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ORIGINAL PAPER

Chemoenzymatic polycondensation of *para*-benzylamino phenol

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para-Benzylamine substituted oligophenol was synthesized via enzymatic oxidative polycondensation of 4-(benzylamino)phenol (BAP). Polymerization involved only the phenolic moiety without oxidizing the *sec*-amine (benzylamine) group. Chemoselective polycondensation of BAP monomer using HRP enzyme yielded oligophenol with *sec*-amine functionality on the side-chain. Effects of various factors including solvent system, reaction pH and temperature on the polycondensation were studied. Optimum polymerization process with the highest yield (63 %) and molecular weight (M_n = 5000, degree of polymerization ≈ 25) was achieved using the EtOH/ buffer (pH 5.0; 1 : 1 vol. ratio) at 25 °C in 24 h under air. Characterization of the oligomer was accomplished by ^{1}H NMR and ^{13}C NMR, Fourier transform infrared spectroscopy (FT-IR), gel permeation chromatography (GPC), ultraviolet-visible spectroscopy (UV-Vis), cyclic voltammetry (CV) and thermogravimetric analysis (TGA). The polymerization process involved the elimination of hydrogen from BAP, and phenolic -OH end groups of the oligo(BAP), confirmed using ^{1}H NMR and FT-IR analyses. The oligomer backbone possessed phenylene and oxyphenylene repeat units, and the resulting oligomer was highly soluble in common organic solvents such as acetone, $CHCl_3$, 1,4-dioxane, *N*,*N*-dimethylformamide (DMF), tetrahydrofurane (THF) and dimethylsulfoxide (DMSO). Oligo(BAP) was thermally stable and exhibited 5 % and 50 % mass loss determined by thermogravimetric analysis at 247 °C and 852 °C, respectively.

Keywords: enzymatic oxidative polymerization, horseradish peroxidase enzyme, oligophenol, hydrogen peroxide

Introduction

Enzymatic oxidative polycondensation of phenols has been extensively studied by many polymer research groups in the last decades. Syntheses of a variety of polyaromatics using aniline and phenol derivatives were accomplished enzymatically using oxidoreductase enzymes under mild reaction conditions providing good yields. This method provides an alternative way to the synthesis of phenolic resins (phenol–formaldehyde resins) by peroxidase-catalyzed coupling of phenols without employing a toxic formaldehyde comonomer (Tonami et al., 1999; Goretzki & Ritter, 1998; Uyama et al., 1997). The possibility to produce phenolic polymers in aqueous ambient conditions provides an important option considering green chemistry aspects. Peroxidase catalyzed polymerization involves direct coupling of phenolic monomers through primarily *ortho* coupling with a possibility of *para* linkage, and carbon–oxygen and carbon–carbon coupling

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of phenols resulting in the formation of oxyphenylene and phenylene repeat units in the product (polyphenol) (Dordick et al., 1987).

Catalytic activity of enzymes is reduced in organic solvents due to their denaturation. Utilization of organic solvents is also not preferred in environmentally benign polymer synthesis (Zhang et al., 2013). Phenolic compounds are weakly soluble in aqueous conditions, which limits the use of aqueous media in the enzymatic oxidative polymerization. Dimers and trimers of phenols formed during the polymerization are even less soluble in water, and precipitation occurs without further polymerization. Therefore, aqueous organic solvents have been used to overcome the low solubility of phenols in water during the polymerization. The effect of the solvent composition on the coupling selectivity (regioselectivity) was investigated, yielding more soluble polyphenols (Uyama et al., 1994). Controlling water content in the organic solvent also provides a significant control over the polydispersity and polymer size of the product (Zheng et al., 2015; Eker et al., 2009; Tanaka et al., 2010).

Enzymes can be derived from renewable feedstocks (for example, horseradish peroxidase (HRP) is found in the roots of horseradish), and they are considered as environmentally benign and green catalysts compared to classical initiators (Vietch, 2004). Enzymatic polycondensation of phenols was first reported by Dordick et al. (1987), who reported enzymatic oxidative polycondensation of phenols using the HRP enzyme in aqueous organic solvents. Various organic and inorganic electron donor compounds including phenols, amines, indoles, phenolic acids and sulfonates can be oxidized using a peroxidase enzyme catalyst and H_2O_2. There is a huge amount of structurally diverse phenols which have been subjected to enzymatic oxidative polycondensation (Uyama & Kobayashi, 2002). Additionally, phenol derivatives having different functional groups can also be chemoselectively polymerized using the enzymatic polycondensation method. For example; when a phenolic compound with a methacryloyl group was subjected to enzymatic polycondensation, chemoselectivity of the enzyme enabled to polymerize only the phenolic moiety without involving the methacryloyl group in the polycondensation process, and a polyphenol with a methacryloyl group on the side-chain was efficiently produced (Uyama et al., 1998). Moreover, enzymatic polycondensation has also been efficiently performed to synthesize conducting polymers (Nabid & Entezami, 2003a, 2003b) and to remove phenol from wastewater (Moulay, 2009; Pradeep et al., 2012; Narayan & Pushpa, 2012).

In literature; aminophenols with primary amine functionalities (for example; 4-aminophenol which contains both phenolic –OH and anilinic $–NH_2$ groups) were polymerized employing the HRP enzyme (Shan et al., 2003). However, amine oxidation was involved in the polycondensation leading to extra branching with an amine group. The present study is focused on enzymatic oxidative polycondensation of *para*-benzylamine functionalized phenol (BAP) with a secondary amine functionality and the determination of its chemoselectivity. Anilinic $–NH_2$ group was protected by a benzyl group to obtain the *sec*-amine functionality. Because the benzyl group is considered to be one of the most important amine protecting groups cleaved by catalytic hydrogenation (Wuts & Greene, 2006), its removal from the oligomer by postpolymerization modification might provide free amine groups for further functionalization of oligophenol.

BAP monomer was chemoselectively polymerized, and the polymerization only involved the phenolic moiety without the *sec*-amine group oxidation as a side reaction. Enzymatic polycondensation was achieved using the HRP enzyme and H_2O_2 as the oxidizer in an organic solvent/buffer (pH 5–8; 1 : 1 vol. ratio) at different temperatures under air. An oligophenol containing the *sec*-amine group on the side-chain with moderate molecular weight (M_n = 5000, degree of polymerization (DP) ≈ 25) was produced in a high yield (63 %). The optimum polymerization process was achieved using the EtOH/buffer (pH 5; 1 : 1 vol. ratio) at 25 ℃ under air. Characterization of the oligomer was accomplished using UV-Vis, FT-IR, ^{1}H NMR and ^{13}C NMR, Thermogravimetric analysis (TGA), and cyclic voltametry (CV) and gel permeation chromatography (GPC) analyses.

Experimental

4-Aminophenol (Merck, Germany), benzaldehyde (Merck), sodium borohydride (Merck), phosphate buffers (pH = 5.0, 6.0, 7.0, 8.0 and 9.0), hydrogen peroxide (Sigma–Aldrich, USA), *N*,*N*-dimethylformamide (DMF, Merck), dimethylsulfoxide (DMSO, Merck), tetrahydrofuran (THF, Merck), chloroform ($CHCl_3$, Merck), dichloromethane (DCM, Merck), acetone (Merck), methanol (Merck), ethanol (Sigma–Aldrich) and 1,4-dioxane (Merck) were used without further purification. HRP enzyme was purchased from AppliChem (Germany) and used as received.

^{1}H NMR and ^{13}C NMR spectra (Bruker-Instrument-NMR Spectrometer DPX-400; Germany) were recorded at 25 ℃ using DMSO-d_6, and TMS was used as an internal standart. Chemical shift is given in δ relative to TMS. FT-IR spectra were recorded on a Perkin–Elmer spectrum 400 (USA) spectrometer equipped with an ATR probe in the region of 4000–400 cm^{-1} at 64 scans per sample. UV-Vis spectroscopy experiments were carried out on a Shimadzu 160A (Japan) instrument using quartz cuvettes (path length: 1 cm, volume: 3 mL, solvent: DMF). Cyclic voltammograms were obtained at RT in tetra-*n*-butylammonium tetrafluoroborate (TBAFB; 0.1 M)/THF electrolyte-solvent coupled with a sys-

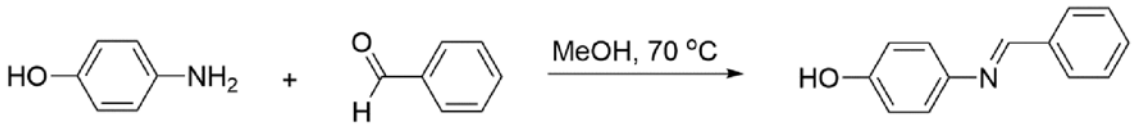

Fig. 1. Synthesis of (*E*)-4-(benzylideneamino)phenol.

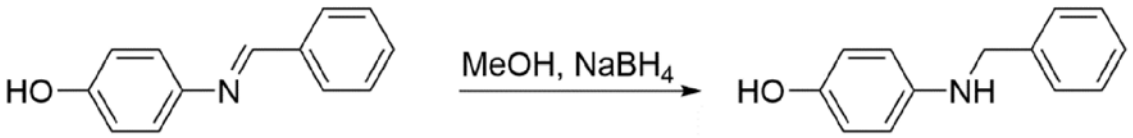

Fig. 2. Synthesis of the BAP monomer.

tem 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. TGAs were performed under N_2 using a Perkin–Elmer Pyrisdiamond 6.0 instrument. About 5–10 mg of each sample were heated at 10 ℃ min^{-1} from RT to 1000 ℃. Gel permeation chromatography (GPC) was performed at 40 ℃ 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 DMF as the mobile phase at the flow rate of 1.0 mL min^{-1}. Calibration was performed with narrow polydispersity polystyrene (PS) standards.

Synthesis of (E)-4-(benzylideneamino)phenol

(*E*)-4-(benzylideneamino)phenol was synthesized according to the procedure reported before (Fig. 1) (Kaya & Gül, 2004). 4-Aminophenol (2.725 g, 25.0 mmol) and benzaldehyde (2.65 g, 25.0 mmol) were stirred in MeOH (50 mL) at RT. The mixture was then refluxed at 70 ℃ for 2 h. After the reaction was completed, the solvent was evaporated. (*E*)-4-(benzylideneamino)phenol was crystallized in MeOH for further purification.

Synthesis of 4-(benzylamino)phenol monomer

BAP (8.0 g, 0.04 mol) was dissolved in MeOH (50 mL), and cooled down to 0 ℃ using an ice-bath. Then, $NaBH_4$ (0.38 g, 0.01 mol) was added to the mixture in portions. The reaction mixture was stirred for 2 h at RT. MeOH was evaporated, and HCl (10 vol. %, 10 mL) was added dropwise to the reaction mixture. The reaction mixture was then extracted with DCM, and washed with brine. The organic phase was dried over magnesium sulfate, and solvent was evaporated and the resulting product was further purified by flash column chromatography (SiO_2 column, elution with hexane/diethylether; $\varphi_r = 8 : 2$; Fig. 2) (Xu & Wang, 2010). BAP was

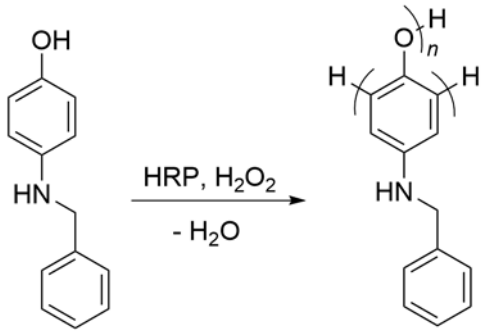

Fig. 3. Chemoselective polymerization of the BAP monomer using the HRP enzyme.

found to be fully soluble in 1,4-dioxane, THF, DMF, DMSO, acetone, chloroform and EtOH and insoluble in water. FT-IR of BAP: 3350 cm^{-1} (-NH stretch), 3000 cm^{-1} (broad, -OH), 1484 cm^{-1} (aromatic –C═C- stretch), 1400 cm^{-1} (-OH bend), 1206 cm^{-1} (aromatic C—N), 1168 cm^{-1} (C—O), 826 cm^{-1} (1,4-*para*-disubstitution), 746 cm^{-1} (aromatic C—H out-of-plane bend) and 699 cm^{-1} (H-bonded O—H out-of-plane bending). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 8.39 (s, Ha), 7.30 (m, Hf, Hg and Hh), 6.50 (d, Hb), 6.42 (d, Hc), 5.58 (s, Hd), 4.16 (s, He). ^{13}C NMR (200 MHz, DMSO-d_6), δ: 48 (C-5), 114 (C-3), 116 (C-2), 127 (C-9), 128 (C-7), 129 (C-8), 141 (C-6), 142 (C-4), 149 (C-1). UV-Vis/nm of BAP in DMSO: 269, 329.

Enzymatic polycondensation of BAP

BAP (0.05 g, 0.25 mmol) and HRP (1 mg) were dissolved in a mixture of EtOH/phosphate buffer (at the desired pH; 1 : 1 vol. ratio; 20 mL) under air. Then, H_2O_2 (70 μL, 34.5–36.5 %) was added to the mixture seven times every 10 min at the desired reaction temperature. After 24 h, oligomer precipitates were collected by vacuum filtration and the product was washed with MeOH and water to remove unreacted BAP and HRP. The obtained oligomer was dried in an oven at 60 ℃ (Fig. 3) (Kumbul et al., 2015).

Table 1. Enzymatic polycondensation of BAP by the HRP enzyme and H_2O_2 at 30 °C under air

Entry[a]	Solvent	Buffer pH	Yield/%	M_n/(g mol^{-1})	PDI
1	MeOH	5.0	58	4500	1.62
2	MeOH	6.0	53	3700	1.46
3	MeOH	7.0	44	2900	1.68
4	MeOH	8.0	48	4300	1.96
5	EtOH	5.0	60	4700	1.25
6	EtOH	6.0	54	4300	1.11
7	EtOH	7.0	54	4200	1.12
8	EtOH	8.0	58	4600	1.35
9	1,4-dioxane	5.0	38	1100	1.74
10	1,4-dioxane	6.0	21	900	1.44
11	1,4-dioxane	7.0	29	800	1.58
12	1,4-dioxane	8.0	38	1000	1.98

a) All polymerization processes were carried out in a mixture of an organic solvent/phosphate buffer (1 : 1 vol. ratio) at 30 °C for 24 h under air; PDI – polydispersity index.

Oligo(BAP) was found to be completely soluble in DMF and DMSO, partially soluble in THF, and insoluble in acetone, MeOH, EtOH, 1,4-dioxane, chloroform and water. FT-IR of oligo(BAP): 3034 cm^{-1} (broad, -OH), 1508 cm^{-1} (aromatic -C═C- stretch), 1233 cm^{-1} (aromatic C—N), 1168 cm^{-1} (C—O), 824 cm^{-1} (1,4-*para*-disubstitution), 697 cm^{-1} (H-bonded O—H out-of-plane bending) (Coates, 2000). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 10.10 (s, Ha), 7.40 (m, Hf, Hg and Hh), 6.90 (s, Hc), 6.50 (s, Hb), 5.80 (s, Hd), 4.83 (s, He). UV-Vis/nm of oligo(BAP) in DMSO: 273, 386.

Results and discussion

Phenolic compounds are weakly soluble in aqueous solutions, which limits the use of aqueous media for the enzymatic polycondensations of phenols. Dimers and trimers of phenols formed during the polymerization are even less soluble in water. Therefore, precipitation of oligophenols occurs without further polymerization. On the other hand, polymerization of phenolic compounds by peroxidase catalysts does not produce polymers in a buffer without using organic solvents. Hence, enzymatic polycondensation of phenols is achieved in an aqueous organic solvent such as acetone/water, 1,4-dioxane/water, etc. (Liu et al., 2000). Addition of organic solvents to the reaction media causes polyphenols solution in the buffer enabling thus polymerization progress.

Phenoxy radicals can be generated through one-electron oxidation of a phenol derivative in the presence of the peroxidase enzyme and H_2O_2 as the oxidizer, and they can undergo radical coupling and transfer reactions generating polymers (Kobayashi & Higashimura, 2003). The obtained polyphenol structures are mainly composed of oxyphenylene and phenylene repeat units formed by carbon–oxygen (oxy–*ortho*), carbon–carbon (*ortho*–*ortho*, *ortho*–*para* or *para*–*para*) coupling of phenols, respectively (Mita et al., 2002).

The aim of this work was to study the enzymatic oxidative polycondensation of a phenol derivative containing the *sec*-amine functionality and the detailed characterization of the resulting oligomer. A phenol derivative with the *para*-benzylamine functionality (BAP) was chemoselectively polymerized under enzymatic oxidative polymerization conditions, and only the phenolic moiety was subjected to the oxidative coupling without involving a side reaction with the *sec*-amine group.

A wide range of buffer pH, different solvent systems and reaction temperatures were studied to determine the optimum conditions of enzymatic polycondensation of BAP using HRP. All polymerization reactions were performed in an organic solvent/phosphate buffer (1 : 1 vol. ratio) under air using H_2O_2 as the oxidizer. An appropriate solvent system and pH enabling enzymatic oxidative polymerization of the monomer were first determined (Table 1). To address the effect of organic solvent, polymerization was performed using three different aqueous organic solvents (MeOH, EtOH and 1,4-dioxane); the results showed that the enzymatic polymerization of BAP is efficiently achieved in the EtOH/buffer (pH 5.0; 1 : 1 vol. ratio) at 30 °C in 24 h (Table 1, entry 5). The other solvent systems provided lower molecular weight and yield of the oligomer. Therefore, EtOH was chosen as the polymerization cosolvent, and reaction pH and temperature were changed to determine their role in the polycondensation.

In order to study the influence of reaction pH, polymerization was performed at four different pH conditions (pH = 5.0, 6.0, 7.0 and 8.0). The HRP enzyme is known to be catalytically inactive at pH $\approx$ 3.0; therefore, pH $<$ 5.0 were not studied (Ikeda et al., 1998). A similar behavior was also reported for the alkaline region and the HRP enzyme exhibits lower catalytic activities at pH $>$ 9.0; thus, neither pH values $>$ 8.0 were not considered for the polycondensation (Zheng et al., 2015). Table 1 also summarizes the influence of pH on the polymerization at 30 °C. Optimum polymerization

Table 2. Influence of reaction temperature on the polycondensation of BAP at pH 5.0

Entry[a]	T/℃	Yield/%	M_n/(g mol^{-1})	PDI
13	20	57	4500	1.18
14	25	63	5000	1.14
15	30	60	4700	1.25
16	35	56	4300	1.56
17	40	42	3400	1.88
18	45	30	1900	1.93
19	55	16	700	2.13

a) All polymerization processes were carried out in EtOH/buffer (pH 5.0; 1 : 1 vol. ratio) for 24 h under air; PDI – polydispersity index.

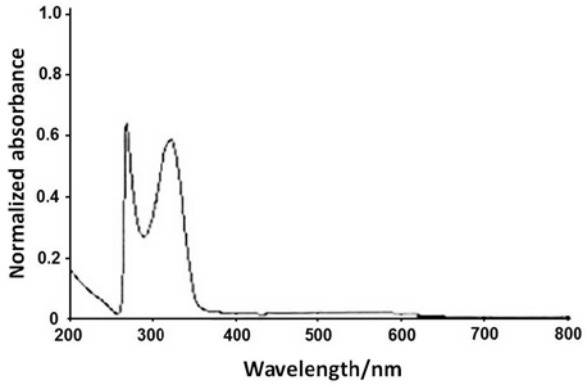

Fig. 4. UV-Vis spectrum of BAP.

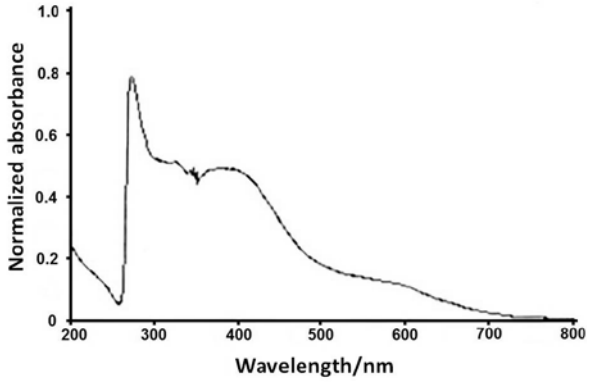

Fig. 5. UV-Vis spectrum of oligo(BAP) (Table 2, entry 14).

pH at 30℃ was found to be pH 5.0 in aqueous EtOH (Table 1, entry 5). Polymerization yield and oligomer molecular weight decreased at pH > 5.0. Lower yields were due to leaving the low molecular weight material in the solution during the work-up procedure to eliminate unreacted BAP and HRP.

After optimization of the reaction pH and solvent system parameters, polymerization temperature was optimized for the EtOH/buffer and pH 5.0. Table 2 summarizes the influence of reaction temperature on the polycondensation process; reaction temperature of 25℃ was found to be the best in terms of the highest yield (63 %) and number average molecular weight (M_n = 5000 g mol^{-1}) for the polymerization of BAP using the EtOH/buffer (pH 5.0; 1 : 1 vol. ratio) and H_2O_2 as the oxidizer (Table 2, entry 14). Higher or lower reaction temperatures decreased both yield and molecular weight of the product. The HRP enzyme is thermally deactivated at ≥ 60℃ (Ghoul & Chebil, 2012); therefore, reaction temperatures higher than 55℃ were not emploied for the polymerization.

Fig. 4 shows the UV-Vis spectrum of the monomer: $n\rightarrow\pi^*$ transition of the amine (-NH-) group and $\pi\rightarrow\pi^*$ transitions of the phenyl rings (-C═C-) of BAP were detected at 269 nm and 329 nm, respectively (Moulay, 2009). UV-Vis spectrum of the oligomer (Table 2, entry 14) illustrated in Fig. 5 displays similar absorption bands at 273 nm and 386 nm. The $n\rightarrow\pi^*$ transition band of the amine (-NH-) group and the $\pi\rightarrow\pi^*$ transition bands of the phenyl rings (-C═C-) of oligo(BAP) were detected as broad absorption bands at 273 nm and 386 nm, respectively. The similar absorption bands obtained for the monomer and the oligomer can be explained by the same resonance-inductive effects. UV-Vis spectrum of oligo(BAP) also displayed an absorption band up to 750 nm which is probably due to the polyaromatic conjugated system in its backbone (Wagner et al., 2002).

Figs. 6 and 7 provide the FT-IR spectra of the monomer and oligo(BAP) obtained via enzymatic polycondensation (Table 2, entry 14), respectively. The broad phenolic -OH stretch at around 3000 cm^{-1} and the secondary amine (-NH-) stretching band at 3350 cm^{-1} confirm the presence of the main functional groups of the monomer (Fig. 6). Similar bond stretching bands were observed also for the oligomer. Overlap of the stretching bands of amine (-NH-) and phenolic -OH groups on the oligomer was observed as a broad band at 3034 cm^{-1}. Disappearance of the band at 746 cm^{-1} from the oligomer spectrum indicates the 1,2-substitution pattern where the repeat units are coupled through *ortho*-positions of the phenolic moiety, verifying thus the polymerization of trisubstituted benzene (Fig. 7) (Kupriyanovich et al., 2008). FT-IR spectra of the monomer and oligomer show the secondary amine N—H wag bands at 699 cm^{-1} and 697 cm^{-1}, respectively (Stuart, 2004). If the secondary amine group had oxidized during the polycondensation, the product would have contained extra tertiary amine C—N stretches in the FT-IR spectrum of the oligomer and the secondary amine N—H peak would have disappeared or be very weak in the ^{1}H NMR spectrum of the product. Moreover, ortho positions of the amine group can also be coupled to produce extra substitution patterns in the structure of the oligomer; however, such coupling was not observed in the FT-IR spectra

Fig. 8 shows the ^{1}H NMR spectrum of the

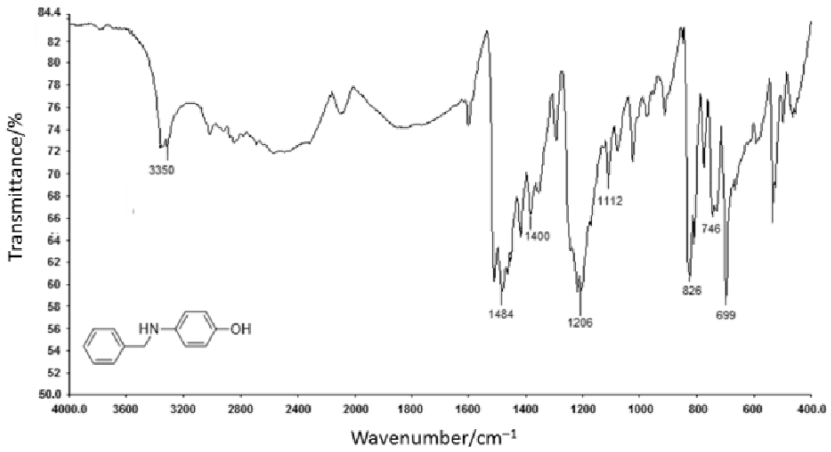

Fig. 6. FT-IR spectrum of BAP.

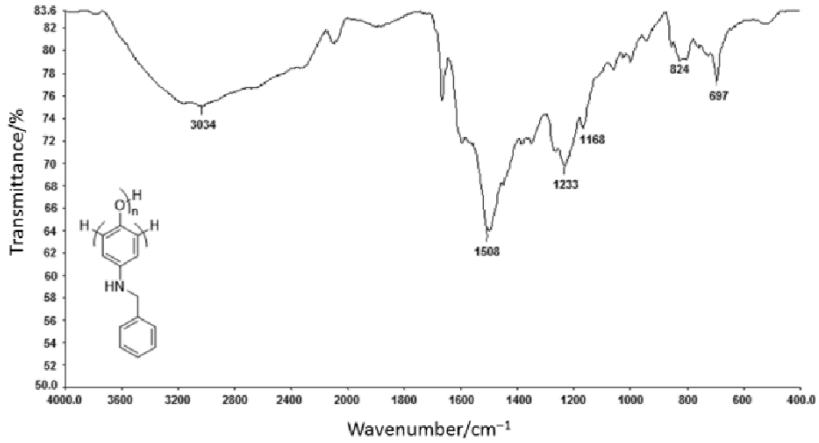

Fig. 7. FT-IR spectrum of oligo(BAP) (Table 2, entry 14).

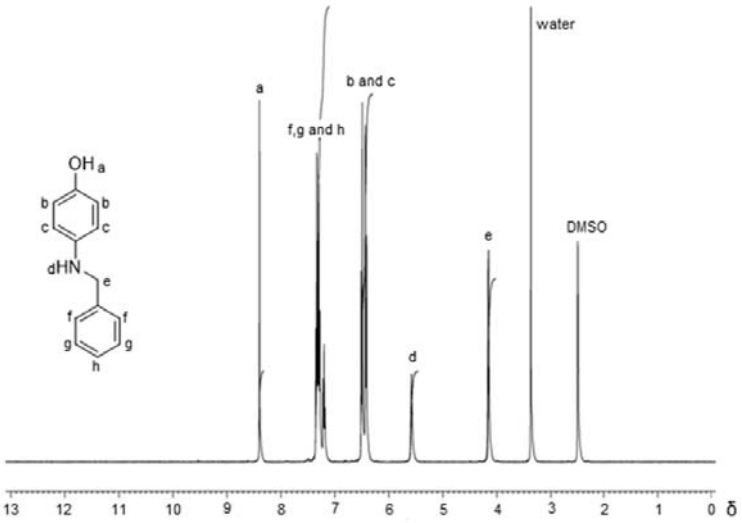

Fig. 8. ^{1}H NMR spectrum of BAP.

monomer. The singlet peak at δ 8.39 was attributed to the phenolic -OH (Ha) proton, the expected benzylamine (-NH-, Hd) and benzylic -CH_2 (He) protons were observed as singlets at δ 5.58 and 4.16,

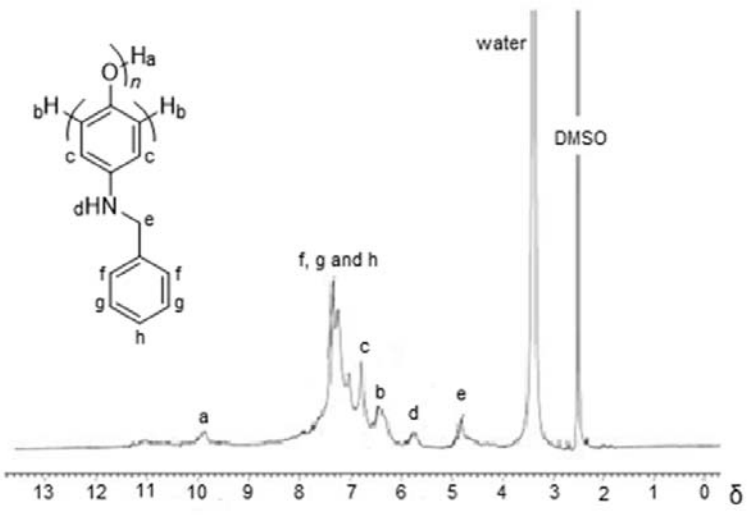

Fig. 9. ^{1}H NMR spectrum of oligo(BAP) (Table 2, entry 14).

Fig. 10. ^{13}C NMR spectrum of BAP.

respectively, verifying the structure of the monomer. The peaks at δ 7.30 (Hf, Hg and Hh), 6.5 (Hb) and 6.42 (Hc) were assigned to aromatic protons of the monomer (Turac et al., 2008). ^{1}H NMR spectrum of the product (Table 2, entry 14) also shows similar proton peaks as its monomer. The peak at δ 10.1 originates from the phenolic -OH (Ha) terminal unit protons (Fig. 9) and the benzylamine (-NH-, Hd) and benzylic -CH_2 protons (He) were observed at δ 5.80 and 4.83, respectively. Aromatic protons of the oligomer were slightly shifted to downfield and observed between δ 7.40, 6.90 and 6.50. Broadening of proton NMR was observed in previous studies and it was attributed to the polymerization reaction. Additionally, the decrease in the intensity of the Hb protons in the oligomer spectrum compared to the monomer indicates that some coupling reactions occur at the *ortho*-positions, which proves that these positions of the phenolic ring are the most favored for coupling.

Fig. 10 displays the ^{13}C NMR spectrum of the monomer (BAP). The peaks at δ 48 and 149 can be recognized as benzylic carbon (-CH_2-, C5) and C1 carbon of phenol forming the monomer. Assignments of other carbons are due to the aromatic carbons on the structure. The ^{13}C NMR data confirmed perfect agreement of the monomer structure with the expected

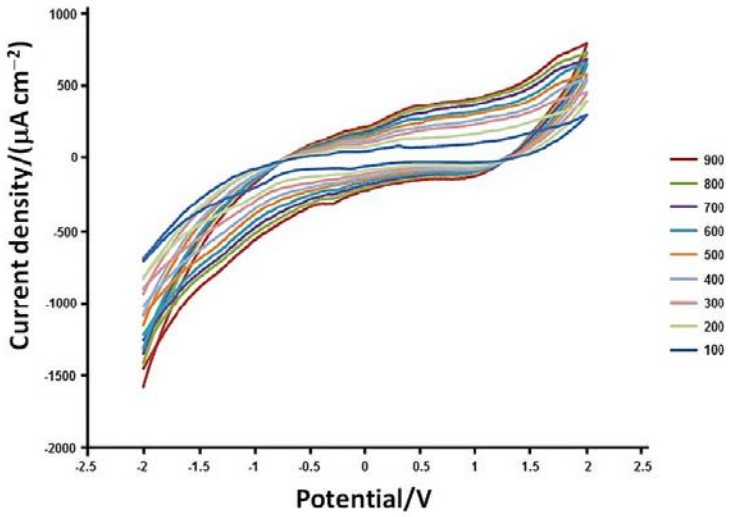

Fig. 11. Cyclic voltammograms of oligo(BAP) (Table 2, entry 14) in monomer-free electrolyte at different scan rates between 100 mV s^{-1} and 900 mV s^{-1}.

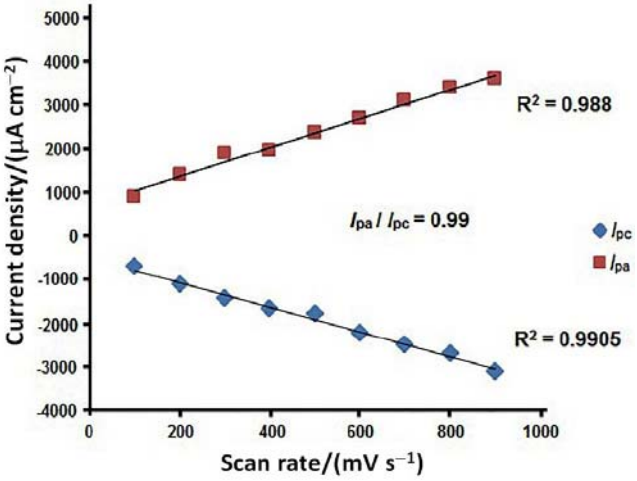

Fig. 12. Relationship between anodic and cathodic peak currents vs. scan rate.

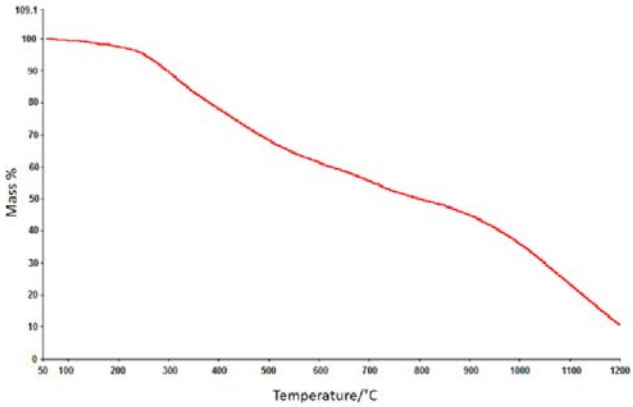

Fig. 13. TGA thermogram of oligo(BAP) (Table 2, entry 14).

chemical shifts (Fig. 10). ^{13}C NMR spectrum of the oligomer could not be taken successfully due to difficult shimming.

Electrochemical characterization of the oligomer (Table 2, entry 14) is shown in Figs. 11 and 12; one oxidation and one reduction peak were observed in the anodic and cathodic regions, respectively, at –0.30 V and 0.42 V vs. Ag/AgCl between –2.0 V and 2.0 V (Fig. 11). The peaks are very well defined and reversible over several cycles (Cheraghi et al., 2009). Anodic and cathodic peak currents (I_{pa} and I_{pc}) showed a significant change at different scan rates between 100 mV s^{-1} and 900 mV s^{-1} (Fig. 12). Peak currents (I_{pa} and I_{pc}) demonstrated a linear dependence on the scan rate indicating that the redox process is surface-controlled and the electrochemical process is reversible even at high scan rates.

Thermal properties of the product (Table 2, entry 14) were evaluated using TGA under N_2 in the range of 25–1200 ℃ (Fig. 13). TGA demonstrated a variety of thermal decomposition responses to increased temperatures. The mass loss of the sample starts at around 100 ℃, which corresponds to the removal of water and indicates that the product was not dried thoroughly before the experiment. Degradation of low molecular weight compounds was not expected at this temperature since the product has a narrow molecular weight distribution. The product mass loss of 5 % at 247 ℃ and of 50 % at 852 ℃ indicates that the oligomer is highly thermostable due to the long conjugated oligomer backbone and that it possesses high thermal stability against thermal decomposition; about 10 % of the initial mass of the sample (carbonaceous residue) remained after its heating up to 1200 ℃ (Kumbul et al., 2015).

Molecular weight of the oligomers was measured using GPC (DMF as the eluent phase with narrow PS standards); number-average molecular weight of the oligomers was in the range of several thousands.

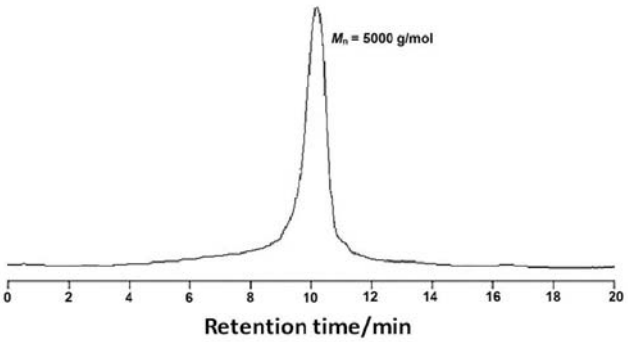

Fig. 14. GPC trace of oligo(BAP) (Table 2, entry 14).

The highest number-average molecular weight (M_n) was detected to be 5000 (DP ≈ 25; Table 2, entry 14) achieved with the EtOH/buffer at pH 5, vol. artio of 1 : 1 and 25 ℃. Its polydispersity index (PDI) was 1.14, which indicates relatively narrow range of the molecular weight of oligomers (Fig. 14) and the formation of oligomers during the polymerization process.

Conclusions

In conclusion, *para*-benzylamine functionalized oligophenol was successfully synthesized via enzymatic oxidative polymerization of BAP in EtOH/buffer (pH 5.0; 1 : 1 vol. ratio) and H_2O_2 as the oxidizer at 25 ℃ under air. The phenolic moiety was chemoselectively polymerized under this reaction conditions and a new class of oligophenols possessing the *sec*-amine side-chain was produced in a high yield (63 %) and with a moderate molecular weight (M_n = 5000, DP ≈ 25). The resulting oligomer contained oxyphenylene and phenylene repeat units. Thermal analysis results obtained for the oligomer showed a 5 % mass loss at 247 ℃ and a 50 % mass loss at 852 ℃, indicating that the oligomer was highly thermostable due to the long conjugated oligomer backbone. About 10 % of the initial mass of the oligomer (carbonaceous residue) remained after its heating to 1200 ℃, proving higher resistance against high temperature. Cyclic voltammetry also confirmed the electroactive nature of the oligomer. Further investigations can explore the applications of the synthesized *sec*-amine-functionalized oligophenols.

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