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Enzymatic polymerization of phenols in room-temperature ionic liquids

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ABSTRACT

Soybean peroxidase (SBP) was used to catalyze the polymerization of phenols in room-temperature ionic liquids (RTILs). Phenolic polymers with number average molecular weights ranging from 1200 to 4100 Da were obtained depending on the composition of the reaction medium and the nature of the phenol. Specifically, SBP was highly active in methylimidazolium-containing RTILs, including 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM(BF_4)), and 1-butyl-3-methylpyridinium tetrafluoroborate (BMPy(BF_4)) with the ionic liquid content as high as 90% (v/v); the balance being aqueous buffer. Gel permeation chromatography and MALDI-TOF analysis indicated that higher molecular weight polymers can be synthesized in the presence of higher RTIL concentrations, with selective control over polymer size achieved by varying the RTIL concentration. The resulting polyphenols exhibited high thermostability and possessed thermosetting properties.

1. Introduction

Phenol–formaldehyde resins have a long history of widespread use in surface coatings, adhesives, laminates, molding and friction materials, and abrasives [1], flame retardants [2], carbon membranes [3], glass fiber laminates [4], fiberboards [5], and protein-based wood adhesives [6]. While severe health and environmental concerns regarding the toxicity of formaldehyde has been noted for quite some time [7], recent legislation in many countries has limited conventional phenol–formaldehyde manufacturing and use [8]. As a result, alternative synthetic approaches have been sought, which do not involve formaldehyde yet provide simple, highly reproducible, and low-cost routes to phenolic polymers with thermosetting properties. One common alternative for formaldehyde-free phenolic polymerization involves free radical polymerization catalyzed by a chemical catalyst such as copper and copper complexes [9,10]. However, this approach is not effective for the polymerization of unsubstituted phenols [11], and still employs the use of toxic chemical catalysts leading to potential environmental problems [12].

An attractive alternative to chemical routes is the use of enzymes as catalysts. Nature provides a clear example of the power of enzymes in the preparation of phenolic polymers through the synthesis of lignin [13], the second most abundant polymer on earth [14]. Plant peroxidases catalyze the one-electron oxidation of phenolic monomers in the presence of H_2O_2, thereby generating free radicals that undergo radical transfer and coupling reactions to build the complex lignin macromolecules found throughout the plant kingdom [15,16]. The mild reaction conditions coupled with the highly reactive and stable peroxidase family of oxidative enzymes is also ideal for synthetic applications. In particular, soybean peroxidase (SBP) and horseradish peroxidase (HRP) have been used to synthesize phenolic polymers and copolymers from a wide range of phenols, including *p*-cresol, *p*-phenylphenol, various naphthols, and phenol itself, along with related anilines [16–18]. Both SBP and HRP yield similar polymeric products, which appear to be representative of the majority of plant peroxidases [19]. Unfortunately, in aqueous solutions, the poor solubility of phenolic monomers and the even lower solubility of the polymeric products result in low yields of oligomeric (predominantly dimers and trimers) that precipitate out of solution [20,21].

In addition to aqueous media, peroxidases are highly active in organic solvents and have been used to catalyze phenolic polymerizations in such milieu [17,22–25]. In some cases significant control over the polymer size and polydispersity has been achieved. For example, Dordick et al. observed changes in polyphenolic M_w from 1000 to over 26,000 Da, using 1,4-dioxane as the solvent at various

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water contents, with an optimal polymer size achieved in 85% (v/v) dioxane [17]. A functionally similar result was obtained by Pizzi et al. where polyphenolic M_w of the dimethylformamide (DMF)- and dimethylsulfoxide (DMSO)-soluble fractions were as high as 35,000 Da [26]. Water-miscible cosolvents have been used to support higher molecular weight polyphenolic synthesis. In addition to 1,4-dioxane–water mixtures, Oguchi et al. performed enzymatic oxidative polymerization of phenol in aqueous methanol solutions using HRP yielding number average molecular weights up to 5200 Da [18].

Despite the aforementioned examples, organic solvents suffer from several drawbacks, including poor solubility of highly polar compounds (including some phenolic monomers), and the relatively low solubility of the polyphenolic products. While highly polar aprotic solvents such as DMF and DMSO are able to solubilize high molecular weight fractions of polyphenols, primarily at very high solvent percentages [18,20,27], enzyme activity in these solvents is low [20,23,27]. RTILs, however, may provide the advantages of a nonaqueous environment that enables high solubility of phenols and their polymerized counterparts, while maintaining sufficient enzyme activity to allow efficient polymerization, all while providing "green" alternatives to volatile organic solvents.

In the present study, we have exploited RTILs as reaction media to support SBP-catalyzed polymerization of phenols. SBP-catalyzed oxidative polymerization reactions were performed in various aqueous RTIL solutions and different phenols were used as substrates for polymer synthesis. Higher molecular weight phenolic polymers were synthesized in solvent systems having higher RTIL contents, owing to the high dissolution capacity of RTILs. Finally, thermal analysis revealed that the resulting polyphenols were highly thermostable and had desirable thermosetting properties.

2. Materials and methods

2.1. Materials

Soybean hull peroxidase was purchased from Sigma–Aldrich (St. Louis, MO) as a solid powder (50 purpurogalin units/mg solid). All phenols, LiBr, and H_2O_2 (30%, w/w solution in water) were also obtained from Sigma–Aldrich and used without further purification. High purity RTILs (>98.5%) were obtained from Fluka (Milwaukee, WI) and polyethylene glycol (PEG) standards were obtained from Polymer Laboratories (Amherst, MA). All other chemicals employed were of the highest purity commercially available.

2.2. Enzymatic reactions

Phenolic polymerizations were carried out in a variety of RTIL/aqueous solutions on an orbital shaker (200 rpm) at 60 °C. Each reaction consisted of 20 mM of a phenolic substrate in the presence of 0.1 mg/ml SBP and 20 mM H_2O_2, the latter being added dropwise to the reaction mixture over a period of 2.5 h, and the reactions stirred for an additional 20 h. Several compositions of RTIL/aqueous solutions were prepared using BMIM(BF_4), and BMPY(BF_4) as the RTIL component ranging from 0 to 90% (v/v) in 10 mM phosphate buffer (pH 7). The pH values of all the aqueous RTILs were independent of the solvent composition and no change in pH was observed upon addition of RTIL (pH 7). Concentrations of phenols were determined by HPLC (Shimadzu LC-VP, Columbia, MD) with detection at 280 nm. Periodically, aliquots from the reaction mixtures were diluted fourfold with acetonitrile and analyzed on a reversed phase C18 column (4.6 mm × 150 mm, 5 μm, Alltech Alltima, Deerfield, IL). The eluent contained 0.1% (v/v) acetic acid in acetonitrile/water solution and was pumped with a linear gradient from 10 to 45% acetonitrile over 10 min and then isocratically at 45% acetonitrile for 20 min with a flow rate of 1 ml/min.

2.3. Gel permeation chromatography

Gel permeation chromatography was employed to determine polymer molecular weight. PLgel 3 μm MIXED-E column (molecular weight cutoff of 30 kDa) (Polymer Laboratories, Amherst, MA) was connected to the HPLC and 0.5 ml/min DMF was used as the mobile phase. PEG standards with peak molecular weights (M_p) of 21,030, 12,140, 8500, 4020, 1010, and 400 were used as molecular weight calibrants and the molecular weight distribution of the polymers was determined based on these PEG standards. LiBr (40 mM) was added to the DMF mobile phase to dissociate molecular aggregates of phenolic polymers during GPC analysis.

2.4. MALDI-TOF analysis

Mass spectra were acquired on an Ultraflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA) in linear and reflector modes. A "smart beam" Nd-YAG laser (λ = 355 nm) was set at ~70% maximum power. Changes in pulse ion extraction (PIE) time in the range of 20–300 ns did not significantly affect the oligomer ion distribution. PIE was set up for standard values of 20 ns in reflector and 100 ns in linear mode. MALDI-TOF analysis involved five different matrixes for sample preparation: 2,5 dihydroxybenzoic acid (DHB), dithranol, 2,4,6-trihydroxyacetophenone (THAP), 6-aza-2-thiothymine, and trans-2-indolacrylic acid. All matrixes produced satisfactory MALDI-TOF mass spectra in positive ion mode. The best negative ion mode mass spectra were obtained with trans-2-indolacrylic acid and THAP as matrixes.

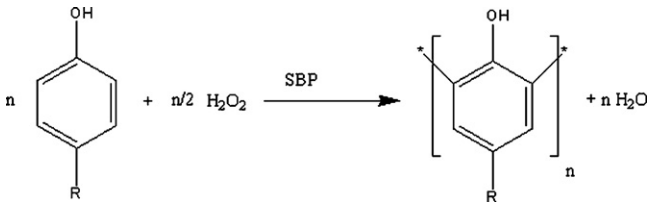
DMF insoluble polymers were pretreated prior to MALDI-TOF analysis. The precipitates were centrifuged and the DMF was removed by washing with water and the solids were dried. The solids were then added to a mixture of DMF and methanol (4:1, v/v) or pure methanol to dissolve the solids. Molecular weights of the soluble fractions were determined based on MALDI-TOF analysis.

2.5. Thermal analysis

Thermal analysis was performed using a thermogravimetric analyzer (TGA) and a differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE). The flow rate of nitrogen was 50 ml/min, the sample size was 1–2 mg for both DSC and TGA, and the heating rate was 10 °C/min.

3. Results and discussion

While a growing literature exists on using enzymes in RTILs [28,29], very little work has been done with peroxidases in these solvents [30], and no reports exist on the polymerization of phenols catalyzed by peroxidases in RTILs. Therefore, we set out to assess the influence of RTILs on SBP catalysis. To that end, we used two different water miscible, commercially available RTILs as reaction solvents: 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM(BF_4)) and 1-butyl-4-methylpyridinium tetrafluoroborate (BMPy(BF_4)). Tetrafluoroborate was chosen as the anion due to its formation of stable RTILs with different cations, and its known capacity to support enzymatic catalysis [29,31]. SBP was used as the model peroxidase due its well-known operational stability, particularly in nonaqueous media [23,32]. A range of simple phenols were used as substrates for the synthesis of high molecular weight polymers, with *p*-cresol (4-methylphenol), employed as the initial model substrate in enzymatic polymerization reactions (Scheme 1).



Scheme 1. Soybean peroxidase (SBP)-catalyzed polymerization of *p*-cresol.

3.1. Influence of reaction medium on SBP-catalyzed polymerization of p-cresol

We initially examined the SBP-catalyzed polymerization of *p*-cresol in aqueous buffer. The reaction mixture containing 20 mM *p*-cresol turned brown and precipitates formed nearly immediately in the presence of 0.1 mg/ml SBP upon addition of H_2O_2. The conversion of the *p*-cresol was 84%, as determined by HPLC. Following the reaction the precipitates were removed by filtration, completely dissolved in DMF, and then subjected to GPC analysis. Low molecular weight oligomeric species were formed (M_n = 720, PDI = 1.3), reflecting an average synthesis of hexamers. This result is consistent with that found in the literature [17,27].

We then performed the SBP-catalyzed polymerization of *p*-cresol in aqueous solutions supplemented with 50% (v/v) BMIM(BF_4) or BMPy(BF_4). As in aqueous solutions, 20 mM *p*-cresol was used along with an equimolar concentration of H_2O_2. The conversion of *p*-cresol reached 80% for both RTILs. No reaction was observed in the absence of enzyme in aqueous RTILs in the presence of 20 mM H_2O_2. As with aqueous media, a precipitate immediately formed during the reaction, which was then filtered and washed with water to remove the enzyme. The solids were fully soluble in DMF and analyzed by GPC. BMIM(BF_4) and BMPy(BF_4) gave polymers with M_n = 1230 Da (PDI = 1.6) and M_n = 1530 Da (PDI = 1.5), respectively, showing higher molecular weights of the phenolic products achieved upon addition of RTIL in the reaction medium. In addition, when the *p*-cresol concentration was doubled in the aqueous RTILs, similar molecular weights (M_n = 1300 Da, PDI = 1.5) were obtained as with lower *p*-cresol concentration. Thus, there does not appear to be a significant effect of the substrate concentration on the molecular weights of the resulting polymers.

3.2. Effect of reaction medium on polymer molecular weights

To assess the effect of RTIL concentration on SBP catalysis, we performed *p*-cresol oxidation in BMIM(BF_4) and BMPy(BF_4) at RTIL concentrations as high as 90% (v/v). Not surprisingly, conversion of *p*-cresol decreased upon an increased RTIL concentration. GPC analysis of the resulting polymers showed declining M_w and M_n above 50% (v/v) RTIL for both RTILs studied (Fig. 1a and b). Indeed at 90% (v/v) of each RTIL, ca. 50% lower M_n was obtained, although the calculated polydispersities were similar at 90 and 50% (v/v) RTIL. GPC was performed only on the DMF soluble polymer fraction, while polymers that formed in both 70 and 90% (v/v) RTILs were only partially soluble in DMF. Thus some higher molecular weight polymers that were insoluble in DMF were likely present in enzyme solutions containing >50% (v/v) RTIL. To determine the molecular weights of DMF insoluble polymers at higher RTIL concentrations, MALDI-TOF analysis was performed.

3.3. Determination of polymer molecular weights based on MALDI-TOF analysis

MALDI-TOF has become a widely accepted analytical tool for polymer molecular weight determination [33–35] and has been

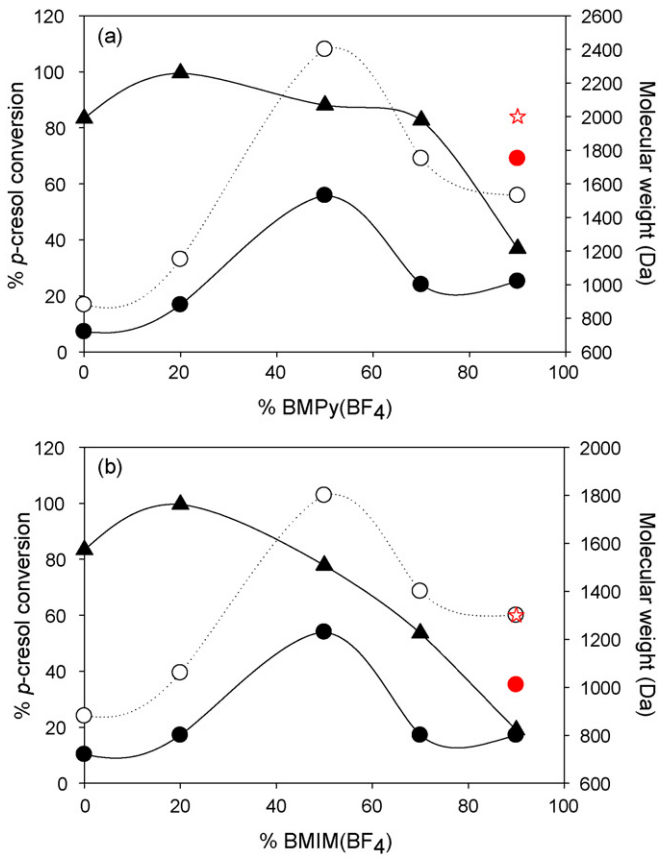
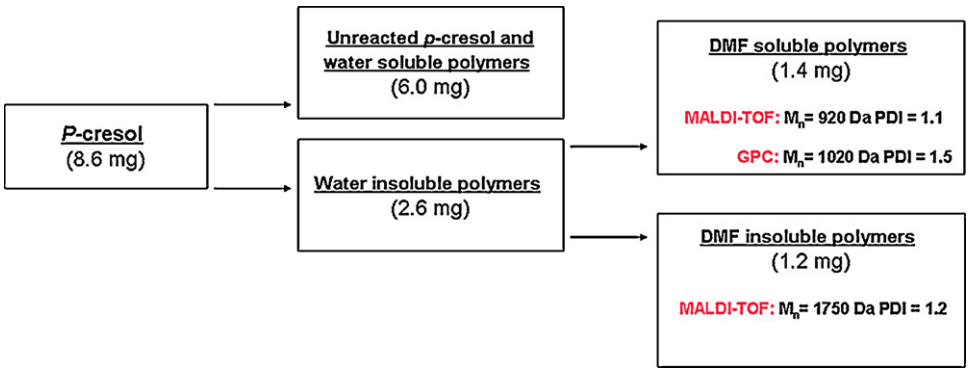


Fig. 1. Influence of With reference to the color information in the artwork, a parenthetical sentence, "For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article." has been incorporated in the caption of Fig. 1. Please check. RTIL concentration on SBP-catalyzed polymerization of *p*-cresol. M_n (closed circles) and M_w (open circles) for DMF soluble poly(*p*-cresol) based on GPC, M_n (red closed circles) and M_w (red star) for DMF insoluble poly(*p*-cresol) at 90% (v/v) RTIL based on MALDI-TOF and *p*-cresol conversion (solid triangles) with 0.1 mg/ml SBP in different concentrations of (a) BMPy(BF_4) and (b) BMIm(BF_4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

used for analysis of phenolic oligomers and polymers [26,33,36]. In this study, we used MALDI-TOF to confirm the molecular weights of polymers analyzed by GPC, and to analyze the DMF insoluble polymer masses that were synthesized at RTIL concentrations >50% (v/v). In the case of 90% (v/v) BMPy(BF_4), *p*-cresol conversion was ca. 30%. Phenolic polymers from the DMF-soluble fraction had a M_n of 1020 Da with a PDI of 1.5, as determined by GPC and relatively similar molecular weights, albeit with lower degrees of polydispersity, were obtained by MALDI-TOF; M_n of 920 Da with a PDI of 1.1. In the case of *p*-cresol polymerization in 90% (v/v) BMPy(BF_4), ca. 14% of the total *p*-cresol (corresponding to nearly 50% of the polymerized phenolic) was DMF insoluble, with the remaining reacted fraction being DMF soluble (Scheme 2). MALDI-TOF analysis was then used to determine the molecular weights of DMF-insoluble polymers, yielding a M_n of 1750 Da and a PDI of 1.2.

Further analysis of the MALDI-TOF spectra revealed information about the structure of the poly(*p*-cresol). Both positive ion mode (Fig. 2a) and negative ion mode (Fig. 2b) spectra were obtained. The former displayed several groups of peaks separated by 108 mass units, as shown for series A and B. Series A could be assigned directly to deprotonated oligomers with molecular weights of $[H(M)_nH-H]^-$, where M represents a *p*-cresol monomer unit. Series B could be assigned to mono-oxidized oligomers, with molecular masses of $[H(M)_nH+O-H]^-$, as shown in Fig. 2a. Mass measurements performed for these ions showed the average mass difference



Scheme 2. Mass balance calculation for SBP-catalyzed poly(*p*-cresol) synthesis at 90% BMPy(BF_4)–10% phosphate buffer (pH 7).

of 16 Da, which corresponds to one oxygen atom, and is likely due to hydroxylation of the *p*-cresol units forming during SBP-catalyzed oxidation. This is consistent with the literature reports of phenol hydroxylation during peroxidase catalysis [11,37]. Series C and D were performed in negative mode, which also yielded peaks separated by 108 Da (Fig. 2b), but corresponding to sodium adducts of oligomers and their mono-oxidized analogs, $[H(M)_nH]Na^+$ and $[H(M)_nH+O]Na^+$, respectively, further conferring the dual measurements consisting of *p*-cresol and hydroxylated *p*-cresol. The optimum solvent composition was found to be 50% RTIL (v/v) for BMPy(BF_4) and BMIM(BF_4) (Fig. 1a and b). However, even at 90% (v/v) RTIL, taking into account the DMF insoluble polyphenolic fractions produced in both RTILs, there was a broad optimum range of RTIL concentration that leads to relatively high molecular weight poly(*p*-cresol). This is shown in Fig. 1a and b where in 90% RTIL, the DMF insoluble fraction (red closed circle) gives M_n values similar to that for the DMF soluble fractions at 50% (v/v) RTIL.

The higher M_w and M_n values in RTILs, vs. aqueous buffer is most likely due to precipitation of the polymers formed in the latter before they reach a high molecular weight, while the high dissolution capacity of the RTIL allows the growing polymer to propagate into larger chains before they fall out of solution, and hence become less reactive to SBP-catalyzed generation of phenoxy radicals (Scheme 3). To confirm that the high dissolution capacity of the RTILs resulted in higher molecular weight polymers, we recovered the insoluble fraction of poly(*p*-cresol) from the aqueous reaction by filtration. These water insoluble polymers were then added to 90% (v/v) BMPy(BF_4) and the polymers fully dissolved, which demonstrated that the intrinsic solubility of higher molecular weight poly(*p*-cresol) is higher in the RTIL than in aqueous buffer. Moreover, the polymers formed at higher RTIL content (70 and 90%, v/v) were also fully soluble in the reaction media during polymerization. Nonetheless, we cannot rule out a molecular basis for increased molecular weights of poly(*p*-cresol) in RTILs

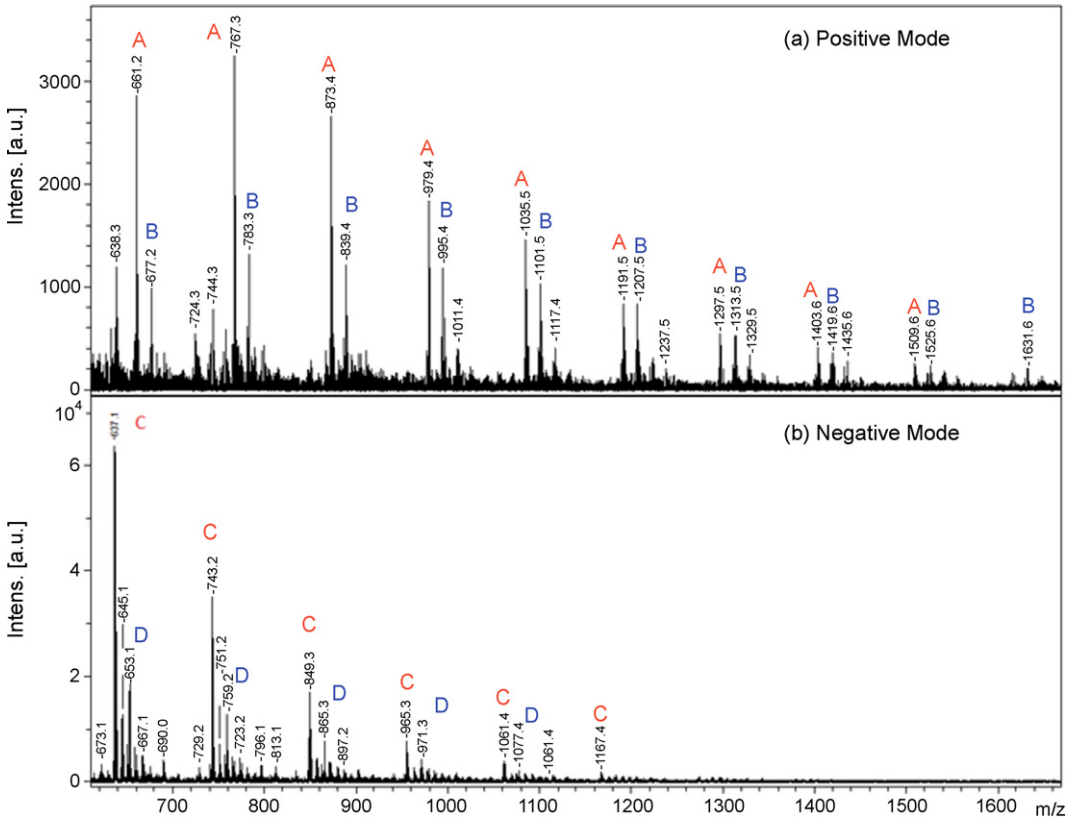
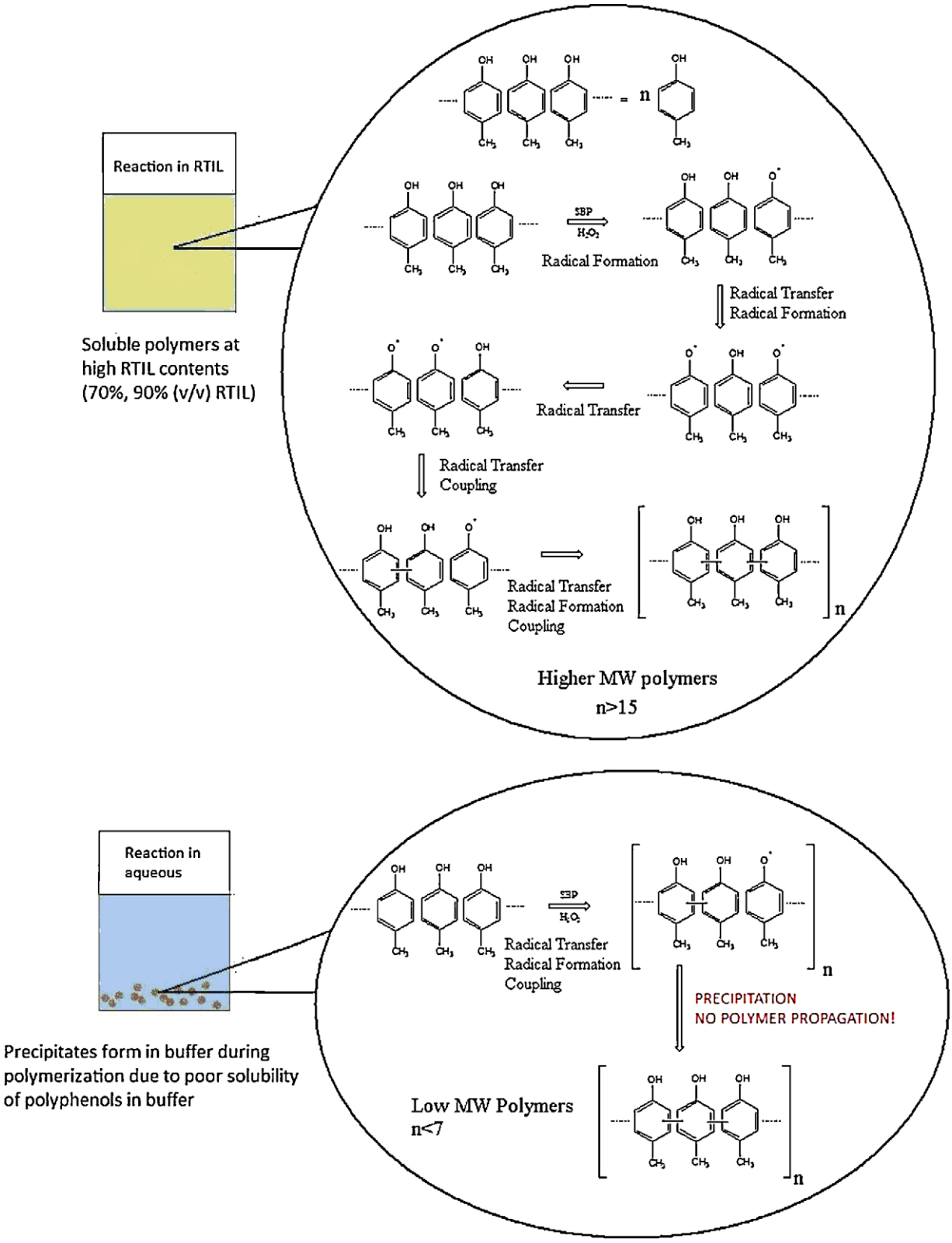


Fig. 2. MALDI-TOF mass spectra of *p*-cresol oligomers in negative (a) and positive (b) ion modes.



Scheme 3. The effect of dissolution capacity of the solvent on polymer molecular weight.

vs. aqueous buffer. Specifically, it may be that *p*-cresol can form molecular clusters in the RTIL due to hydrogen bonding between itself and the anion of the RTIL (BF_4^-), which could result in a higher effective local concentration of *p*-cresol, and therefore, lead to enhanced radical transfers and coupling reactions and, ultimately higher molecular weights. Such a hypothesis has been advanced by Oguchi et al. [38] for poly(*m*-cresol) in methanol–water systems. Regardless of the mechanism, polymer size could be controlled simply by adjusting the RTIL concentration in water (Fig. 1).

3.4. Polymerization of other phenols

The high polyphenolic solubilization capacity of RTILs and the well-known breadth of substrate specificity of SBP [23,39], led us to consider other phenols as substrates for SBP-catalyzed polyphenol synthesis. To that end, we examined the polymerization of hydrophobic phenols (*p*-phenylphenol, 1-naphthol, and 2-naphthol), as well as phenol, itself. In the case of *p*-phenylphenol, conversions in 70% (v/v) BMIM(BF_4) and 70% (v/v) BMPy(BF_4) exceeded 95%, indicating that SBP was highly efficient in catalyzing the oxidation of this hydrophobic phenolic. This conversion was similar to that obtained in aqueous buffer containing 10% (v/v)

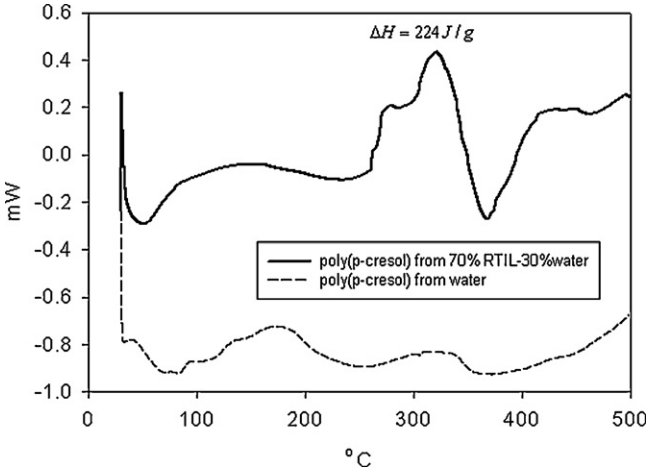


Fig. 3. DSC profiles for poly(*p*-cresol) synthesized in 70% RTIL–30% buffer and synthesized in buffer.

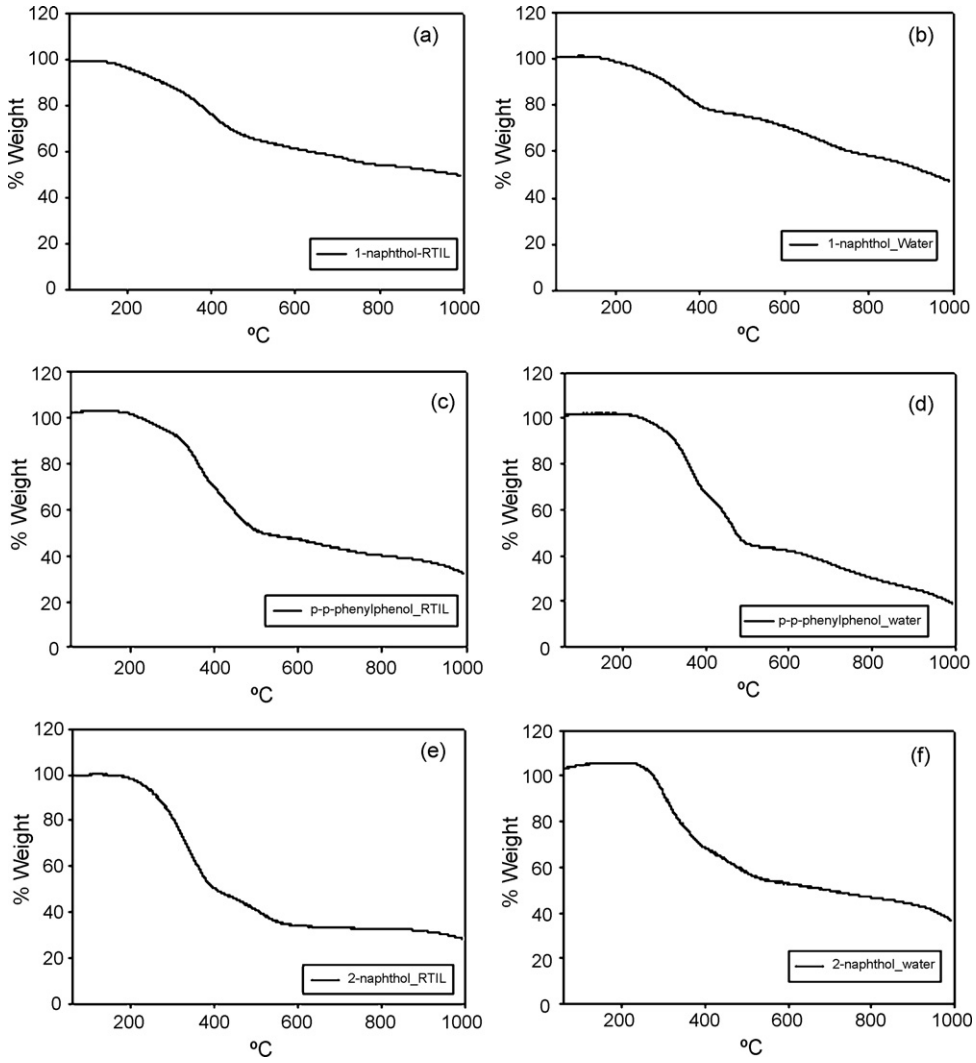


Fig. 4. TGA profiles for (a) poly(1-naphthol) synthesized in 70% RTIL–30% water; (b) poly(1-naphthol) synthesized in 10% RTIL–90% water; (c) poly(*p*-phenylphenol) synthesized in 70% RTIL–30% water; (d) poly(*p*-phenylphenol) synthesized in 10% RTIL–90% water; (e) poly(2-naphthol) synthesized in 70% RTIL–30% water; and (f) poly(2-naphthol) synthesized in 10% RTIL–90% water.

Table 1
SBP-catalyzed polymerization of *p*-phenylphenol in different ionic liquids.

Solvent	% Conversion	% Yield DMF soluble	% Yield DMF insoluble	MALDI-TOF		
				M_w	M_n	PDI
70% BMIMBF$_4$–30% aqueous	96.2 ± 0.3	96.2 ± 0.3	–	1820	1400	1.3
70% BMPy BF$_4$–30% aqueous	95.0 ± 1.2	95.0 ± 1.2	–	1860	1550	1.2
10% BMPyBF$_4$–90% aqueous	99.7 ± 0.1	99.7 ± 0.1	–	737	670	1.1

Table 2
Polymerization of different phenols in 70% BMPy(BF$_4$)–30% buffer.

Substrate	% Conversion	% Yield DMF soluble	% Yield DMF insoluble	DMF soluble MALDI-TOF			DMF insoluble MALDI-TOF		
				M_w	M_n	PDI	M_w	M_n	PDI
Phenol	60 ± 0.5	50 ± 0.0	10 ± 0.0	1100	1000	1.1	2900	1650	1.75
1-Naphthol	83 ± 0.5	69 ± 0.0	14 ± 0.0	2010	1670	1.2	2600	1700	1.5
2-Naphthol	75 ± 10	75 ± 10	0	1170	970	1.2	–	–	–
p-Phenylphenol	95 ± 1.2	95 ± 1.2	0	1860	1550	1.2	–	–	–

BMPy(BF$_4$), which was used to enable sufficient solubility of the hydrophobic phenol. While the molecular weight of the polymer obtained in aqueous buffer was low (M_n = 600), substantially higher molecular weights (>2.5-fold) were obtained in the RTIL (Table 1).

In the case of the naphthols, relatively high conversions (>70%) were achieved, with most remaining soluble in DMF. Poly(phenol) was also mostly DMF soluble, showing relatively lower conversion (60%) than naphthols and *p*-phenylphenol. The M_w values of the DMF soluble polymer fractions as determined by MALDI-TOF were between ca. 1000 and 1700 Da with low PDI (1.1–1.2). DMF insoluble fractions of poly(1-naphthol) and poly(phenol) showed highly polydisperse polymers with M_w up to 2900 Da (Table 2). These results suggest that high molecular weight polyphenolics are readily synthesized by SBP catalysis in RTILs from a diverse range of phenolic monomers. Moreover, the polymer size is strongly controlled by both solvent composition and the nature of the phenolic monomer.

3.5. Thermal properties

Differential scanning calorimetry and thermogravimetric analysis were used to study thermal properties of some of the polymers synthesized in 70% (v/v) RTIL and compared to those synthesized in aqueous buffer. Since *p*-phenylphenol, 1-naphthol, and 2-naphthol have negligible water solubility, the reactions in aqueous buffer with these phenols contained 10% (v/v) BMPy(BF$_4$). The DSC scan of poly(*p*-cresol) synthesized in 70% (v/v) BMPy(BF$_4$) was considerably different from that synthesized in aqueous buffer (Fig. 3). Specifically, the former exhibited an exotherm around 320 °C ($\Delta H \sim 224$ J/g), while the polymer generated in aqueous buffer showed essentially no features on the DSC. This heat flow for the polymer generated in the RTIL was absent in a second heating (data not shown), which demonstrated that poly(*p*-cresol) acted as a thermoset, as expected [40]. Similar DSC profiles were obtained for other phenols (data not shown) and all showed thermosetting properties. In addition, all of the phenolic polymers synthesized in both RTIL and aqueous buffer, did not show a clear glass transition temperature (T_g).

TGA revealed that at least 20% of the initial weight of all the polymers remained after heating to 1000 °C, and >50% of the mass remained above 500 °C indicating that the polyphenols were highly thermostable (Fig. 4). In the case of poly(1-naphthol) and poly(2-naphthol), there was a 40–50 °C increase in decomposition temperature when they were synthesized in 70% (v/v) BMPy(BF$_4$) which presumably was due to the higher molecular weight compared to poly(naphthols) synthesized in aqueous buffer and possibly structural changes of the polyphenols in the RTIL as compared to aqueous buffer. With respect to the latter, recent studies have shown that polyphenols obtained by peroxidase catalyzed polymerization reactions in aqueous–organic solvent mixtures, are structurally composed of a mixture of phenylene and oxyphenylene units, which are formed by C–C and C–O couplings of phenols, respectively [18,41]. The regioselectivity of phenol polymerization is mainly affected by the solvent composition and the nature of the phenol [42]. These factors may also result in structural differences of the phenols synthesized in RTILs vs. aqueous buffer. Such structural studies are currently underway to elucidate the effects of the RTIL on the resulting polyphenolic structure.

4. Conclusion

In conclusion, we have found that RTILs are effective solvents for SBP-catalyzed oxidative polymerization of phenols. Selective control over polymer size can be obtained by varying the RTIL concentration. Higher molecular weight polymers can be synthesized in the presence of higher RTIL concentration based on GPC and MALDI-TOF analysis. Thermal analysis showed that the polymers synthesized in RTILs are highly thermostable and function as thermosets.

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References

[1] G.L. Brode, in: Kirk-Othmer Encyclopedia of Chemical Technology, Phenolic resins, John Wiley and Sons, New York, 1982, pp. 384-416.
[2] S. Levchika, A. Piotrowskia, E. Weilb, Q. Yaob, Polym. Degrad. Stabil. 88 (2005) 57–62.
[3] N. Kishorea, S. Sachana, K.N. Raib, A. Kumar, Carbon 41 (2003) 2961–2972.
[4] J.E. Shafizadeh, S. Guionnet, M.S. Tillman, J.C. Seferis, J. Appl. Polym. Sci. 73 (1999) 505–514.
[5] B.D. Park, B. Riedl, E.W. Hsu, J. Shields, Wood Sci. Technol. 35 (2001) 311–323.
[6] I. Yang, M. Kuo, D.J. Myers, A. Pu, Wood Sci. Technol. 52 (2006) 503–508.
[7] J.J. Clary, J.E. Gibson, R.S. Waritz, Formaldehyde: Toxicology, Epidemiology, Mechanisms, Dekker, New York, 1983.
[8] J.L. Niu, J. Burnett, Environ. Int. 26 (2001) 573–580.
[9] A.S. Hay, H.S. Blanchard, G.F. Endres, J.W. Eustance, J. Am. Chem. Soc. 81 (1959) 6335.
[10] A.S. Hay, J. Polym. Sci. 58 (1962) 581.
[11] A.S. Hay, J. Polym. Sci., Polym. Chem. Ed. 36 (1998) 505.
[12] R.A. Gross, A. Kumar, B. Kalra, Chem. Rev. 101 (2001) 2097–2124.
[13] K.V. Sarkanen, C.H. Ludwig, Lignins: Occurrence, Formation, Structure and Reactions, Wiley, New York, 1971.
[14] N.G. Lewis, E. Yamamoto, Annu. Rev. Plant Physiol. Plant Mol. Biol. 41 (1990) 455–496.
[15] A. Hüttermann, C. Mai, A. Kharazipour, Appl. Microbiol. Biotechnol. 55 (2001) 377–384.

[16] M. Reihmann, H. Ritter, Adv. Polym. Sci. 194 (2006) 1–49.
[17] J.S. Dordick, M.A. Marletta, A.M. Klibanov, Biotechnol. Bioeng. 30 (1987) 31–36.
[18] T. Oguchi, S. Tawaki, H. Uyama, S. Kobayashi, Macromol. Rapid Commun. 20 (1999) 401.
[19] R.D. Schwartz, D.B. Hutchinson, Enzyme Microb. Technol. 3 (1981) 361–363.
[20] J.S. Dordick, M.A. Marletta, A.M. Klibanov, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 6255–6257.
[21] J.A. Akkara, K.J. Senecal, D.L. Kaplan, J. Polym. Sci. Polym. Chem. 29 (1991) 1561.
[22] K. Ryu, J.S. Dordick, Biochemistry 31 (1992) 2588–2598.
[23] A.L. Serdakowski, I.Z. Munir, J.S. Dordick, J. Am. Chem. Soc. 128 (2006) 14272–14273.
[24] S. Kobayashi, H. Kurioka, H. Uyama, Macromol. Rapid Commun. 17 (1996) 503–508.
[25] H. Uyama, N. Maruichi, H. Tonami, S. Kobayashi, Biomacromolecules 3 (2002) 187–193.
[26] A. Pizzi, H. Pasch, C. Simon, K. Rode, J. Appl. Polym. Sci. 92 (2004) 2665–2674.
[27] M.S. Ayyagari, K.A. Marx, S.K. Tripathy, J.A. Akkara, D.L. Kaplan, Macromolecules 28 (1995) 5192–5197.
[28] F. van Rantwijk, R. Madeira-Lau, R.A. Sheldon, Trends Biotechnol. 21 (2003) 131–138.
[29] Z. Yang, W. Pan, Enzyme Microb. Technol. 37 (2005) 19–28.
[30] S. Sgalla, G. Fabrizi, S. Cacchi, A. Macone, A. Bonamore, A. Boffi, J. Mol. Catal. B: Enzym. 44 (2007) 144–148.
[31] U. Kragl, M. Eckstein, N. Kaftzik, Curr. Opin. Chem. Biol. 13 (2002) 565–571.
[32] J.J. Kraus, I.Z. Munir, J.P. McEldoon, D.S. Clark, J.S. Dordick, Appl. Biochem. Biotechnol. 80 (1999) 221–230.
[33] P. Xu, J. Kumar, L. Samuelson, A.L. Cholli, Biomacromolecules 3 (2002) 889–893.
[34] A.K. Burkoth, K.S. Anseth, Macromolecules 32 (1999) 1438–1444.
[35] M.W.F. Nielen, Mass Spectrom. Rev. 18 (1999) 309–344.
[36] H. Mandal, A.S. Hay, Polymer 38 (1997) 6267–6271.
[37] R.Z. Kazandjian, A.M. Klibanov, J. Am. Chem. Soc. 107 (1985) 5448–5450.
[38] T. Oguchi, A. Wakisaka, S.-I. Tawaki, H. Tonami, H. Uyama, S. Kobayashi, J. Phys. Chem. B 106 (2002) 1421–1429.
[39] I.Z. Munir, J.S. Dordick, Enzyme Microb. Technol. 26 (2000) 337–341.
[40] A.M. Blinkovsky, J.S. Dordick, J. Polym. Sci. Polym. Chem. 31 (1993) 1839–1846.
[41] H. Uyama, H. Kurioka, I.,S.K. Kaneko, Chem. Lett. 23 (1994) 423–426.
[42] N. Mita, S. Tawaki, H. Uyama, S. Kobayashi, Chem. Lett. 31 (2002) 402–403.