



Synthesis of ultrahigh molecular weight phenolic polymers by enzymatic polymerization in the presence of amphiphilic triblock copolymer in water

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ABSTRACT

An enzymatic oxidative polymerization of phenols was investigated in the presence of poly(ethylene glycol) (PEG)–poly(propylene glycol) (PPG)–poly(ethylene glycol) (PEG) triblock copolymer (Pluronic) in water. The formation of micellar aggregate of phenol and Pluronic by hydrogen bonding interaction in an aqueous solution was verified by DLS measurement. The PEG content of Pluronic greatly affected the polymerization behaviors. Using Pluronic with high PEG content improved the regioselectivity of the polymerization of phenol to give the polymer mainly consisting of phenylene unit. The polymerization in the presence of Pluronic F68 (EG₇₆–PG₂₉–EG₇₆) produced the phenolic polymer with ultrahigh molecular weight ($M_w > 10^6$). From other phenols, high molecular weight polymers were also obtained. In addition, the FT-IR, DSC, and XRD analyses exhibited the formation of miscible complex between the phenolic polymer and Pluronic by hydrogen bonding interaction.

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1. Introduction

Recently, enzymatic syntheses of phenolic polymers [1–5] have received much attention as an alternative method for the production of conventional phenolic resins (novolak and resol resins), which usually involves use of toxic formaldehyde. So far, a new class of useful and high-performance phenolic polymers have been prepared by utilizing characteristic catalysis of enzymes, most of which cannot be obtained by conventional chemical methods [6–10].

In the enzymatic polymerization of phenol and phenol derivatives, using mixed solvents of polar organic solvents and buffer often produced the polymers effectively [11,12]; the polymerization of phenol in water scarcely proceeded to give the polymer in a very low yield. From the standpoint of green chemistry, development of the efficient polymer production process without use of organic solvents is highly required. The enzymatic polymerization of phenols in an aqueous medium proceeded in the presence of cyclodextrins to give the polymers in high yields; however, a much amount of cyclodextrins are required for the efficient polymer production [13,14]. Recently, the enzymatic template polymerization of phenol using poly(ethylene glycol) (PEG) as template has been developed [15,16]. The presence of PEG enabled the

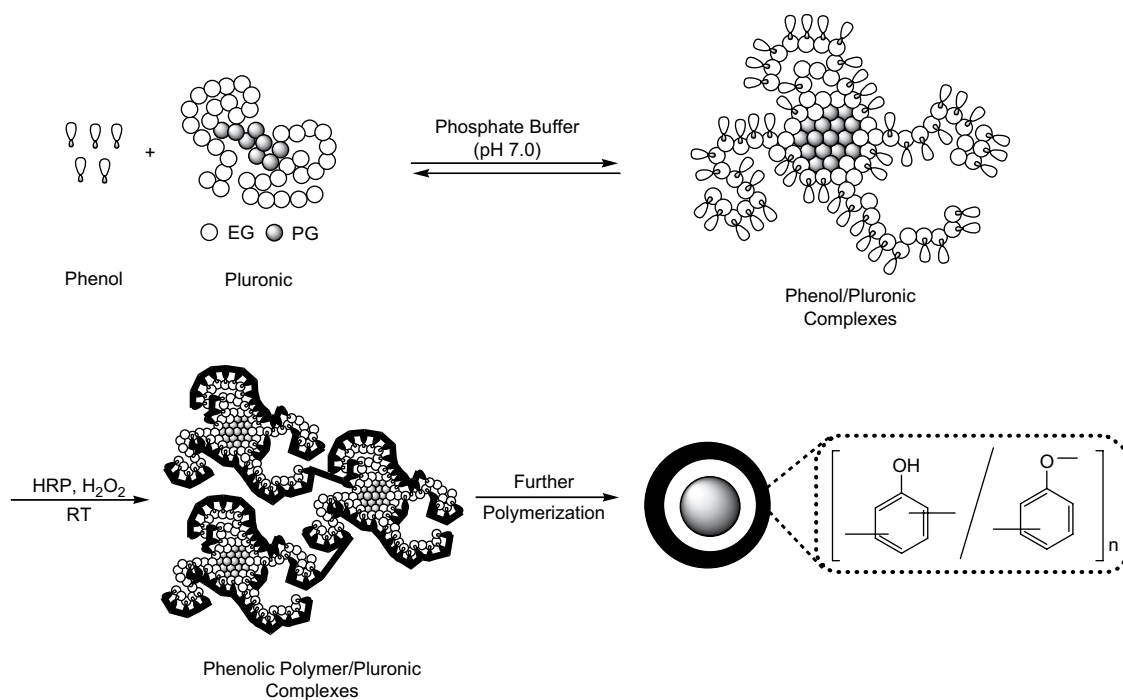
polymerization in water to produce the polymer in a high yield. Furthermore, the regioselectivity of the polymerization was greatly improved, yielding the polymer mainly consisting of phenylene unit [16].

PEG–poly(propylene glycol) (PPG)–PEG triblock copolymers, known as Pluronics, are nonionic polymer surfactants and widely used in various industrial fields. Their complex aggregation behaviors in an aqueous solution have been extensively investigated [17,18]. These amphiphilic characteristics depend critically on both molecular architecture of Pluronics (block size and block sequence) and thermodynamic parameters such as temperature and pressure, causing self-assembly of the triblock copolymers with a variety of structures including spherical, rod-like, pancake-shaped micelles, and complex-structured fluids [19]. Around ambient temperature, the micellar aggregates form a hydrophobic core consisting mainly of weakly hydrated PPG blocks which are surrounded by an outer shell of almost hydrated PEG blocks [17,18].

In most of the enzymatic polymerization of phenols [1–5], the resulting product immediately precipitated, resulting in the formation of the polymers with molecular weight of several thousands. The precipitate formation may prevent the propagation of the polymer during the reaction. In this study, the enzymatic polymerization of phenol has been examined in the presence of Pluronics in aqueous medium (Scheme 1). When Pluronic F68 (EG₇₆–PG₂₉–EG₇₆) was used as additive, the polymerization proceeded homogeneously without the formation of the precipitated polymer, leading to the production of the phenolic polymer with

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Scheme 1.

molecular weight higher than 10^6 . This is the first example of the preparation of ultrahigh molecular weight polymers by the enzymatic oxidative polymerization.

2. Experimental section

2.1. Materials

Horseradish peroxidase (HRP) was purchased from Wako Pure Chemical Industries, Ltd. and used without further purification. Pluronics (PEG–PPG–PEG triblock copolymers) were kindly donated by Asahi Denka Co., Ltd. Their structural description is presented in Table 1. Other reagents and solvents were commercially available and were used as-received.

2.2. Enzymatic oxidative polymerization of phenol in the presence of Pluronic

A typical run was as follows (entry 4 in Table 2). Phenol (0.47 g, 5.0 mmol) and Pluronic F68 (EG₇₆–PG₂₉–EG₇₆, 0.47 g) were dissolved in 0.1 M phosphate buffer (pH 7.0) (25 ml). Then, the solution of HRP (2.0 mg, 440 units) in 0.1 M phosphate buffer (5 ml) was added. To this solution, 3.4 ml of 5% hydrogen peroxide (5.6 mmol) was added dropwise for 2 h. The mixture was stirred at room temperature under air. After 1 h, the solution was poured into methanol (150 ml) and the resulting precipitates were collected by

centrifugation, and washed with methanol repeatedly, followed by drying in vacuo to give 0.62 g of the product. ¹H NMR (DMSO-*d*₆): δ = 1.1 (d, CH₃), 3.3 (d, CH(CH₃)CH₂O), 3.4 (m, CH(CH₃)CH₂O), 3.5 (s, CH₂CH₂O), 6.4–7.5 (br, Ar), 9.2–9.5 (br, ArOH). ¹³C NMR (DMSO-*d*₆): δ = 18 (CH₃), 70 (CH₂CH₂O), 72 (CH(CH₃)CH₂O), 75 (CH(CH₃)CH₂O), 112–121 (Ar), 127–134 (Ar), 152–163 (Ar). FT-IR (KBr): 3275 (ν O–H), 2864 (ν CH of alkane), 1586, 1485, 1445 (ν C=C of Ar), 1204 (ν C(Ar)–O–H), 1091 cm^{−1} (ν CH₂–O–CH₂).

2.3. Measurements

Size exclusion chromatographic (SEC) analysis was carried out by using a Tosoh GPC-8020 apparatus equipped with refractive index (RI) and UV detectors under the following conditions: TSKgel α -3000 and α -M columns and DMF containing 0.10 M LiCl eluent at a flow rate of 1.00 ml/min at 60 °C. The calibration curves were obtained using the polystyrene standard. NMR spectra were recorded on a Bruker DPX400 spectrometer. Fluorescence excitation spectra were measured by a Hitachi F-2500 spectrometer at 25 °C. Dynamic light scattering (DLS) measurements were performed using an Otsuka DLS-6006US spectrophotometer equipped with a 75 mW argon-ion laser at a scattering angle of 90°. FT-IR measurements were carried out with a Perkin–Elmer Spectrum One. DSC analysis was performed using a Seiko SSC5200–DSC220 apparatus at a heating rate of 10 °C/min in a nitrogen flow rate of 30 ml/min. X-ray diffraction (XRD) measurements were carried out

Table 1
Properties and aggregate characteristics of Pluronic and phenol–Pluronic complex

Pluronic	Molecular weight ^a	Formula	Average aggregate size of Pluronic ^b (nm)	Average aggregate size of phenol–Pluronic complex ^b (nm)
L61	2000 (10)	EG ₂ –PG ₃₀ –EG ₂	58.2 ± 4.7	277.4 ± 11.3
L62	2500 (20)	EG ₆ –PG ₃₄ –EG ₆	44.3 ± 6.1	182.1 ± 9.1
L64	2900 (40)	EG ₁₃ –PG ₃₀ –EG ₁₃	25.2 ± 2.6	158.2 ± 9.1
F68	8400 (80)	EG ₇₆ –PG ₂₉ –EG ₇₆	3.4 ± 0.5	14.9 ± 2.0

^a Data from the supplier. In parentheses, weight content (wt%) of the PEG block in Pluronic.

^b Average aggregate size determined by DLS measurement in the absence and presence of the same amount of phenol to Pluronic at 25 °C in 0.1 M phosphate buffer (pH 7.0).

Table 2
Polymerization of phenol in the presence of Pluronic^a

Entry	Pluronic	Yield ^b (%)	M_n^c ($\times 10^3$)	M_w/M_n^c	Composition ratio ^d (polymer:PEG)	Ph/Ox ^e
1	L61	14	5.4	2.6	1:0.28	66/34
2	L62	70	4.1	2.6	1:0.41	70/30
3	L64	95	3.9	2.4	1:0.64	74/26
4	F68	96	582	2.1	1:0.63	86/14

^a Polymerization of phenol (0.47 g, 5.0 mmol) using HRP catalyst (2.0 mg) in the presence of Pluronic (0.47 g) in 0.1 M phosphate buffer (pH 7.0) at room temperature under air.

^b Yield of polymer based on phenol in the complex product, determined by ¹H NMR spectroscopy using acetone as internal standard.

^c Determined by SEC using DMF as eluent with polystyrene standards.

^d Molar ratio of monomer unit between phenolic polymer and PEG of Pluronic determined by ¹H NMR spectroscopy using acetone as internal standard.

^e Unit ratio of phenylene/oxyphenylene (Ph/Ox) determined by the titration of hydroxyl group.

to characterize the crystalline phase of the phenolic polymer and Pluronic with a Rigaku X-ray diffractometer RINT 2500 with Cu K α radiation at 50 kV/300 mA. The samples were placed in a glass plate and set inside the chamber of the apparatus in order to perform the measurement. The diffractograms were scanned in a 2θ range of 10° – 40° at a rate of 2° /min.

3. Results and discussion

3.1. Aggregate formation of triblock copolymer

In this study, four Pluronic samples with different contents of PEG were employed such as L61, L62, L64 and F68 (Table 1). At first, the aggregate size of Pluronic in the absence and presence of phenol was determined by DLS measurements in a phosphate buffer as shown in Table 1. According to a previous report [20,21], Pluronics with a high PPG content formed large and loosely connected aggregates, resulting that Pluronic L61 (EG₂–PG₃₀–EG₂) exhibited the formation of the biggest aggregate (58.2 nm). Moreover, the aggregate size of Pluronics increased by the addition of phenol, suggesting the hydrogen bonding interaction of the flexible outer shell (PEG block) of Pluronic with the hydroxyl group of phenol [22]. A similar behavior was observed for the combination of phenol and PEG in water [16]. By adding PEG to the buffer containing phenol, UV–vis spectra were changed, due to the formation of a phenol–PEG complex by the hydrogen bonding interaction.

3.2. Polymerization of phenols in the presence of triblock copolymer

The HRP-catalyzed polymerization of phenol was performed in a phosphate buffer (pH 7.0) in the presence of Pluronics at room temperature under air (Table 2). The amount of Pluronic used in this polymerization was over the critical micelle concentration (cmc). The cmc of Pluronic was determined by using pyrene as a fluorescence probe [23,24], which was in the range from 0.17 g/l for Pluronic L61 to 0.80 g/l for Pluronic F68, relying on the PEG/PPG ratio. The polymerization started by the addition of hydrogen peroxide as an oxidizing agent. In the presence of Pluronics L61, L62, or L64, polymeric powders were immediately precipitated during the polymerization, on the other hand, a homogeneous polymer solution was formed in the case of Pluronic F68. ¹H NMR analysis showed that the product was a complex of the phenolic polymer and Pluronic.

The difference of the polymerization behaviors greatly affected the molecular weight of the resulting polymer, estimated by size exclusion chromatography (SEC) with refractive index (RI) and UV (wavelength = 340 nm) detectors (Fig. 1). The peak separation of the phenolic polymer and Pluronic was confirmed by a RI detector

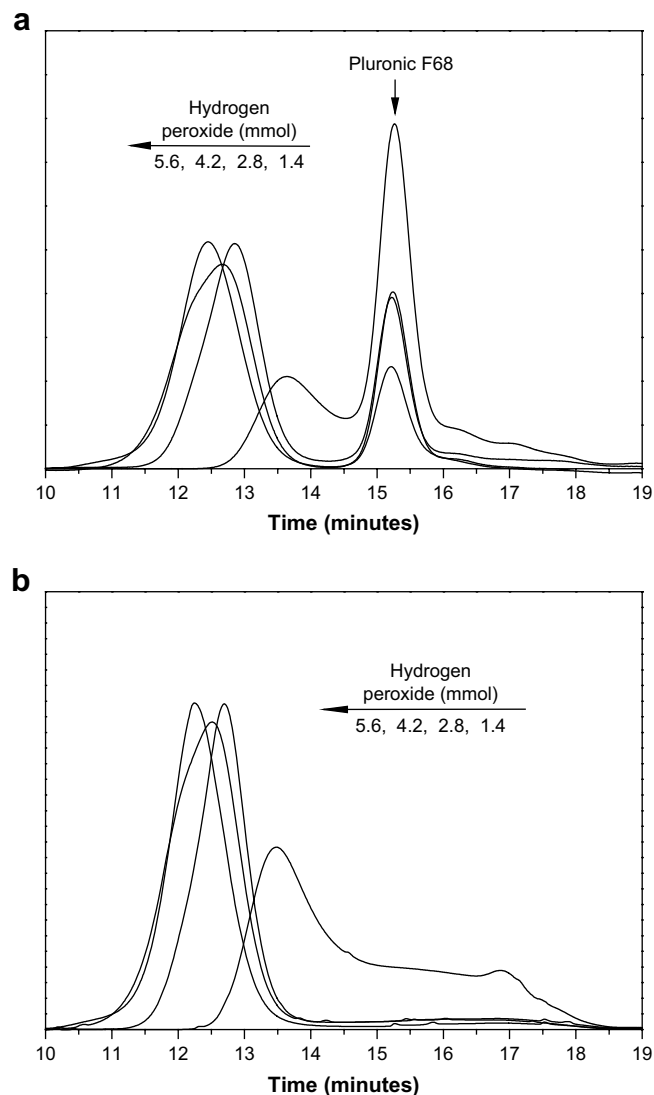


Fig. 1. SEC traces of the phenolic polymer complex with Pluronic F68 synthesized under the conditions of entry 7 in Table 2 using (a) RI and (b) UV (wavelength = 340 nm) detectors.

and the phenolic polymer was only detected by a UV detector. The unimodal peak was observed in all cases examined. In using Pluronic with high PEG content (Pluronic F68), the molecular weight of the polymer reached 5.8×10^5 , while the addition of other Pluronics having the PEG content below 40% gave the polymers in the range of molecular weights from 3900 to 5400. The homogeneous reaction system probably provided the high molecular weight polymer, which is also strongly related to the size (Table 1) and stability of the aggregate; the aggregate of phenol and Pluronic F68 was much smaller and more stable in comparison to the ones obtained with other Pluronics, resulting in no formation of the polymeric precipitates [20]. These data clearly show that Pluronic F68 was the most effective for the synthesis of high molecular weight phenolic polymer.

In addition, the Pluronic structure strongly affected the regioselectivity of the polymerization; the higher PEG content of Pluronic caused the higher ratio of the phenylene unit. When Pluronic F68 was used, the regioselectivity reached 86%, which is close to that obtained by the polymerization in the presence of PEG in water [16]. The unit ratio of the phenolic polymer and Pluronic in the product depended on the Pluronic structure.

Table 3
Polymerization of phenol with different amounts of Pluronic F68^a

Entry	Amount (g)	Yield ^b (%)	$M_n^c (\times 10^3)$	M_w/M_n^c	Composition ratio ^d (polymer:PEG)	Ph/Ox ^e
4	0.47	96	582	2.1	1:0.63	86/14
5	0.60	92	352	1.9	1:0.61	84/16
6	0.80	91	202	1.4	1:0.64	85/15
7	1.00	93	184	1.5	1:0.63	87/13

^a Polymerization of phenol (0.47 g, 5.0 mmol) using HRP catalyst (2.0 mg) in the presence of Pluronic F68 in 0.1 M phosphate buffer (pH 7.0) at room temperature under air.

^b Yield of polymer based on phenol in the complex product, determined by ¹H NMR spectroscopy using acetone as the internal standard.

^c Determined by SEC using DMF as eluent with polystyrene standards.

^d Molar ratio of monomer unit between phenolic polymer and PEG of Pluronic F68 determined by ¹H NMR spectroscopy using acetone as internal standard.

^e Unit ratio of phenylene/oxyphenylene (Ph/Ox) determined by the titration of hydroxyl group.

The polymerization with different amounts of Pluronic F68 was carried out (Table 3), in which precipitated polymers were hardly observed during the reaction. The Pluronic amount also affected the polymerization behavior. The molecular weight of the polymer decreased as the ratio of Pluronic F68 to phenol increased. In all cases, the unit molar ratio of the phenolic polymer and PEG of Pluronic F68 was ca. 1:0.6.

To examine the mechanism of the formation of the ultrahigh molecular weight phenolic polymer in the presence of Pluronic F68, the polymerization was monitored by SEC under the conditions given in entry 7 of Table 3. The peak of the phenolic polymer was gradually shifted to lower elution times as a function of the hydrogen peroxide amount (Fig. 1), suggesting that the oxidatively coupled products of phenol, which are formed in the early stage of the reaction, were further reacted with each other during the polymerization, leading to the high molecular weight. This tendency is in marked contrast to that of the HRP-catalyzed polymerization in a mixture of polar organic solvents and buffer without any additives [25]; during the polymerization, the molecular weight hardly changed. This is probably owing to the homogeneous reaction system without the formation of the precipitated polymer in the presence of Pluronic F68.

The absolute molecular weight of the polymer was examined by a combination of SEC with an on-line viscometer (VISC) and a right angle laser light scattering (RALLS) detector. The molecular weight (M_w) values in the entries 6 and 7 of Table 3 were 3.2×10^6 and

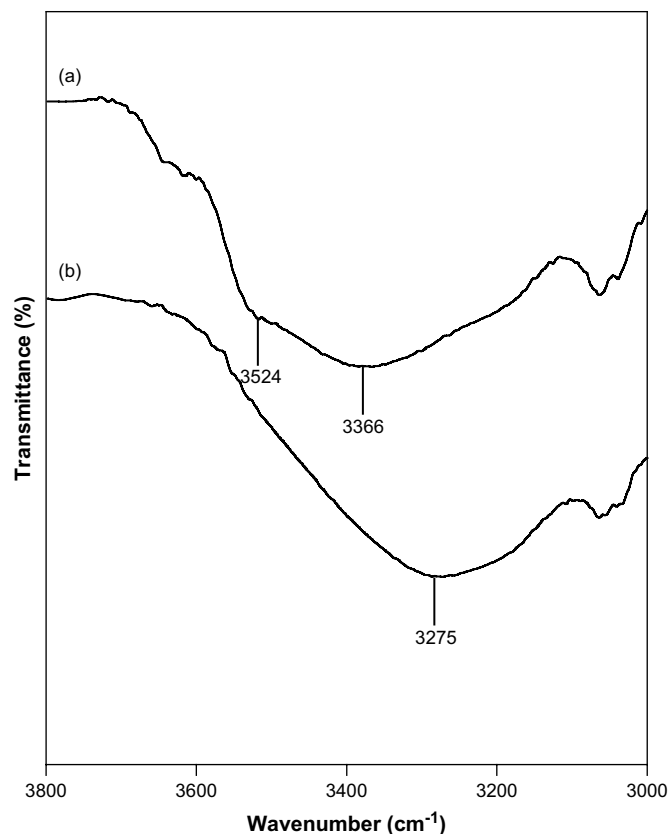


Fig. 3. FT-IR spectra of (a) phenolic polymer synthesized in a mixture of methanol and buffer, and (b) phenolic polymer–Pluronic complex synthesized in phosphate buffer.

2.5×10^6 , respectively. To the best of our knowledge, this is the first example of the synthesis of phenolic polymers with ultrahigh molecular weight by the enzymatic oxidative polymerization.

In order to explain the growth of the molecular weight of the phenolic polymer with rising the hydrogen peroxide amount, the change of the aggregate size of the resulting polymer–Pluronic complex as a function of the hydrogen peroxide amount was measured by DLS under the conditions given in Table 3. As shown in Fig. 2, the aggregate size enlarged with increasing the amount of hydrogen peroxide. This can be associated to the self-assembly process triggering further propagation between the precursor

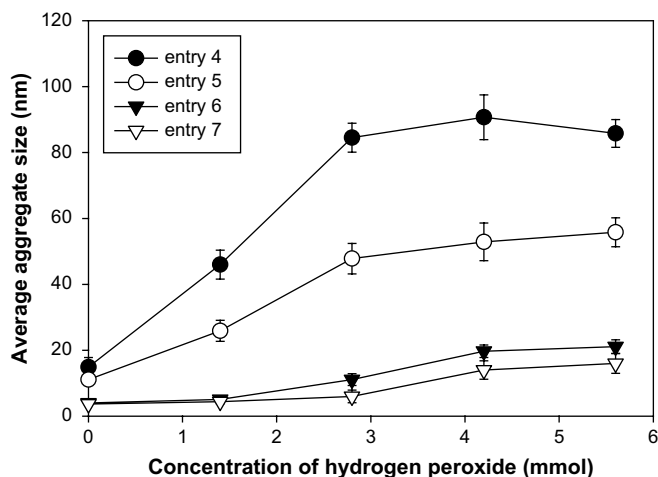


Fig. 2. Change of the aggregate size during the polymerization in the presence of Pluronic F68, $n = 3$.

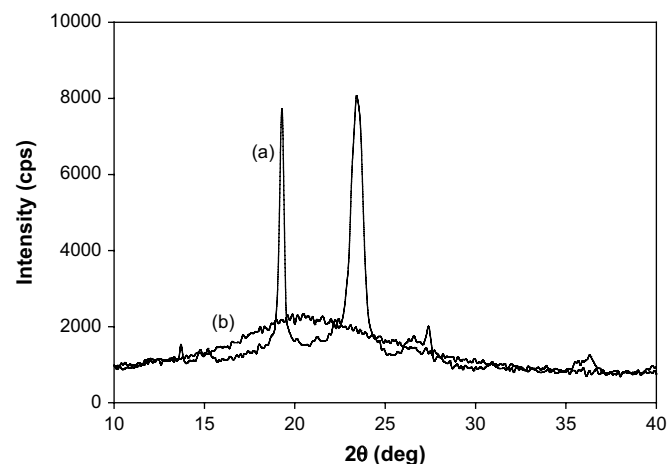


Fig. 4. X-ray diffraction patterns of (a) Pluronic and (b) phenolic polymer–Pluronic complex.

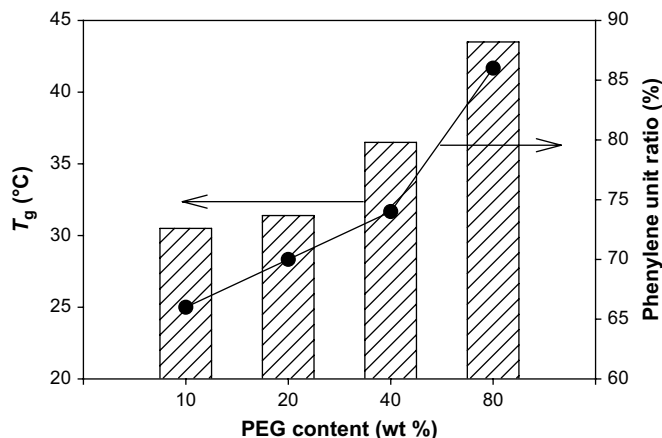


Fig. 5. Effect of PEG content of Pluronic on glass transition temperature (T_g) and regioselectivity. PEG contents of 10%, 20%, 40% and 80% denote Pluronic L61, L62, L64 and F68, respectively.

aggregates, which can arise from the fact that Pluronic form rather dynamic aggregates in which the individual polymer chains constantly move from one aggregate to another [20–22]. Furthermore, the aggregate size of the polymers decreased with increasing the amount of Pluronic F68 (Fig. 2).

In the present reaction system using Pluronic F68 as additive, other phenols were also polymerized to give the high molecular weight polymers. Bisphenol A and *m*-cresol were polymerized to give the corresponding polymers in yields higher than 90%. The molecular weights estimated by SEC were 3.2×10^5 and 2.3×10^5 , respectively. These data strongly suggest that the addition of Pluronic F68 is highly useful for the synthesis of high molecular weight phenolic polymers.

3.3. Properties of phenolic polymer complexes with triblock copolymer

The complex formation between the resulting polymer and Pluronic by the hydrogen bonding interaction was confirmed by FT-IR (Fig. 3). Two peaks centered at 3366 cm^{-1} and 3524 cm^{-1} were observed for the phenolic polymer synthesized in an aqueous solution without Pluronic; the former was due to self-associated phenolic O–H group and the latter was due to the non-associated free hydroxyl group. In the case of the phenolic polymer complex with Pluronic synthesized in phosphate buffer, these characteristic peaks disappeared and a peak at 3275 cm^{-1} , ascribed to phenolic O–H hydrogen bonding with the PEG block of Pluronic, newly appeared [26–29]. Crystallographic analysis using XRD exhibited the clear crystalline diffraction peaks of Pluronic at $2\theta = 19^\circ$ and 24° (Fig. 4). For the complex, on the other hand, the crystalline diffraction peaks of the Pluronic disappeared and a broad peak appeared at $2\theta = 21^\circ$. These data clearly support that the phenolic polymer synthesized in the buffer with Pluronic was a miscible complex with Pluronic possessing a homogeneous amorphous phase.

The DSC analysis of the present complex verified the miscibility between the phenolic polymer and Pluronic. In all the samples prepared in this study, the melting point due to Pluronic disappeared and a single glass transition temperature was observed.

These data clearly indicate that there exists a rather strong intermolecular interaction between both polymers in the complex [26–29], and they are fully miscible with each other in homogeneous amorphous phase. Moreover, the glass transition temperature was elevated by the increase of the PEG content of Pluronic (Fig. 5), which is closely related to the phenylene content of the phenolic polymer.

4. Conclusion

In this study, we have synthesized the phenolic polymers with ultrahigh molecular weight ($M_w > 10^6$) by the HRP-catalyzed polymerization of phenol using Pluronic F68 for the first time. Besides, the addition of Pluronic F68 enormously improved the regioselectivity to give the polymer mainly consisting of phenylene unit. The present study clearly shows that the highly stabilized aggregate of the phenol monomer and Pluronic F68 can prevent the formation of the polymer precipitates during the polymerization, leading to the production of the phenolic polymers with ultrahigh molecular weight. Furthermore, an environmentally benign system for the production of phenolic polymers without use of organic solvents was developed by the addition of Pluronic, which will be useful for potential applications of high-performance phenolic polymers with ultrahigh molecular weight.

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