

# CNT Templated Regioselective Enzymatic Polymerization of Phenol in Water and Modification of Surface of MWNT Thereby

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**ABSTRACT:** Carbon nanotubes (CNTs) are used as templates to synthesize regioselective polymers from enzymatic polymerization of phenol in water. About 90% of total polymeric units in the obtained polymers are the highly thermally stable oxyphenylene units. The polymer-yields are dependent on the quantities of CNTs used. On the basis of MWNT-templated enzymatic polymerization of phenol, covalent attachment of polyphenol chains to the surface of MWNT by way of a linking molecule, hydroquinone, is achieved. This approach supplies a novel way for producing high-performance polymers and for functionalization of the surface of CNT. © 2009 Wiley Periodicals, Inc. *J Polym Sci Part A: Polym Chem* 47: 1627–1635, 2009

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## INTRODUCTION

Poly(phenylene oxide) (PPO) is one of high-performance thermally stable engineering plastics. PPO is usually synthesized from oxidative coupling polycondensation of 2,6-substituted phenols.<sup>1</sup> The synthesis of PPO moieties directly from the simplest unsubstituted phenol is still a challenge to polymer chemists, because that unsubstituted phenol is a multifunctional monomer in an oxidative polymerization. Few successful regioselective polymerization of 2,6-unsubstituted phenols has been reported. The only example reported by Higashimura et al. was that a peroxo dicopper(II) complex,<sup>1,2</sup> a copper-dioxygen model

complex, could catalyze highly regioselective oxidative polymerization of 4-phenoxyphenol to poly(1,4-phenylene oxide) with a conversion varying from 9 to 17% and weight-average molecular weight from 1100 to 29,100 dependent on different organic solvents and reaction times.<sup>3</sup>

Another possible approach to produce PPO moieties from phenol is regioselective oxidative polymerization of phenol catalyzed by peroxidase, including horseradish peroxidase (HRP), soybean peroxidase, and palm tree peroxidase.<sup>3b,4</sup> Horseradish peroxidase (HRP) is the most popularly used peroxidase to catalyze coupling of a number of phenol and aniline derivatives using hydrogen peroxide as oxidant. The polymer chains obtained from enzymatic polymerization of phenol may contain two kinds of repeating units, that is, phenylene and oxyphenylene.<sup>5</sup>

However, peroxidase catalyzed polymerization of phenol in water is limited by two factors (1). A

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major limitation of the enzymatic polymerization in pure aqueous solutions is that only dimers or trimers are formed. Thus, mixed solvents play an important role because the HRP is water-soluble and the polymers are only organic solvent-soluble. Many research studies indicate the influence of the solvent on the reaction. Organic solvents that are miscible in water, for example, dioxane,<sup>6</sup> acetone,<sup>2</sup> methanol,<sup>7</sup> must be used, despite the decrease in the activity of HRP (2). The control of the polymeric structure to afford soluble toward organic solvents and regioselective materials is an important issue in the enzymatic oxidative polymerization of phenolic compound. Micelles,<sup>8</sup> mesoporous materials,<sup>1c</sup> polymer templates such as PEG,<sup>9</sup> Langmuir-Blodgett films (LB films),<sup>10</sup> have been used for the design of polymers with controlled structures. The ratio of phenylene and oxyphenylene units (regioselectivity) can also be improved by changing the solvent composition ("solvent engineering").<sup>4</sup> However, nearly all reports on the regioselective enzymatic oxidative polymerizations of phenolic compounds produce phenylene-structure-prevailing polymers, perhaps because that phenylene structures with free-standing —OH groups show high affinity to aqueous environments. To our knowledge, no report on enzymatic approach to produce oxyphenylene-structure-prevailing polymers in water is found in the literature.

Carbon nanotubes (CNTs) have been regarded as a miracle material for polymer composites, nano-electrics, and many other modern applications. Carbon nanotubes (CNTs) are demonstrated as a good support for the immobilization of enzymes.<sup>11</sup> In the course of studying on the functionalization of CNTs,<sup>12</sup> we realize that confinement in interfacial thin films over CNT surfaces due to adsorption will be helpful for controlling the polyphenol's structures.

In this work, we demonstrate the use of multi-walled carbon nanotubes (MWNT) for controlling the regioselectivity in the enzymatic polymerization of unsubstituted phenol in water to produce thermally stable PPO, which has not been reported in the literature. Furthermore, attachment of the polyphenol to CNT sidewall is achieved through a linking molecule by *in situ* enzymatic polymerization of unsubstituted phenol. Modification of gold surfaces or the outer walls of CNTs by peroxidase-catalyzed *in situ* polymerization of many different monomers have been reported.<sup>13</sup> Grafting polymers to the surface of MWNT may find many important

applications in nano-composites, bio-sensors, and so forth.

## EXPERIMENTAL

### Materials

Single-walled carbon nanotube (SWNT, purity > 90%), multi-walled carbon nanotube (MWNT, (purity > 95%)) and carboxylic acid-functionalized multi-walled carbon nanotube (MWNT-COOH, (purity > 95%)) of high purity are obtained from Chengdu Organic Chemicals Co., Chinese Academy of Sciences. The MWNT has outside diameters of 8–15 nm, inside diameter of 3–5 nm. MWNT-COOH contains 1% mol. (surface carbon atom) of carboxylic acid groups. The SWNT has outside diameters of 1–2 nm, and tube-lengths of about 50  $\mu\text{m}$ . The SWNT, MWNT, and MWNT-COOH are purified by mixing in THF overnight and filtered, and then thoroughly washed with ethanol and then THF before usage. Hydroxyl-functionalized MWNT (MWNT-OH) is synthesized according to our previous work, with 29% mol. (surface atom) of hydroxyl groups, measured by XPS.<sup>13c</sup> Horseradish peroxidase (HRP, E.C. 1.11.1.7, RZ = 3, activity = 250 u/mg) and dicyclohexylcarbodiimide (DCC, 99%) are purchased from Sigma. Phenol, hydroquinone, hydrogen peroxide (30 wt % aqueous solution), anhydrous  $\text{K}_2\text{CO}_3$ , and DMF, DMSO, THF, and pyridine are purchased from local markets. Organic solvents are distilled and kept in the presence of 4 Å molecular sieves.

### Measurements

Fourier transform infrared (FTIR) spectra are carried out with on a Bruker VECTOR-22 IR spectrometer. The 300 MHz  $^1\text{H}$  NMR and 75 MHz  $^{13}\text{C}$  NMR measurements were performed on a Bruker Avance 300 spectrometer using TMS as an internal reference.

A Finnigan LCQ Advantage MAX LC/MS/MS ion trap mass spectrometer (ESI-MS; Thermo Finnigan, San Jose, CA, USA) was used in the electrospray ionization (ESI) mode. The spray voltage was 2.4 kV with a current of about 20 mA. Samples were introduced to the source by direct insert probe. MALDI-TOF-MS is performed on an Applied Biosystems Voyager-DE-STR (Foster City, CA). The matrix used is 2,5-dihydroxybenzoic acid (DHB).

Thermal gravimetric analyses (TGA) are performed on a diamond TG/DTA (Perkin–Elmer) with heating rate of 10 K/min under N<sub>2</sub> atmosphere. Differential scanning calorimetry (DSC) is obtained on a Perkin–Elmer Pyris Diamond DSC with a temperature gradient of 10 K/min. Polymer molecular weight and polydispersity index (PDI) are estimated by gel permeation chromatography (GPC) using a Waters 2414 with polystyrene as the calibration standard and THF as the eluent at a flow rate of 1.0 mL/min.

### Enzymatic Polymerization of Phenol in Presence of MWNT in Water

MWNTs (0.1 g) and phenol (0.1 g) are dispersed in 0.1 M phosphate buffer (pH 7.0, 20 mL) with sonication for 30 min. Then, a fresh enzyme solution of HRP (10 mg) in 0.1 M phosphate buffer (5 mL) is added. To this solution, hydrogen peroxide (5%, 3.4 mL, 5.6 mmol) was added dropwise for 2 h. The mixture is stirred rigorously at room temperature for 24 h. After that, the black solution is vacuum-filtered through a 0.22  $\mu$ m Millipore PVDF membrane and subsequently washed with deionized water thoroughly to remove HRP, residual H<sub>2</sub>O<sub>2</sub>, and phenol. The black filtrate (MWNT and polyphenol) is vacuum-dried. The black powder is redispersed in THF, and the raw polyphenol is collected as a THF solution by centrifugation at 10,000 g, followed by passing through a 0.22  $\mu$ m Millipore PVDF membrane. The raw polyphenol is purified by passing through a plug of silica gel, with ethanol eluent at first to give lower molecular weight fractions with 3–10 repeating units according to MALDI-TOF-MS analysis, followed by THF eluent to give a higher molecular weight part (0.042 g). The higher molecular weight part is collected for characterization. The characteristic of 300 MHz <sup>1</sup>H NMR of the products is a broad peak centered at about 7.0 ppm. 75 MHz <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 157.9, 156.7, 154.4, 150.0, 130.2, 128.9, 123.8, 118.9, 115.8, 115.1 ppm. FTIR: 3429 cm<sup>-1</sup>, 1628 cm<sup>-1</sup>, 1562 cm<sup>-1</sup>, 1472 cm<sup>-1</sup>, 1178 cm<sup>-1</sup>.

### Acetation Procedure of Polyphenol

To a dried 50 mL flask with polyphenol (0.1 g) and pyridine (20 mL) is charged with acetic anhydride (1.02 g, 100 mmol). The mixture is stirred at room temperature for 24 h. After that, the solution is condensed and the remainder is precipitated in deionized water. The polymer is collected and

washed with deionized water repeatedly. A light yellow solid is obtained after being vacuum-dried at 60 °C.

### Preparation of Hydroquinone-Functionalized MWNT (MWNT-HQ)

MWNT-COOH (0.150 g) is dispersed in 15 mL of DMF by a 30 min sonication treatment. Hydroquinone (0.220 g, 2 mmol) and DCC (0.412 g, 2 mmol) are added to the dispersion of MWNTs-COOH at room temperature. The mixture is then stirred for 12 h at ambient conditions. The resulting solid is then separated by vacuum-filtration using 0.22  $\mu$ m Millipore PVDF membrane filter and repeatedly washed with DMF and THF. Finally, the products are vacuum-dried overnight at 60 °C to give MWNT-HQ (0.172 g). FTIR: MWNT-HQ, 1626.0, 1564.3, 1396.5, 875.7; MWNT-COOH, 1704.0, 1560.4, 1475.5, 1174.6, 846.0.

### Preparation of Polyphenol-Functionalized MWNT (MWNT-g-PPO)

In this process, MWNT-HQ (0.100 g) and phenol (0.94 g, 10.0 mmol) are dispersed in 0.1 M phosphate buffer (pH 7.0) (20 mL) with sonication for 30 min. Then, a fresh enzyme solution of HRP (4.0 mg, about 1000 units) in 0.1 M phosphate buffer (5 mL) is added. To this solution, 6.8 mL of 5% hydrogen peroxide (11.2 mmol) is added dropwise for 2 h. The mixture is stirred rigorously at room temperature for 24 h. After that, the black solution is vacuum-filtered through a 0.22  $\mu$ m Millipore PVDF membrane and repeatedly washed with deionized water to remove water soluble enzyme, salts and phenol, and then with THF to remove freely standing polymers (0.018 g). For the purpose of removing any residual homopolymer and unreacted monomer from the functionalized CNTs, the black filtrate is redispersed in THF by sonication and is subjected to centrifugation at 10,000 g. After five cycles of dispersion-centrifugation, the black powder is collected, and is vacuum-dried overnight at 60 °C, generating MWNT-g-PPO (0.139 g).

### Cleavage of Polyphenol from MWNT-g-PPO

MWNT-g-PPO (0.080 g), THF (40 mL), and anhydrous K<sub>2</sub>CO<sub>3</sub> (3 g) are placed in a round-bottom flask. The mixture is stirred and refluxed for 72 h. The product is filtered, and the resulting filtrate

**Table 1.** Polymerization of Phenol on the Template of Different CNTs in 24 h

	MWNT	MWNT-OH	MWNT-COOH	SWNT
Yield (% wt) <sup>a</sup>	40 ± 3	56 ± 4	54 ± 4	48 ± 4
$M_n^b$	1880	2300	2200	n.d.
PDI	1.39	1.61	1.29	n.d.
PPO (% mol) <sup>c</sup>	89 <sup>d</sup>	90 <sup>d</sup>	86, <sup>d</sup> 89 <sup>e</sup>	n.d.

<sup>a</sup>The yield is that of polyphenol based on CNTs used.

<sup>b</sup>Measured by GPC.

<sup>c</sup>The content of oxyphenylene structure.

<sup>d</sup>% wt. measured by TG.

<sup>e</sup>% mol. measured by acetation and NMR.

is condensed and precipitated in distilled water. The precipitate is filtered and vacuum-dried, generating the cleaved polyphenol (0.024 g).

## RESULTS AND DISCUSSION

### Enzymatic Polymerization of Phenol in the Presence of MWNT in Water

Without any structure-controlling techniques, enzymatic polymerization of phenol in water solutions cannot yield soluble polymers. According to our results in this work, peroxidase-catalyzed polymerization of phenol in aqueous solutions (pH 7.0-buffer) gives rise to the formation of dimers and trimers. A trace of a black tarry material precipitates from the aqueous reaction system and is insoluble in common organic solvents or water. This is also observed in other researchers' work.<sup>7a,14</sup>

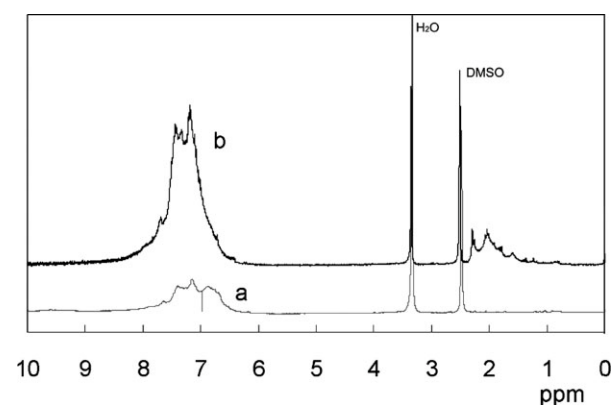
CNTs including SWNT, MWNT, MWNT-OH and MWNT-COOH are tested as structure-regulating materials for the enzymatic polymerization of phenol in aqueous solutions. Very careful gravimetry analysis showed that the usage of any a kind of CNT affords soluble materials toward THF, DMF or DMSO. According to gravimetry results, about 95% of the polymerization products are soluble toward organic solvents, for example DMF, THF. The left 5% is perhaps because of adsorption on CNTs or experimental errors.

It is found that the yields of soluble polyphenol depend linearly on the quantities of CNTs used, however, not on the quantities of phenol at all. MWNT-OH, MWNT-COOH, and SWNT yield more soluble polymers than does MWNT. About 0.4 g of soluble polymer is yielded per gram of MWNT. One gram of MWNT-OH yields 0.56 g of soluble polymer in average (Table 1). These results indicate that adsorption on the carbonaceous solid surface is an important factor affecting

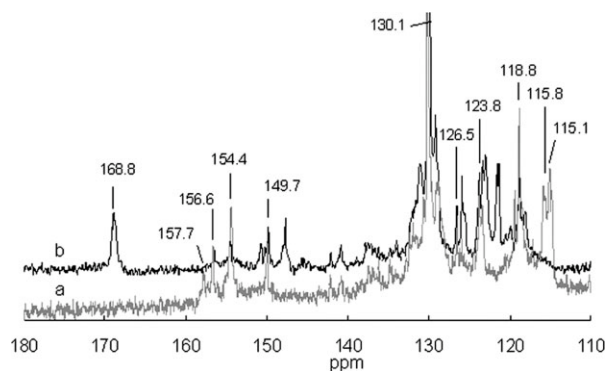
the yields. The number-average molecular weights ( $M_n$ ) and polydispersity (PDI) of the polymers obtained under templates of different CNTs are listed in Table 1.

The polymers obtained under a template of MWNT-COOH are characterized by using <sup>1</sup>H and <sup>13</sup>C NMR, TGA, MALDI-TOF, and ESI MS. Figure 1(a) is the proton NMR of the obtained polyphenol, and (b) is that of the acetated sample for evaluating the oxyphenylene contents. The signals of protons of acetates are around 2.0 ppm. According to the integration ratio of the NMR spectrum, the oxyphenylene content is about 89% mol. Although there still exists phenylene units of 11% mol, the phenol proton gives rise to a very weak peak at about 9.5 ppm in the NMR spectrum, perhaps because of two reasons: the phenol protons usually give rise to wide and weak peaks, and protons exchange between the phenols and deuterium solvents.

Figure 2 illustrates the <sup>13</sup>C NMR of the polyphenol (a) and its acetated sample (b). The signals in the <sup>13</sup>C NMR of the polyphenol can be grouped

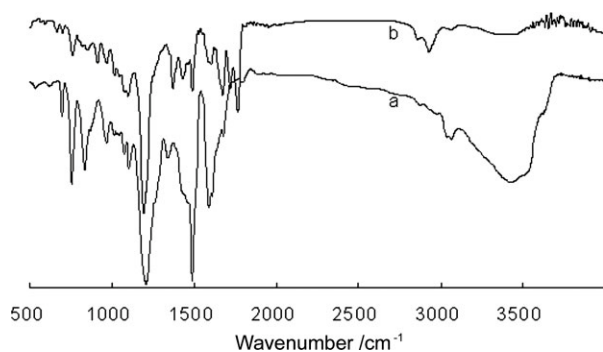


**Figure 1.** 300 MHz <sup>1</sup>H NMR spectra of the polymers: (a) polyphenol produced under a template of MWNT-COOH; and (b) the acetated sample of (a).



**Figure 2.** 75 MHz  $^{13}\text{C}$  NMR spectra of the polymers: (a) polyphenol produced under a template of MWNT-COOH; and (b) the acetylated sample of (a).

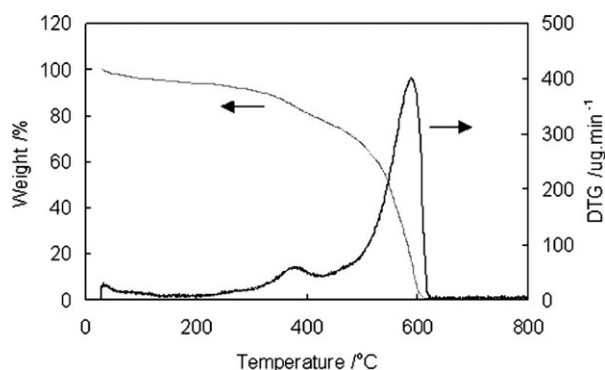
into two classes, one is the aromatic carbons adjacent to  $-\text{O}-$  groups, resonant peaks appearing above 140 ppm; and another one is the aromatic *ortho*-, *meta*-, *para*-carbons to the  $-\text{O}-$  groups, resonant peaks appearing between 140 and 110 ppm. Acetylation can just affect the signals arising from carbons in phenylene units with free  $-\text{OH}$  groups, whereas the carbons in the oxyphenylene units are not affected by acetylation. After acetylation, both of the carbon adjacent to the  $-\text{OH}$  group and the *meta*-carbon to the  $-\text{OH}$  group shift to lower chemical shifts, whereas the *ortho*-, and *para*-carbons shift to higher chemical shifts. The peak at 168.8 ppm arises from the acetate. According to Figure 2, the strong peaks at 130.1, 156.6, 154.4, 149.7, 126.5, 123.8, 118.8 ppm do not change. The peaks around 115 ppm belong to the phenylene units, because they disappear and up-shift. The  $^{13}\text{C}$  NMR result provides another evidence for an oxyphenylene-unit prevailing structure.



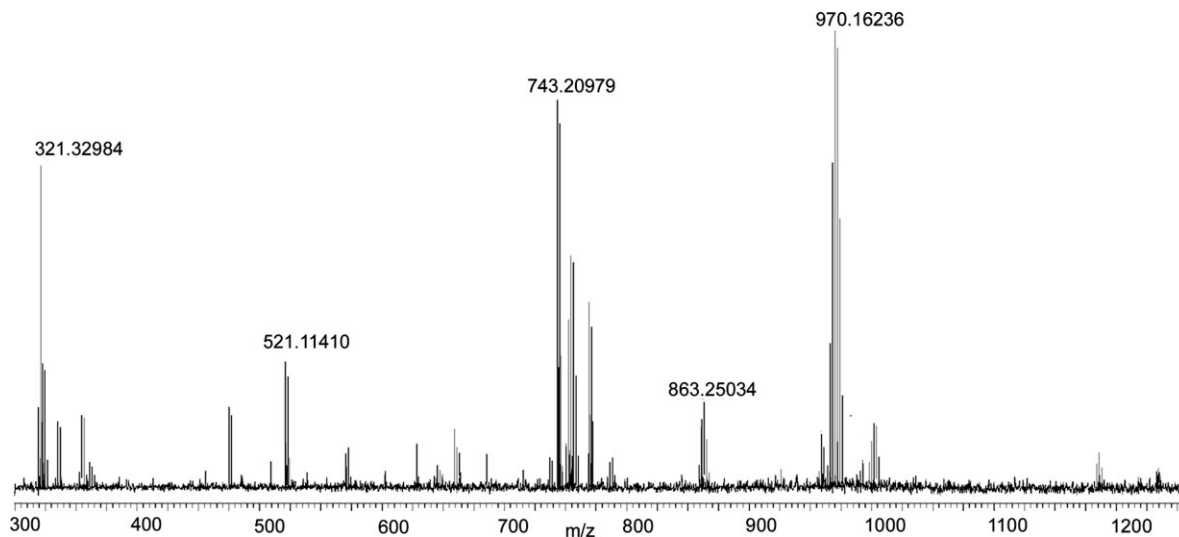
**Figure 3.** FTIR spectra of polyphenol produced under a template of MWNT-COOH (a) and its acetylated sample (b).

Figure 3 shows the FTIR spectra of the polyphenol (a) and its acetylated sample (b). In the FTIR of the acetylated sample, two peaks at 1726 and 1766  $\text{cm}^{-1}$  are assigned the stretching vibration bands of carbonyl groups. The strongest peaks at 1200  $\text{cm}^{-1}$  in both Figure 3(a,b) are due to the stretching vibration of  $\text{C}-\text{O}$ . The carbonyl stretching is much weaker than that of  $\text{C}-\text{O}$  in Figure 3(b), which also supports an oxyphenylene-unit prevailing structure.

Thermal analysis of the same sample is performed. Figure 4 is the TGA results of the same sample as that used in Figure 1. According to the literature, the TGA of the phenylene-prevailing samples showed rapid degradation at temperatures of 378  $^{\circ}\text{C}$ .<sup>7a</sup> The TGA of the sample obtained under a template of MWNT-COOH curve demonstrates two peaks of rapid degradation, and the first one is observed at 378  $^{\circ}\text{C}$ , and the second one at 594  $^{\circ}\text{C}$ . The rapidest decomposition peak at 594  $^{\circ}\text{C}$  arises from the decomposition of poly(phenylene oxide).<sup>15</sup> The first rapid decomposition peak at 378  $^{\circ}\text{C}$  arises from the decomposition of polyphenylene units. According to the TGA result, the oxyphenylene content of the sample is about 86 wt %, which is 86% mol. because the molecular masses of oxyphenylene and phenylene unit are the same. The differences between the results of TGA and NMR are acceptable. Thus, we measure the oxyphenylene content of other samples under templates of other CNTs by TGA for the sake of convenience. The results are listed in Table 1. It seems that the samples obtained under templates of MWNT and MWNT-OH have higher oxyphenylene contents than that obtained with MWNT-COOH, perhaps because of the  $-\text{COOH}$  groups on the surface of MWNT.

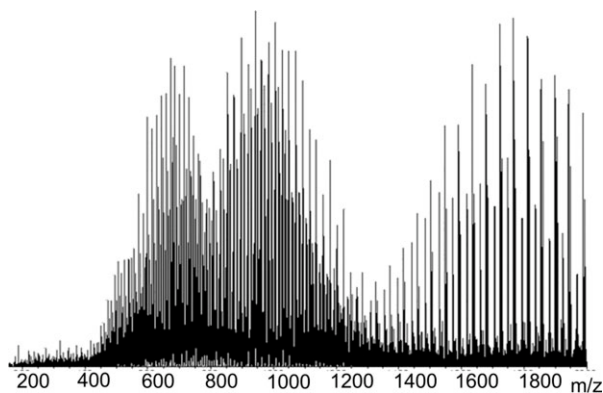


**Figure 4.** TGA and DTG curves of the polyphenol on a template of MWNT-COOH.



**Figure 5.** MALDI-TOF MS of the low-molecular-weight fractions of the polymer obtained under a template of MWNT-COOH.

The polyphenols obtained by a template of MWNT-COOH are further characterized by MALDI-TOF and ESI MS. Usually, ESI MS can measure higher molecular weight compounds than MALDI-TOF MS due to multiple charging that is an intrinsic feature of ESI of macromolecules. The lower molecular weight fractions of purification process by silica gel chromatograph is measured by using MALDI-TOF MS, from which it is found that in the low molecular weight fraction are oligomers with repeating units ranging from 3 to 10 (Fig. 5). The MALDI-TOF MS result is helpful for the analysis of ESI MS result. Figure 6 shows the ESI MS spectrum of a polymer obtained under a template of MWNT-COOH, whose  $M_n$  is about 2200 measured by GPC. It is hard to interpret the ESI MS of a polymer with a



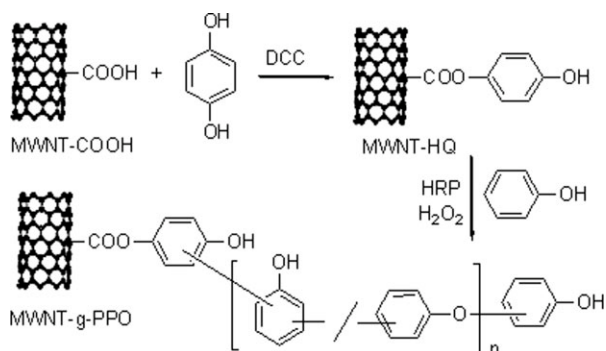
**Figure 6.** ESI MS of the polymer obtained under a template of MWNT-COOH.

polydispersity. In the  $m/z$  range from 400 to 1400 may include species with 2–4 charges.

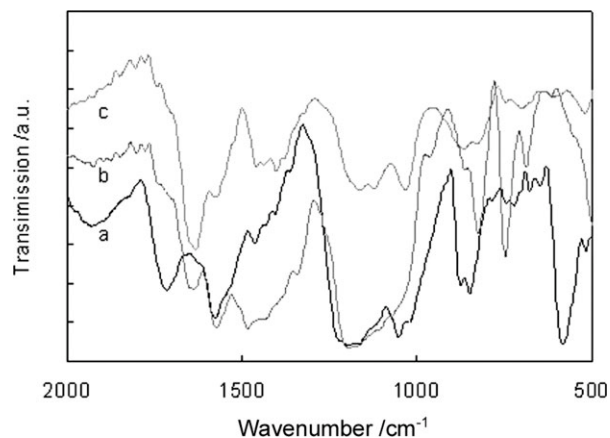
#### Grafting Polyphenol to the Surface of MWNT

As previously demonstrated in this work, MWNT can be used to regulate the polyphenol structures to achieve regioselective PPO unit-prevailing polymers. Thus, we try to graft polyphenol chains to the surface of MWNT by way of hydroquinone molecules that are covalently attached to the surface of MWNT. Scheme 1 illustrates the mechanism. The polymer chains may contain two kinds of repeating units, that is, phenylene and oxyphenylene. Obviously, the polyoxyphenylene units have lower contents of hydroxyl groups and higher thermal stability than polyphenylene units.

According to gravimetry results, the grafting degree of polyphenol to MWNT-HQ is about 28 wt %



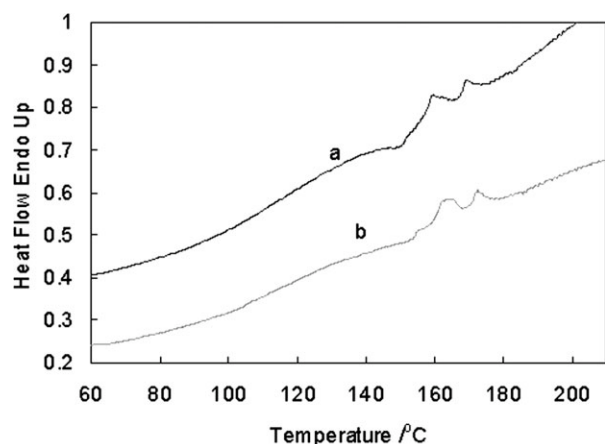
**Scheme 1.** Modification of MWNT by enzymatic polymerization of phenol.



**Figure 7.** FTIR of (a) MWNT-COOH, (b) MWNT-g-PPO, and (c) MWNT-HQ.

in the MWNT-g-PPO. The polymer brushes are cleaved for analysis purpose. The polymer cleaved from MWNT has a  $M_n$  of 2560 and PDI of 1.58 measured by GPC. The structure of the cleaved polymer brushes is analyzed by  $^1\text{H NMR}$ , which is nearly the same as that illustrated in Figure 1(a).

Figure 7 compares the FTIR between MWNT-COOH, MWNT-HQ, and MWNT-g-PPO. The characteristic peaks of MWNT-g-PPO are the strong peaks at 1626.0 and 1396.5  $\text{cm}^{-1}$  arising from the benzene skeletons. Figure 8 demonstrates the DSC curves of MWNT-g-PPO (a) and the polyphenol cleaved from MWNT-g-PPO (b). Two peaks appear in both samples: 160.4 and 170.3  $^\circ\text{C}$  for MWNT-g-PPO, and 163.2 and 173.7  $^\circ\text{C}$  for PPO cleaved from MWNT-g-PPO. The former peaks are perhaps crystalline points and the latter ones are melting points. The appearance of crystallization peaks coincides with the regioselective

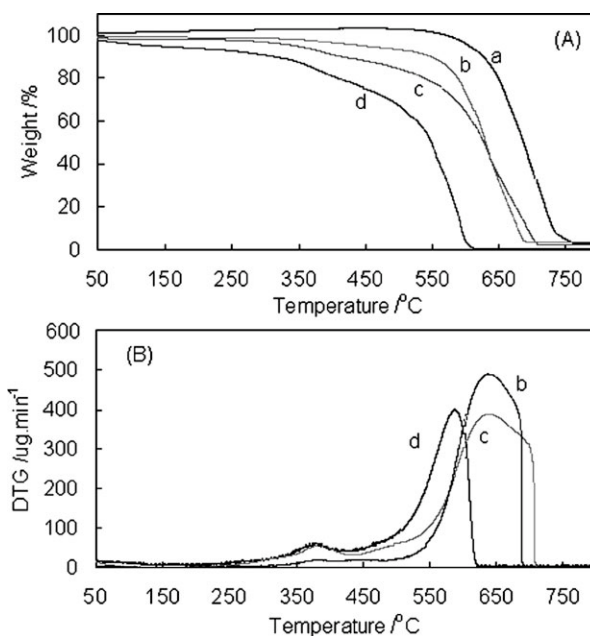


**Figure 8.** DSC of (a) MWNT-g-PPO and (b) the PPO cleaved from MWNT-g-PPO.

behaviors of the CNT templated enzymatic polymerization of phenol, because that PPO is a rigid polymer with high tendency to crystalline.

Thermal stability of the MWNT-g-PPO and the grafted polyphenol chains are evaluated by TGA (Fig. 9). As shown in Figure 9, two rapid decomposition processes are observed in the samples of MWNT-HQ, MWNT-g-PPO, and the PPO obtained on the template of MWNT-COOH, and the first one at the same 378  $^\circ\text{C}$ , whereas the second one is different for different samples. For MWNT-g-PPO and MWNT-HQ, the rapidest decomposition occurs at about 640  $^\circ\text{C}$ , which is lower than the rapid decomposition temperature of MWNT-COOH at about 718  $^\circ\text{C}$ , but higher than 594  $^\circ\text{C}$  for the homopolymer PPO. The TGA data suggest that MWNT has dramatic influence on the thermostability of the covalent grafted polymer.

Because of the covalent attachment of polyphenol chains, the affinity of MWNT-g-PPO toward THF is significantly improved. MWNT-g-PPO can be well dispersed in THF for months. MWNT-HQ can be suspended in THF with obvious sedimentation. Quick sedimentation is observed for the raw MWNTs in THF. The obvious improvement of dispersibility of MWNT-g-PPO also support the fact



**Figure 9.** TGA (A) and DTG (B) plots for (a) MWNT-COOH; (b) MWNT-HQ; (c) MWNT-g-PPO; (d) freely-standing polyphenol on a template of MWNT-COOH.

that polyphenol has been covalently grafted to the surface of MWNTs.

### Discussion on MWNT-Templated Enzymatic Polymerization of Phenol

Study of the effects of CNT on a bio-synthesis process is a very interesting and challenging work. In this work, we find that CNTs in the aqueous medium help to improve polyphenol regioselectivity. Under the conditions of an oxidative coupling reaction of unsubstituted phenol, only a black tarry material was obtained, because phenol is multifunctional and a combination of C–C and C–O coupling reactions as well as hydroxylation reactions can take place.<sup>1a</sup> To obtain relative high molecular weight poly(phenylene oxide) from unsubstituted phenol, Higashimura et al. designed a catalyst that selectively generated phenoxy free radicals.<sup>2</sup> To obtain poly(phenylene oxide) from monosubstituted phenols, one successful effort was to use catalysts with bulky ligands to shield the ortho positions from attack, by which high molecular weight polymers were obtained from *o*-cresol.<sup>16</sup>

It is found in this work that the yields of soluble polyphenol depend linearly on the quantities of CNTs used, however, not on the quantities of phenol at all. Adsorption by CNTs is a demanding factor in the CNT templated regioselective enzymatic polymerization of phenol in water. Two classes of researches carried out by other groups must be paid attention (1). Direct electron transfer between adsorbed HRP and carbonaceous electrodes has been demonstrated in a number of publications starting in 1978.<sup>17</sup> Effective and stable direct electron transfer between HPR and CNT was also observed by Cai and coworkers<sup>18</sup> (2). Ruzgas et al.<sup>17</sup> found that the rate of the reaction of H<sub>2</sub>O<sub>2</sub> with HRP adsorbed on the graphite surface was 385 times slower than that in solutions.

Considering enzymatic polymerization of phenol in the presence of CNTs, three factors possibly work: (1) conductive CNT may affect the redox voltage of peroxidase; (2) adsorption to CNTs may prevent polymeric compounds from being precipitated from reaction mixture, and help to grow longer polymer chains; (3) the *para* or *ortho* positions of phenol are possibly shielded from attack by adsorption onto the surface of CNT, thus, phenoxy free radicals are formed preponderantly to propagate the polymerization in a regioselective manner. However, to find the true mechanism needs further work.

### CONCLUSIONS

CNTs are used as templates to achieve highly regioselective polymer from enzymatic polymerization of phenol in water. The obtained polymers have a number-average molecular weight of about 2000 with about 90% of highly thermally stable oxyphenylene units. The polymer yields depend on the quantities of CNT used, which suggests that adsorption to the surface of CNT is an important factor in the enzymatic polymerization. On the basis of CNT-templated enzymatic polymerization of phenol, covalent attachment of PPO chains to the surface of MWNT by a linking molecule hydroquinone is achieved. This approach supplies a novel way for functionalization of the surface of CNTs.

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