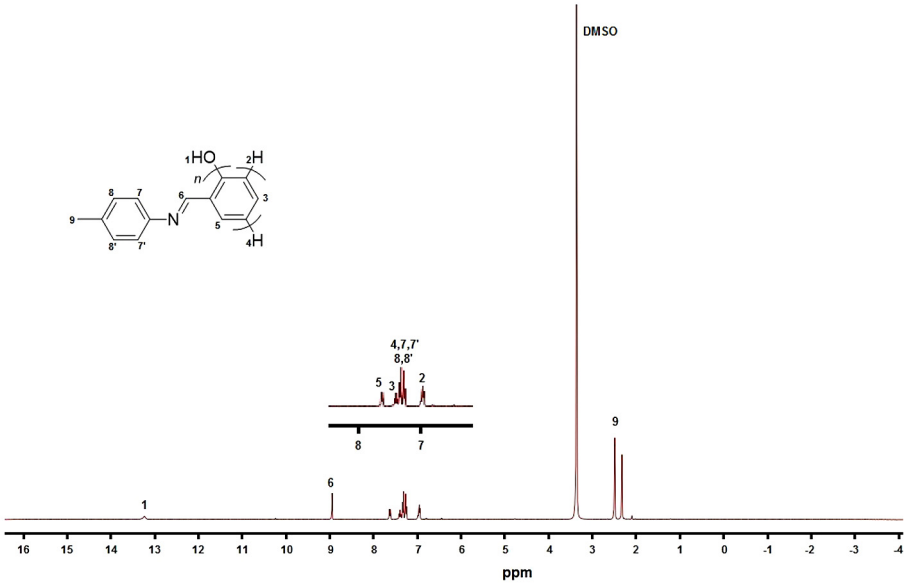

Figure 4 ^{1}H NMR spectrum of oligo(PTIMP) (entry 27, Table 2).

min. Calibration was performed with narrow polydispersity polystyrene (PS) standards. Cyclic voltammograms were obtained at RT in tetra-*n*-butylammonium tetrafluoroborate (TBAFB; 0.1 M)/DMSO electrolyte-solvent couple with a system consisting of an electrochemical analyzer (CH Instruments 660 B, USA) and a CV cell containing Pt plates as the working and counter electrodes, and an Ag wire as the pseudo reference electrode.

3. Results and discussion

The aim of this work was to investigate enzymatic polymerization of a Schiff base (imine) functionalized phenol and to show detailed characterization of the obtained material. *Ortho*-imine functionalized phenol derivative, (*E*)-2-((*p*-tolylimino)methyl) phenol (PTIMP), was synthesized according to the literature in high purity [21]. It was then chemoselectively polymerized in the presence of HRP enzyme and H_2O_2 oxidant. Chemoselectivity of HRP enabled to polymerize only the phenolic group without involving the imine functionality during the process and an oligophenol with an imine group in the side chain was successfully produced. During the synthesis of oligophenol, the reaction mixture became purple and then dark brown (blackish), indicating the formation of oligophenol after addition of H_2O_2. The obtained oligomer is a black powdery material and soluble in most of the solvents (such as

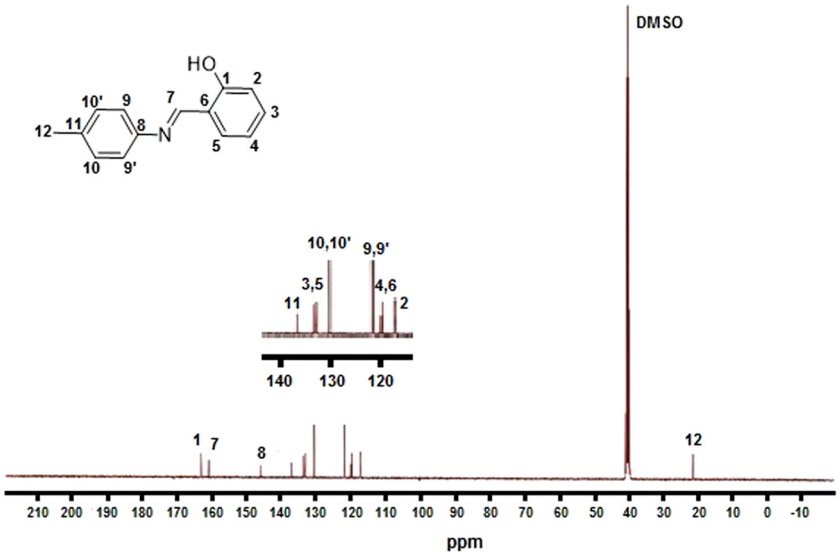

Figure 5 ^{13}C NMR spectrum of PTIMP.

THF, DMF, DMSO and etc.) and insoluble in water, which is similar to that of polyphenols synthesized from the mixture of phosphate buffer and water miscible organic solvents.

The effects of buffer pH value, organic solvents and reaction temperature upon the polymerization were investigated. Even though adding organic solvents in the reaction medium decrease in the activity of HRP enzyme [23], it is necessary to use water miscible organic solvents such as acetone, dioxane, methanol and etc. Therefore, we initially decided to evaluate effects of the solvent system on the polymerization, and we used five different water miscible solvents: methanol (MeOH), ethanol (EtOH), tetrahydrofuran (THF), acetone and 1,4-dioxane. It was found that employing ethanol as a cosolvent greatly affected the oligomer yield and molecular weight. Ethanol–water mixture (50:50 vol.%) was used as the optimized solvent.

The influence of buffer pH value on HRP catalyzed polymerization was then assessed. The results were summarized in Table 1. To address the impact of the buffer pH on the polymer molecular weight and reaction yield, polymerization was performed in five different pH conditions (pH 5.0, 6.0, 7.0, 8.0 and 9.0). The results have shown that buffer pH value highly affected the yield and polymer molecular weight. Polymerization in pH 6.0 condition produced oligophenol with moderately high yield and molecular weight, whereas the polymerization hardly proceeded in the buffers of pH $>$ 6.0 and pH $<$ 6.0 conditions. On the basis of these results, we have selected ethanol in phosphate buffer solution at pH 6.0 condition for further polymerizations. The reason of obtaining low yields could be a result of the low solubility of the obtained oligomer in the solvent system, thereby preventing further polymerization to get higher yields and molecular weights.

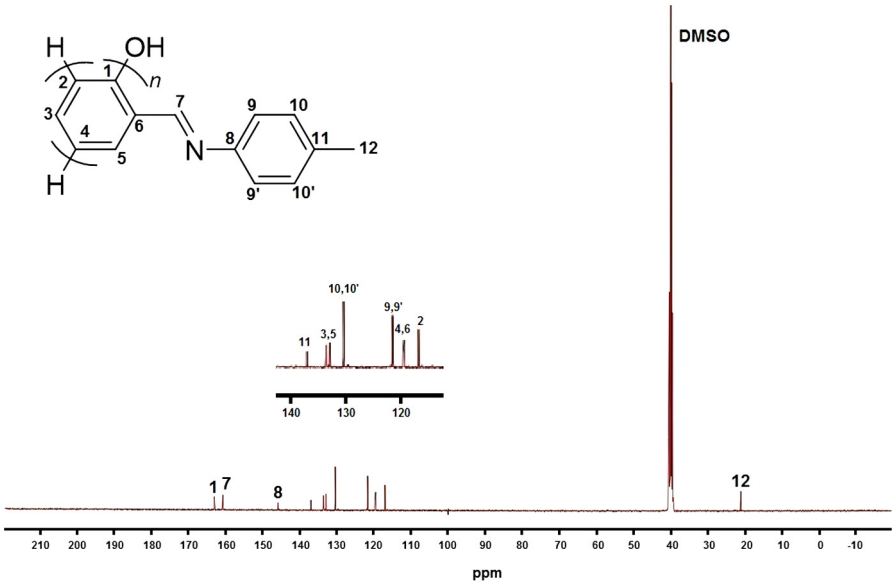

Figure 6 ^{13}C NMR spectrum of oligo(PTIMP) (entry 27, Table 2).

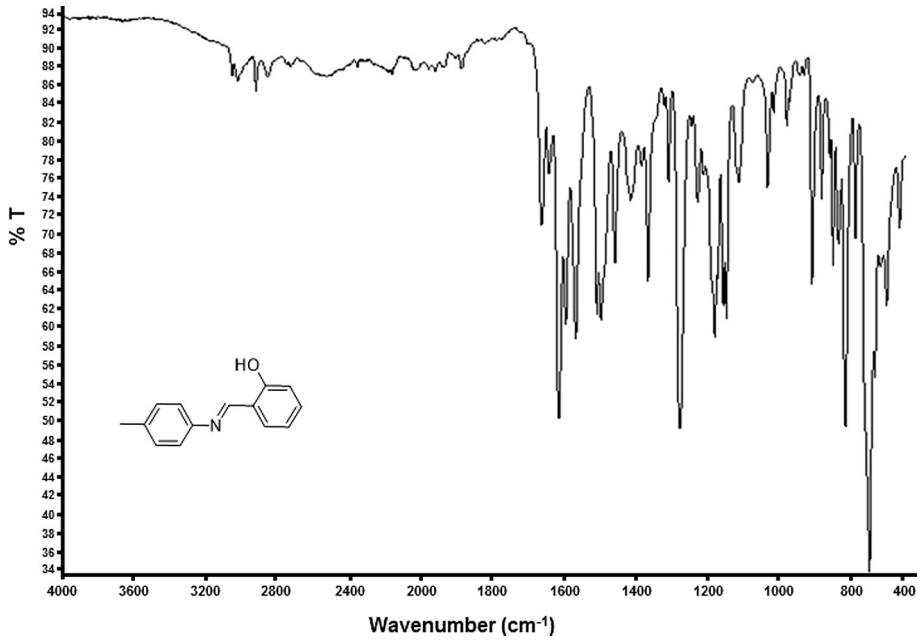

Figure 7 FT-IR spectrum of PTIMP.

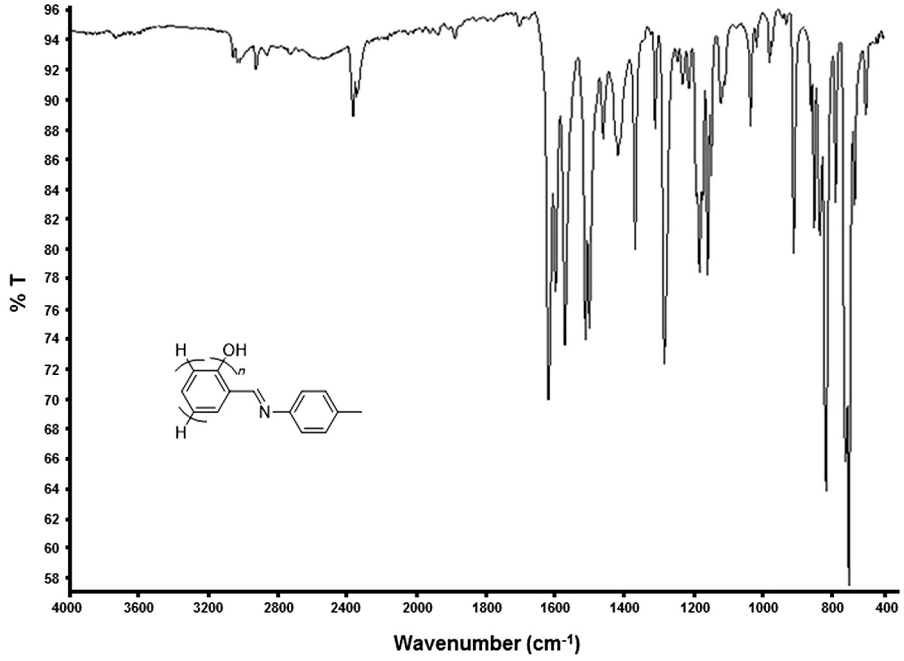

Figure 8 FT-IR spectrum of the oligo(PTIMP) (entry 27, Table 2).

Next, the influence of reaction temperature on polymerization was investigated and results are summarized in Table 2. In order to determine optimum polymerization temperature, all reactions were examined in EtOH/pH 6.0 buffer (50:50 vol. %) in 24 h under air, and 25 °C reaction temperature was found to be the optimum polymerization temperature. The reaction yield and oligomer molecular weight decreased with increasing reaction temperatures. It is known that HRP enzyme is thermally deactivated at ≥60 °C; therefore, higher temperatures were not performed for polymerization [24,25].

3.1. ^1H NMR spectra studies

The structures of both PTIMP and oligo(PTIMP) were first analyzed by ^{1}H NMR spectroscopy in Figs. 3 and 4, respectively. The peaks appeared at δ = 13.2, and 8.9 ppm were

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assigned to the imine (—CH═N—) and phenol (—OH) protons of PTIMP (Fig. 3). The peak at δ = 2.33 ppm was the signal of the methyl protons connecting to phenyl ring. The signals between 6.9 and 7.6 ppm belonged to the phenyl ring protons. The oligomer sample possessed similar ^{1}H NMR chemical shifts with its monomer. The ^{1}H NMR spectrum of the oligomer presents overlapping of some signals with its monomer (Fig. 4). The signals belonging to the phenolic (—OH) and azomethine (—CH═N—) protons of the oligomer are observed respectively at 13.3 and 8.9 ppm. The signals between 6.9 and 7.6 ppm belonged to the protons of the oligomer phenyl rings. Methyl protons connecting to the phenyl ring was observed as a singlet at 2.31 ppm. The similar observations of the proton signals of the monomer and the oligomer in the ^{1}H NMR spectra prove that both are successfully prepared.

3.2. ^{13}C NMR spectra studies

The ^{13}C NMR spectra further confirmed the structures of both PTIMP and oligo(PTIMP). The presence of the imine group of the monomer was observed at 161 ppm (Fig. 5). The peaks between 116 and 161 ppm belonged to the carbons of the phenyl rings of the monomer. Methyl carbon signal connected to the phenyl ring of both the monomer and the oligomer was observed at 21 ppm. ^{13}C NMR spectrum of the oligo(PTIMP) gives similar carbon chemical shifts as the monomer (Fig. 6). Azomethine carbon peak was located at 161 ppm. Aromatic carbons of the oligomer were observed between 117 and 161 ppm.

3.3. FT-IR analyses

FT-IR spectra of PTIMP and oligo(PTIMP) are demonstrated in Figs. 7 and 8, respectively. PTIMP monomer exhibited characteristic absorption peaks centered at 2916 and 1605 cm^{-1} attributed to the imine group (Fig. 7). Absorptions at 1587, 1504 ve 1441 cm^{-1} were ascribed to C═C vibrations of phenyl rings. Absorption band at 1280 cm^{-1} is due to C—O vibration. Based on these absorption bands, it was verified that the monomer has been successfully synthesized. The FT-IR spectrum of the oligomer has very similar characteristic absorption bands. Stretching vibrations of the imine group of oligo (PTIMP) are observed at 2921 and 1580 cm^{-1} (Fig. 8). Absorptions at 1512, 1479, 1445 cm^{-1} are attributed to C═C vibration modes of aromatic rings. The absorption at 1211 cm^{-1} corresponds to C—O—C asymmetric stretching vibration of the aromatic ether bonds. Disappearance of a band at 850 cm^{-1} from oligo(PTIMP) FT-IR spectrum indicates the 1,2,4-substitution pattern where the repeat units are coupled through *ortho*- or *para*-positions of the phenolic moiety, verifying thus the polymerization of trisubstituted phenyl ring [16]. Therefore, oligo(PTIMP) was considered to comprise the *ortho–ortho*-, *ortho–para*- and oxy-*ortho*-connected phenyl repeat units.

3.4. Uv–Vis spectra results

UV–Vis spectroscopy was performed for the monomer and the oligomer (entry 27, Table 2) shown in Figs. 9 and 10, respectively. The UV–Vis spectra of PTIMP and oligo(PTIMP) were quiet similar. Absorption bands at 271 and 291 nm of the monomer spectrum were interpreted by $\pi \rightarrow \pi^*$ transitions of (—C═C—) phenyl rings and imine group (—N═CH—), respectively. A shoulder appeared at 339 nm was due to $n \rightarrow \pi^*$ transition of the imine group (Fig. 9). The spectrum of the oligomer is red shifted from the monomer spectrum. $\pi \rightarrow \pi^*$ transition of (—C═C—) phenyl rings and imine functionality (—N═CH—) of the oligomer were observed respectively at 271 and 288 nm. $n \rightarrow \pi^*$ absorption of the imine group of the oligomer was observed at 302 nm. Absorption band was also extended up to 540 nm in the spectrum of the oligomer (Fig. 10). This is an evidence for the increase in conjugation in the oligomer backbone [26,27].

3.5. CV studies

Electrochemical characterization of the product was investigated with cyclic voltammetry and the result is shown in Fig. 11. The voltammetric measurement of oligo(PTIMP) (entry 27, Table 2) was carried out in dimethylsufoxide (DMSO). Two oxidations at −0.7 and +0.5 V and two reductions at −1.0 and −0.6 V for oligo(PTIMP) were obtained respectively in the anodic and cathodic regions versus Ag/AgCl between −1.5 and +1.5 V for 0.2 V/s scan rate. On recording the cyclic voltammogram at different scan rates between 100 and 800 mV/s, it was observed that there is a noteworthy change in the anodic and cathodic peak currents (I_{pa} and I_{pc}). Anodic and cathodic peak currents demonstrate a linear relationship as a function of the scan rates indicating that the electrochemical process is controlled by diffusion [28–30].

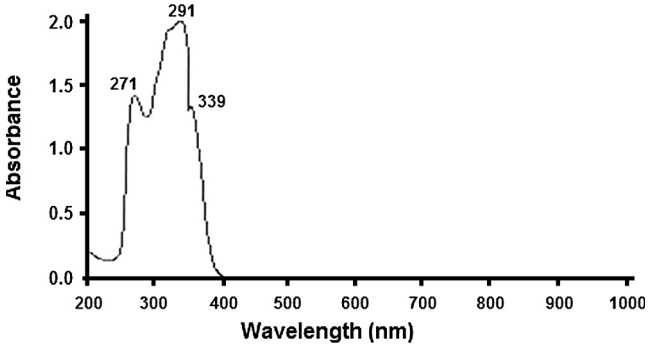


Figure 9 UV–Vis spectrum of PTIMP.

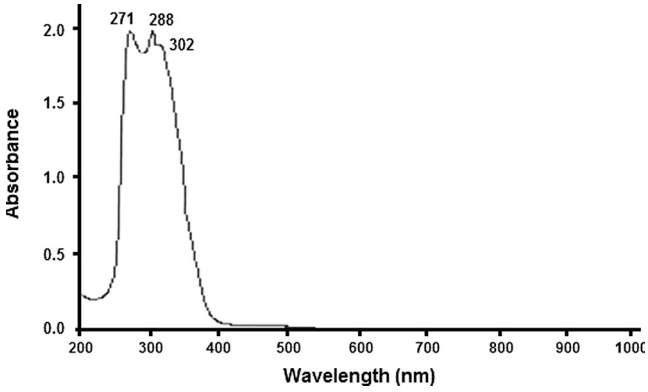


Figure 10 UV–Vis spectrum of oligo(PTIMP) (entry 27, Table 2).

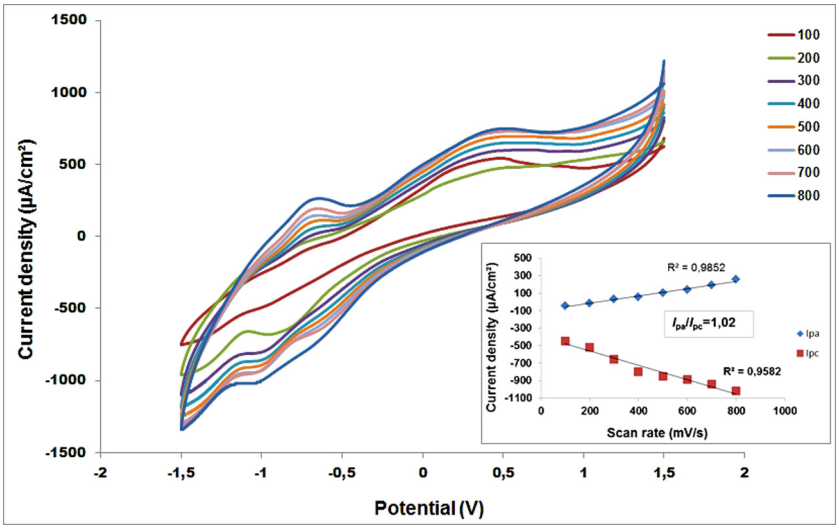


Figure 11 Cyclic voltammograms of oligo(PTIMP) (entry 27, Table 2) in monomer-free electrolyte at different scan rates between 100 and 800 mV/s. Relationship between anodic and cathodic peak currents vs. scan rate.

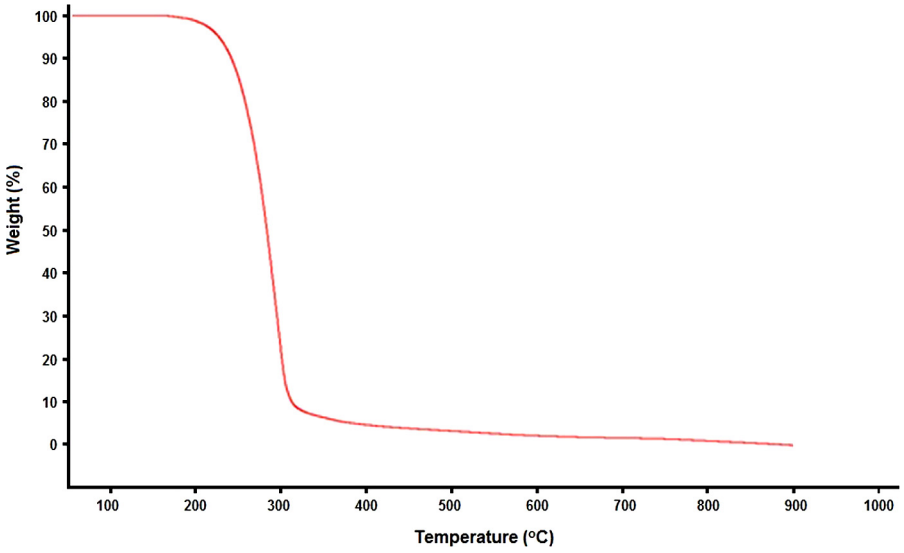
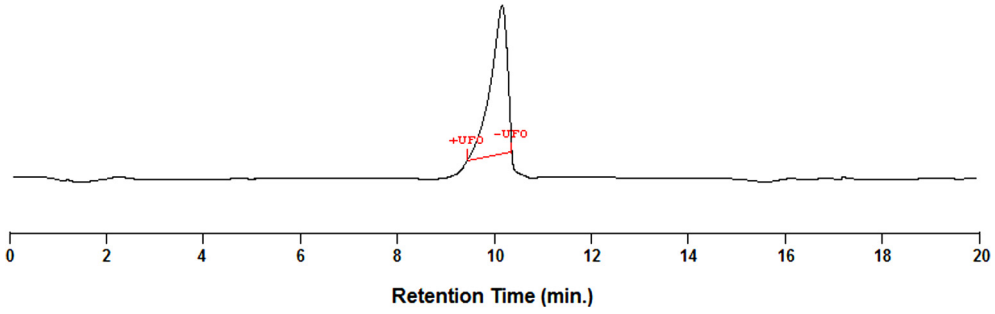


Figure 12 TGA thermogram of oligo(PTIMP) (entry 27, Table 2).



Peak Molecular Weight Report

Peak	Component	M_w	M_n	M_w/M_n	Start M_w	End M_w
1	Oligo(PTIMP)	6628	6093	1.09	16569	3822

Figure 13 GPC trace of oligo(PTIMP) (entry 27, Table 2).

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The slope of these curves indicated that the oligomer was electroactive and well adhered to the electrode [31,32].

3.6. TGA analysis

Thermal stability of oligo(PTIMP) was studied by thermogravimetric analysis (TGA). Fig. 12 shows TGA trace of oligo(PTIMP) (entry 27, Table 2) at 10 °C/min heating rate under nitrogen atmosphere. It can be seen that oligo(PTIMP) lost 5% of its weight at 220 °C and lost 90% of its weight at 310 °C. This implies that oligomer sample had relatively high thermal stability with long conjugation since it increases the delocalization of π electrons. Oligo(PTIMP) completely decomposes at about 900 °C [26]. TGA results show that synthesized oligomer has relatively high thermal stability, and therefore, it could be used for the applications in high temperature stable materials.

3.7. GPC results

The number-average molecular weights (M_n) and polydispersities (PDI) of the products measured by GPC analysis in DMF with commercially available polystyrene standards for calibration. Oligo(PTIMP) with the highest M_n was found to be 6100 g/mol and its polydispersity was 1.09 (Fig. 13). Obtaining low PDI indicates a relatively narrow range of the molecular weight due to removal of low molecular weight oligomers during the workup procedure.

4. Conclusions

In conclusion, oligophenol having *ortho*-imine functionality was efficiently produced through enzymatic polymerization of (*E*)-2-((*p*-tolylimino)methyl)phenol (PTIMP) in EtOH/pH 6.0 buffer (50:50 vol.%) and H_2O_2 oxidizer at 25 °C under air. The monomer (PTIMP) was chemoselectively polymerized under this reaction condition and a new type of oligophenol possessing *ortho*-imine side-chain was obtained in 65% of yield with M_n = 6100 g/mol (DP ≈ 29, PDI = 1.09). The resulting oligomer possessed oxyphenylene and phenylene repeat units. According to thermal analysis results, the product lost 5% of its mass around 220 °C and lost 50% of its mass around 310 °C showing that oligo(PTIMP) was not highly thermostable compared to the previous reports. Oligo(PTIMP) also completely decomposed around 900 °C. Cyclic voltammetry study also confirmed the electroactive nature of the product. Further investigations can explore the applications of the synthesized imine functionalized oligophenols.

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