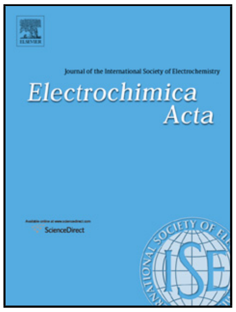

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Comparison of a series of laccase mediators in the electro-oxidation reactions of non-phenolic lignin model compounds

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Highlight

The electro-oxidations of non-phenolic lignin model compounds with laccase mediators (NHPI, HBT, VLA, TEMPO, ABTS) have been investigated under the same reaction conditions.

The reaction selectivity of the mediators in the electrolytic mediator system was basically reflected to their reaction mechanism in the laccase mediator systems.

NHPI was the best mediator for selective Cα-carbonylation of non-phenolic β-O-4 structures in lignin in the present system.

ABSTRACT

The electro-oxidations of non-phenolic lignin model compounds in an electrolytic mediator system (EMS) have been investigated with several laccase mediators, including *N*-hydroxyphthalimide (NHPI), 1-hydroxybenzotriazole (HBT), violuric acid (VLA), 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), under the same reaction conditions. In the bulk electrolyses of the monomeric model compound [1-(4'-ethoxy-3'-methoxyphenyl)ethanol (1G)], oxidation with NHPI gave the corresponding C_α=O product (2G) in high yield, whereas the oxidations with HBT, VLA, and TEMPO afforded 2G in moderate yields. The highest reaction selectivity for the guaiacyl-units was found in the oxidation conducted in the presence of ABTS, although the yield was low. In the bulk electrolyses of the dimeric model compound [4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (3G)], the oxidation with NHPI gave the corresponding C_α=O product (4G) in high yield, whereas the oxidations with HBT, VLA, and TEMPO gave 4G in low yields. In contrast, the oxidation with ABTS gave a C_α-C_β cleavage product (5G) in 5.5% yield. The selectivity of the mediators in the EMS reaction effectively reflected the mechanisms of their reactions, as reported for the laccase mediator system. NHPI was confirmed to be the best mediator in the present system for the selective C_α-carbonylation of the non-phenolic β-O-4 structures in lignin.

Keywords

Cα-carbonylation, Cα-Cβ cleavage, electrolytic mediator system (EMS), laccase mediator, lignin

1. Introduction

Kraft pulping is one of the most important manufacturing processes in the pulp and paper industry. During the pulping treatment, strong alkali is used in the presence of a sodium sulfide catalyst for the cleavage of the lignin. Unfortunately, the application of such forcing conditions can lead to appreciable levels of degradation in the carbohydrates, and the development of a pretreatment process to facilitate the delignification would therefore be desirable for high-yield pulping. The cleavage of the non-phenolic β-O-4 linkages of lignin is believed to be the rate-determining step during the main delignification process [1]. In contrast, it has also been reported that the alkali cleavage of non-phenolic β-O-4 linkages can be significantly accelerated by the presence of C_{α}-carbonyl groups [2, 3]. Selective C_{α}-carbonylation in non-phenolic β-O-4 substructures is therefore a target of considerable interest for the development of a pretreatment process for kraft pulping.

The oxidation reactions of lignin in the laccase- and electrolytic-mediator systems (LMS and EMS, respectively) have been proposed as eco-friendly processes for kraft pulping (Fig.1) [4-7]. In these systems, lignin is indirectly oxidized thorough a mediator. To date, many papers have been published concerning the oxidation of non-phenolic lignin model compounds in an LMS [8-11]. A variety of different compounds have been reported as laccase mediators, including *N*-hydroxyphthalimide (NHPI), 1-hydroxybenzotriazole (HBT), violuric acid (VLA), 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) (Fig. 2). The laccase mediators can be divided into three different groups based on their reaction mechanisms in the LMS, which include (1) hydrogen atom transfer (HAT) mechanism-type mediators such as NHPI, HBT, and VLA; (2) ionic mechanism-type mediators such as TEMPO; and (3) electron transfer (ET) mechanism-type mediators such as ABTS [12-14]. It is well known that the C_α-carbonylation process competes with C_α-C_β cleavage during the oxidation of non-phenolic substrates in the LMS. Oxidations involving HAT mechanism type mediators (e.g., NHPI, HBT, and VLA) and ionic mechanism type mediators (e.g., TEMPO) preferentially afforded the C_α-carbonyl product in the LMS, whereas oxidations involving ET mechanism type mediator (e.g., ABTS) gave the C_α-carbonyl product together with the C_α-C_β cleavage products [9].

The use of EMS has two advantages over LMS, in that (1) a wide range of pH and temperature conditions can be selected; and (2) a mediator with a high redox potential can be used. The mediators that have been proposed for LMS can be also used in EMS. Only a few reports concerning the oxidation of non-phenolic lignin model compounds in EMS, however, have been published in the literature [15-19]. The oxidation reactions of a non-phenolic β-O-4 dimer in the EMS have been reported using several different mediators, including NHPI [15], VLA [16], HBT and ABTS [17], and tris(4-bromophenyl)amine [19]. Furthermore, the oxidation of veratryl alcohol has been reported with HBT and ABTS [18]. The mediators that have been proposed for use with LMS could be also used in the EMS, where they are expected to afford similar results to those achieved in the EMS. Interestingly, however, the EMS and LMS oxidations of a non-phenolic β-O-4 dimer with the same mediator gave different results, in that the oxidation of non-phenolic β-O-4 dimer in LMS with HBT gave a C_α-carbonyl product exclusively, whereas the oxidation in the EMS with HBT gave a mixture of the C_α-carbonyl and C_α-C_β cleavage products [17]. A satisfactory comparison of the laccase mediators has been hampered by the different electro-oxidation conditions. A systematic investigation of laccase mediators in both the EMS is therefore needed. Herein, a comparison of several representative laccase mediators, including NHPI, HBT, VLA, TEMPO, and ABTS has been conducted with NHPI under the same electro-oxidation conditions described in the previous paper [15].

2. Experimental

The substrates [1-(4’-ethoxy-3’-methoxyphenyl)ethanol (guaiacyl type, 1G), 1-(4’-ethoxy-3’,5’-dimethoxyphenyl)ethanol (syringyl type, 1S), 1-(4’-ethoxyphenyl)ethanol (*p*-hydroxyphenyl type, 1H), and 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (3G)] and the authentic samples (Fig. 3) were prepared as described by Shiraishi et al. [20]. The laccase mediators and all of the other chemicals used in the study were obtained from commercial sources and used as received (Nacalai Tesque Inc., Kyoto, Japan). In contrast to our previous publications [15], where a 0.1 M $LiClO_4$ solution in CH_3CN was predominantly used as the electrolyte for the cyclic voltammetry (CV) measurements and bulk electrolyses, a 0.1 M $LiClO_4$ solution in a mixture of CH_3CN and H_2O (7/3 - v/v) was used in the current study because VLA and ABTS were insoluble in CH_3CN.

The CV measurements were performed with an ALS electrochemical analyzer (ALS 650B; BAS, Tokyo, Japan) in an undivided cell (5 mL) [working electrode: 1.6 mm diameter platinum disk; reference electrode: Ag/Ag^+ reference electrode (0.1 M $LiClO_4$, 0.01 M $AgNO_3$ in CH_3CN), counter electrode: platinum wire electrolyte: 0.1 M $LiClO_4$ in CH_3CN/H_2O = 7/3 (v/v), concentration of the mediator: 5 mM, 2,6-lutidine: 25mM, compound 1G: 25 mM].

Bulk electrolyses of the non-phenolic monomer 1G and the dimer 3G with the laccase mediator at fixed potentials (Tables 1 and 3) were carried out with the ALS electrochemical analyzer equipped with a power booster (ALS 680) in the anode chamber of a divided cell, until the current value dropped to about 1 mA, unless otherwise noted. [The divided cell: in the anode chamber, a carbon felt working electrode (2.4 × 3.0 cm^2 for 1G or 1.5 × 2.0 cm^2 for 3G), the Ag/Ag^+ reference electrode, a substrate (5 mM), a mediator (1 mM), 2,6-lutidine (25 mM), and 0.1 M $LiClO_4$ in CH_3CN/H_2O = 7/3 (v/v) (20 mL for 1G or 10 mL for 3G); in the cathode chamber, the platinum plate electrode, 0.1 M tetra-*n*-butyl ammonium perchlorate (TBAP) in CH_3CN/H_2O = 7/3 (v/v) (5 mL)]. Upon completion of the electrolysis, CH_3CN (0.5 ml) containing an internal standard (36 μmol benzhydrol for 1G or 18 μmol benzophenone for 3G) was added to the anode chamber. The anolyte was extracted three times with EtOAc, and the combined organic layers were washed with distilled water, dried over Na_2SO_4, and concentrated to dryness to give the products as colorless oil. The products were subjected to gas chromatography for quantification of the different components as previously described in the literature for the oxidations of 1G [20] and 3G [15].

The bulk electrolyses of a mixture of non-phenolic monomers 1G, 1S and 1H (2.5 mM in each case) were performed in accordance with the procedure described above for the electrolyses of 1G. The relative reactivity ratios (k_G/k_S and k_G/k_H, see Eq. 1) of the mediators were calculated from the yields of the oxidation products [21].

$$\begin{aligned} &1\mathrm{G} + \mathrm{Med}_{\mathrm{OX}} \xrightarrow{k_G} 2\mathrm{G} + \mathrm{Med} \\ &1\mathrm{S} + \mathrm{Med}_{\mathrm{OX}} \xrightarrow{k_S} 2\mathrm{S} + \mathrm{Med} \\ &1\mathrm{H} + \mathrm{Med}_{\mathrm{OX}} \xrightarrow{k_H} 2\mathrm{H} + \mathrm{Med} \end{aligned} \tag{1}$$

3. Results and discussion

3.1. Cyclic voltammetry (CV) studies

Cyclic voltammetry (CV) measurements of the laccase mediators were performed to allow for the determination of the applied potential in the bulk electrolysis and for simple qualitative analysis of the electrolysis. Fig. 4A–F show the CVs of the laccase mediators in the presence and in the absence of the base catalyst (2,6-lutidine) in a 0.1 M $LiClO_4$ solution in CH_3CN/H_2O = 7/3 (v/v). In the CVs of NHPI (Fig.4A), the oxidation peak potential (0.90 V of peak (a)) of NHPI in the absence of the catalyst in CH_3CN/H_2O was different from that in CH_3CN (1.78 V, data in the previous paper [15]), indicating that the oxidation potential was significantly influenced by the reaction conditions in the EMS. The oxidation peak potentials of *N*-hydroxylamine derivatives are known to be shifted in a negative direction in the presence of a base [22, 23]. A negative shift in the oxidation peak potential of NHPI was also observed in the CVs in CH_3CN/H_2O (from 0.90 V of the shoulder peak (a) to 0.54 V of peak (c)), as well as in the CVs conducted in CH_3CN [15]. These shifts could be attributed to the deprotonation of NHPI by 2,6-lutidine to give the NHPI-anion. The anion could then be oxidized on the electrode at 0.54 V to give the phthalimide-*N*-oxyl (PINO) radical (Fig. 5). A reduction peak was not observed in the CV of NHPI conducted in the presence of 2,6-lutidine, suggesting that the PINO radical was unstable in the system. In the CVs of HBT (Fig. 4B), a negative shift was observed in the oxidation peak potential (from 0.72 V of peak (d) to 0.50 V of peak (e)) in the presence of 2,6-lutidine. The reduction peak was not found in the CV with 2,6-lutidine, suggesting that the benzotriazole-*N*-oxyl (BTNO) radical was unstable in the system. The poor stability of the BTNO radical was also reported by Bourbonnais et al. [18]. The oxidation peak potentials in the absence (peak (f)) and presence (peak (h)) of 2,6-lutidine were 0.58 and 0.37 V, respectively, in the CVs of VLA (Fig. 4C), clearly indicating the occurrence of a negative shift. A reduction peak (peaks (i)) was clearly observed in the CV conducted in the presence of 2,6-lutidine, suggesting that the VLA radical was stable even in the presence of 2,6-lutidine, and this observation was consistent with data reported elsewhere

[16,24]. A relatively low oxidation peak potential (peak (j) 0.32 V) was found for TEMPO in the CV conducted in the absence of 2,6-lutidine (Fig. 4D), whereas no shift was observed in the CV conducted in the presence of 2,6-lutidine. A reduction peak (peak (k)) was clearly observed in this particular case. Furthermore, peak (j) was attributed to the single-electron oxidation of TEMPO to the corresponding oxoammonium ion ($TEMPO^+$), whereas the reduction peak (k) was attributed to the reduction from $TEMPO^+$ to TEMPO, suggesting that $TEMPO^+$ was stable in the system. It was also reported in the CV of TEMPO-poly (acrylic acid) modified glassy carbon disk electrode [25].

In the CV of ABTS conducted in the absence of a catalyst at the standard scan rate (0.05 Vs^{-1}), two oxidation peaks were observed at (l) at 0.28 V and (m) at 0.73 V (Fig. 4E), corresponding to the single-electron oxidations from ABTS to $ABTS^{+\bullet}$ and from $ABTS^{+\bullet}$ to $ABTS^{2+}$, respectively. No negative shift was observed in this particular case. Two reduction peaks (n) at 0.35 V and (o) at 0.24 V were also found. The peak (n) was not considered the reduction peak from $ABTS^{2+}$ to $ABTS^{+\bullet}$ because of the big difference between the oxidation peak (m) and reduction peak (n), whereas peak (o) was attributed to the reduction of $ABTS^{+\bullet}$ to ABTS. Bourbonnais et al. [18] reported that the reduction peak of $ABTS^{2+}$ was not observed in the CV conducted in the presence of a catalyst at a slow scan rate (0.20 Vs^{-1}). They also explained the lack of a reduction peak on the basis of the enhanced intensity of the reduction peak of $ABTS^{+\bullet}$ from the comproportionation reaction between ABTS and $ABTS^{2+}$. Peak (n) might be considered the peak corresponding to the comproportionation reaction, but further investigation is required.

Fig. 4F shows the CV of ABTS in the absence of the catalyst at an enhanced scan rate (0.5 Vs^{-1}). The CV possessed a profile consistent with those previously reported in the literature [18]. The CV contained a peak (p) corresponding to the reduction of $ABTS^{2+}$ to $ABTS^{+\bullet}$ and there was also a reduction in the intensity of peak (n), suggesting that $ABTS^{2+}$ was relatively unstable. The current data therefore suggested that $ABTS^{2+}$ was spontaneously converted to an unknown ABTS intermediate, and that the intermediate was reduced to $ABTS^{+\bullet}$ at the potential of peak (n) in this particular CV at the standard scan rate.

It is well known that the catalytic efficiency of a mediator can be evaluated based on the CVs of the mediator conducted in the presence and in the absence of a substrate [18]. The catalytic efficiency of a mediator can be expressed by the ratio i_k/i_c, where i_k is the anodic peak current of the mediator in the presence of a substrate and i_c is the anodic peak current of the mediator in isolation [26, 27]. Fig. 4G-K show the CVs of the laccase mediators with 2,6-lutidine in the absence and presence of a substrate (compound 1G) in a 0.1 M $LiClO_4$ solution of CH_3CN/H_2O = 7/3 (v/v). The anodic current was enhanced in the CVs of NHPI and HBT (Fig. 4G and H), suggesting that NHPI and HBT were reactive towards compound 1G. The catalytic efficiencies of NHPI and HBT were 3.6 and 1.4, respectively. In contrast, the CVs of VLA and TEMPO showed no significant change in the presence of compound 1G (Fig. 4I and J), suggesting that VLA and TEMPO were not useful mediators in the current system. No enhancement was observed in the anodic current in the CVs of ABTS (Fig. K) following the addition of compound 1G. Interestingly, Bourbonnais et al. [18] reported that the anodic current was enhanced in the presence of veratryl alcohol in a 0.05 M sodium citrate buffer solution (pH 4) [18]. These results confirmed that the catalytic efficiency of the mediators was significantly influenced by the reaction conditions in the EMS. Consequently, the results of the CV studies revealed that NHPI was the most suitable mediator in the current system, when used in conjunction with 2,6-lutidine in a 0.1 M $LiClO_4$ solution of

CH_3CN/H_2O = 7/3 (v/v).

3.2. Bulk electrolyses of the non-phenolic monomers 1G, 1S and 1H

The laccase mediators were submitted to the bulk electrolyses of non-phenolic monomer 1G with 2,6-lutidine in a 0.1 M $LiClO_4$ solution of CH_3CN/H_2O = 7/3 (v/v). Several different applied potentials were used, including 0.70 V for NHPI, 0.50 V for HBT, 0.40 V for VLA, 0.40 V for TEMPO, and 0.30 and 0.75 V for ABTS. The values for NHPI and HBT were decided on the basis of the potentials with the highest catalytic efficiencies, whereas the other three were decided on the basis of their oxidation potentials. The quantitative results are summarized in Table 1. The HBT-mediated electro-oxidation of compound 1G afforded compound 2G in 50.5% yield, whereas the NHPI-mediated electro-oxidation gave compound 2G in 94.1% yield. The moderate yield in the former case was attributed to the short lifetime of the BTNO radical. The behavior observed in the current VLA-mediated electro-oxidation was particularly unusual. Although a significant reduction was observed in the current within the first seconds to ca. 2 mA, with a further gradual reduction to 1 mA over the following 15 min, compound 2G was not detected. Based on the CV studies of the VLA described above, the VLA radical was expected to be stable. When the reaction time was increased from 15 min (i.e. until the current dropped to 1 mA) to 3 days (i.e. until the current dropped to 0.02 mA), the yield of compound 2G increased to 47.6%, suggesting that the VLA radical was stable, but less reactive towards compound 1G. TEMPO-mediated electro-oxidation of compound 1G afforded compound 2G in 60.2% yield, suggesting that the benzylic alcohol was easily oxidized in the TEMPO-mediated electro-oxidation as well as in the TEMPO-mediated oxidation with Laccase [28]. The ABTS-mediated electro-oxidation of compound 1G with

2,6-lutidine was initially conducted at 0.30 V (corresponded to the oxidation peak (l) for the conversion of ABTS to ABTS$^{+\bullet}$). The current fell to 1 mA within several seconds, suggesting that all of the electricity was consumed in the formation of ABTS$^{+\bullet}$, although no further reactions were detected. This result suggested that the ABTS$^{+\bullet}$ provided a similar level of reactivity to the veratryl alcohol [18]. The ABTS-mediated electro-oxidation was then conducted both in the absence and in the presence of 2,6-lutidine at 0.75 V (corresponding to the oxidation peak (m) for the conversion of ABTS$^{+\bullet}$ to ABTS^{2+}), affording compound 2G in 3.0 and 16.2% yields, respectively. 2,6-Lutidine effectively improved the yield of compound 2G, likely by accelerating the deprotonation of the radical cation intermediate formed via the single electron transfer of compound 1G according to the ET mechanism, as well as by the direct electro-oxidation of compound 1G [20]. The yield of compound 2G, however, was low, even when 2,6-lutidine was used, and this was attributed to the rapid conversion of ABTS^{2+} to the unknown ABTS intermediate, as described in the discussion of the CV studies involving ABTS.

The substrate selectivity in the EMS was also an important variable for the laccase mediators. The relative reactivity ratios were determined from the yields of compounds 2G, 2S and 2H upon competition of the electrolyses of the mixtures of 1G, 1S and 1H [21]. The results are shown in Table 2. In all of the electro-oxidations, the k_G/k_S and k_G/k_H values were greater than 1, indicating that the preferential oxidation of the guaiacyl-type compound 1G had proceeded. Although the substrate selectivity for the guaiacyl-type compound in the ABTS-mediated electro-oxidation was very high, the yields of the corresponding C$_\alpha$-carbonyl compounds were low. The preference for the oxidation of the guaiacyl-type compound over the syringyl-type and *p*-hydroxyphenyl-type compounds could be attributable to the higher ionization potential of the non-phenolic syringyl-type compound [29, 30] and the higher oxidation potential of compound 1H (1.4 V for 1H and 1.0 V for 1G [20]), respectively. Other factors potentially affecting the preference of the laccase mediators for oxidation should also be considered, such as the polar effects of the radical transition state in the NHPI-mediated electro-oxidation [31, 32]. Further investigations would be required to develop a clearer understanding of the observed substrate selectivity.

3.3. Bulk electrolyses of the non-phenolic dimer 3G

The laccase mediators were subjected to bulk electrolyses of the non-phenolic β-O-4 dimer 3G in the presence of 2,6-lutidine in a 0.1 M $LiClO_4$ solution in CH_3CN/H_2O = 7/3 (v/v). The gas chromatograms of the products in the bulk electrolyses are shown in Fig. 6A-E, and the quantitative results are summarized in Table 3. In the NHPI-mediated electro-oxidation of compound 3G, nearly all of the starting material 3G was consumed (Fig. 6A) and a C_α=O product (compound 4G) was obtained in 92.3% yield [15]. In the HBT- and VLA-mediated electro-oxidations of compound 3G, compound 4G was obtained in 14.1 and 11.5% yields, respectively, with most of the starting material 3G being recovered in both cases (Fig. 6B and C). These results indicated that the PINO radical was reactive towards compound 3G, and that the BTNO and VLA radicals were not as reactive, as expected from the CV studies of the mediators. Although the peak (q) observed at 4.4 min did not correspond to the peak derived from compound 5G in the gas chromatogram of the products in the electro-oxidation with HBT (Fig. 6B), it appeared to correspond to the peak from compound 5G. The formation of a C_α-C_β cleavage product (compound 5G) was not detected in the electro-oxidation reactions involving the *N*-hydroxylamine compounds (i.e. NHPI, HBT and VLA). The reaction selectivity observed for the *N*-hydroxylamine compounds was consistent with the results expected on the basis of their reaction mechanism (HAT mechanism) in the LMS. The TEMPO-mediated electro-oxidation of compound 3G also preferentially afforded compound 4G in 2.0% yield, with most of the starting material 3G also being recovered (Fig. 6D), suggesting that TEMPO was less reactive towards compound 3G. Further, a much lower yield was observed for the electro-oxidation of dimer 3G in this particular case than in the oxidation of monomer 1G. These results were in agreement with the data observed in the LMS conducted with TEMPO [28]. The low yield in this case could be attributed to the steric hindrance afforded by the C_α-OH moiety of the substrate. The electron donating effect of the B-ring in intermediate 6G (Fig. 7) could also have an impact on the yield of the reaction, because electron donating groups have been reported to oppose the deprotonation of TEMPO-adducts [28]. Although the use of TEMPO as a selective oxidation catalyst for the oxidation of primary alcohols has been well documented in the literature [33], no C_γ-oxidation products were observed in the current electro-oxidation reactions using TEMPO. The ABTS-mediated electro-oxidation of compound 3G gave compounds **4** and **5** in 2.8 and 5.5% yields, respectively, suggesting that the C_α-C_β cleavage reaction proceeded preferentially. The results suggested that ABTS operated via an ET mechanism in the LMS. Overall, the results demonstrated that the reaction selectivities of the laccase mediators in the EMS were in agreement with those observed in the LMS using the same mediators [9], and that NHPI was the best mediator for the C_α-carbonylation in the present system.

4. Conclusions

The oxidations of non-phenolic lignin model compounds were investigated under the same conditions in the EMS with a variety of different laccase mediators, including NHPI, HBT, VLA, TEMPO, and ABTS.

The CV studies of the mediators showed that NHPI and HBT demonstrated a high level of catalytic efficiency towards the non-phenolic monomer 1G in the system with 2,6-lutidine, and that the VLA radical and $TEMPO^+$ were stable. In the bulk electrolyses of the non-phenolic monomer 1G, the NHPI-mediated electro-oxidation afforded a C_α=O product (compound 2G) in high yield, whereas the HBT-, VLA-, TEMPO- and ABTS-mediated electro-oxidations afforded 2G in only moderate or low yields. The pronounced lifetime of the VLA radical was also confirmed by the extended reaction time of the VLA-mediated electro-oxidation reaction. The guaiacyl-units were found to be more reactive than the syringyl- and *p*-hydroxyphenyl-type units in the current system. Although the highest reaction selectivity for the guaiacyl-units was found in the ABTS-mediated electro-oxidation reaction, the conversion efficiency was low. In contrast, in the bulk electrolyses of the non-phenolic dimer 3G, the NHPI-, HBT-, VLA-, TEMPO-mediated electro-oxidation reactions afforded a C_α=O compound (compound 4G) exclusively, but the yield was extremely low in the TEMPO-mediated electro-oxidation. The ABTS-mediated electro-oxidation reaction gave a C_α-C_β cleavage product (compound 5G) preferentially in a low yield. The reaction selectivity of the mediators in the EMS effectively reflected their reaction mechanisms, as reported in the LMS. Consequently, the current study confirmed that NHPI was the best of the mediators tested in the current system [0.1 M $LiClO_4$/2.6-lutidine/(CH_3CN/H_2O=7/3 by vol.)] for the selective C_α-carbonylation of non-phenolic β-O-4 structures in lignin, although optimum conditions for the HBT-, VLA-, TEMPO-, and ABTS-mediated electro-oxidation reactions should be investigated in greater detail.

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Table 1 Bulk electrolysis of 1-(4'-ethoxy-3'-methoxyphenyl)ethanol (1G) with different mediators

Mediator		Potential	2,6-Lutidine	Yield of 2G[a]	Electricity	CE[b]
	(eq.)	(vs Ag/Ag / V)	(eq.)	(%)	(F mol^{-1})	(%)
NHPI[c]	0.2	0.70	5	94.1	2.23	79.8
HBT	0.2	0.50	5	50.5	1.38	73.0
VLA	0.2	0.40	5	-	0.22	-
VLA	0.2	0.40[d]	5	47.6	2.86	33.3
TEMPO	0.2	0.40	5	60.2	1.56	59.7
ABTS	0.2	0.30	5	-	0.16	-
ABTS	0.2	0.75	5	16.2	1.23	26.3
ABTS	0.2	0.75	0	3.0	0.55	10.9

[a] Yields (based on 1G) were determined by GC using benzhydrol as an IS; [b] current efficiency; [c] data in the previous paper [15]; [d] until current dropped to 0.02 mA (for 3 days)

Table 2 Reactivity ratio of the mediators in the bulk electrolyses of a mixture of 1-(4'-ethoxyphenyl)ethanols (1G, 1S and 1H)

Mediator	Potential	Reactivity ratio[a]			Yield[b] (%)			Electricity
	(vs Ag/Ag+ / V)	k_G/k_S	k_G/k_H	k_S/k_H	2G	2S	2H	(F mol^{-1})
NHPI	0.70	2.6	3.1	1.2	53.5	25.3	22.3	0.67
HBT	0.50	1.7	2.9	1.7	32.7	21.6	13.1	0.60
VLA	0.40	1.9	1.3	0.7	40.5	24.4	33.0	0.88
TEMPO	0.40	3.8	1.3	0.3	36.5	10.9	29.9	0.34
ABTS	0.75	13.0	4.2	0.3	19.1	2.3	4.7	0.46

[a] Reactivity ratio was determined by the method reported by d'Acunzo et al. [21];
[b] Yields (based on 1G, 1S and 1H, respectively) were determined by GC using benzhydrol as an IS.

Table 3 Bulk electrolyses of 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (3G)

Mediator		Potential	2,6-Lutidine	Yield[a] (%)		Electricity	CE[b]
	(eq.)	(vsAg/Ag+ / V)	(eq.)	4G	5G	(F mol^{-1})	(%)
NHPI[c]	0.2	0.70	5	92.3	n.d.[e]	2.23	82.7
HBT	0.2	0.50	5	14.1	n.d.[e]	0.75	37.4
VLA[d]	0.2	0.40[c]	5	11.5	n.d.[e]	0.79	29.2
TEMPO	0.2	0.40	5	2.0	n.d.[e]	0.41	1.9
ABTS	0.2	0.75	5	2.8	5.5	1.03	1.3

[a] Yields (based on 3G) were determined by GC using benzhydrol as an IS; [b] current efficiency; [c] data in the previous paper [15]; [d] until current dropped to 0.02 mA (for 12 h); [e] not detected

Figure 1 Laccase mediator system (LMS) and electrolytic mediator system (EMS)

Figure 2 Laccase mediators

Figure 3 Non-phenolic lignin model compounds (substrates and authentic samples) used in the current study

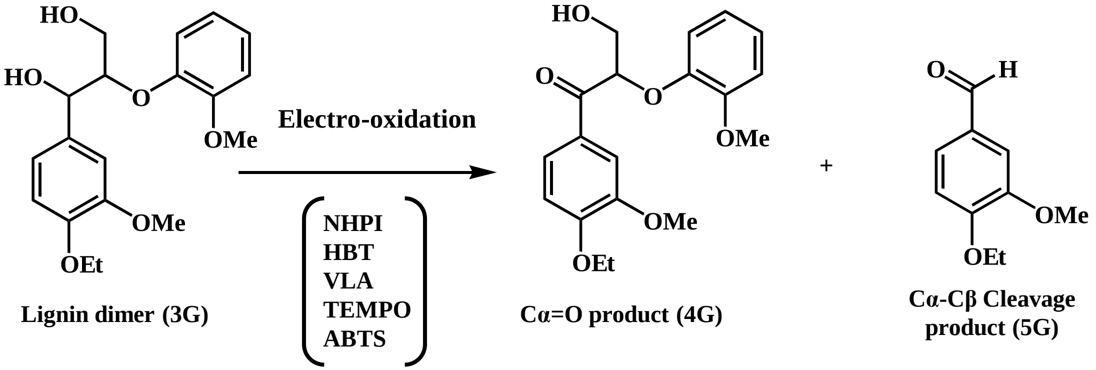
Figure 4 Cyclic voltammograms of NHPI (A, G), HBT (B, H), VLA (C, I) TEMPO (D, J) and ABTS (E, F, K) reactions in the presence (solid line) or absence (dashed line, pH 6) of 2,6-lutidine (A-F, pH 8), as well as in the presence (solid line) or absence (dashed line) of compound 1 (G-K); 0.1 M $LiClO_4$ in CH_3CN/H_2O = 7/3 by (v/v); scan rate 0.05 V s^{-1} (A-E), 0.5 V s^{-1} (F), 0.01 V s^{-1} (G-K) at 28° C

Figure 5 Reaction mechanism for the NHPI-mediated electro-oxidation

Figure 6 Gas chromatograms of the products observed in the electro-oxidation reactions conducted in the presence of NHPI (A), HBT (B), VLA (C) , TEMPO (D) and ABTS (E).

Figure 7 Proposed reaction mechanism of the TEMPO-mediated electro-oxidation of compound 3G

Graphical Abstract



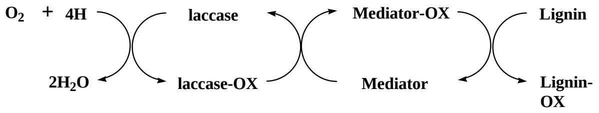
Lignin dimer (3G)

Cα=O product (4G)

Cα-Cβ Cleavage product (5G)

Figure(s)

(a) Laccase mediator system (LMS)



(b) Electrolytic mediator system (EMS)

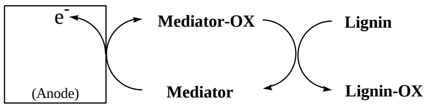


Figure 1 Laccase mediator system (LMS) and electrolytic mediator system (EMS)

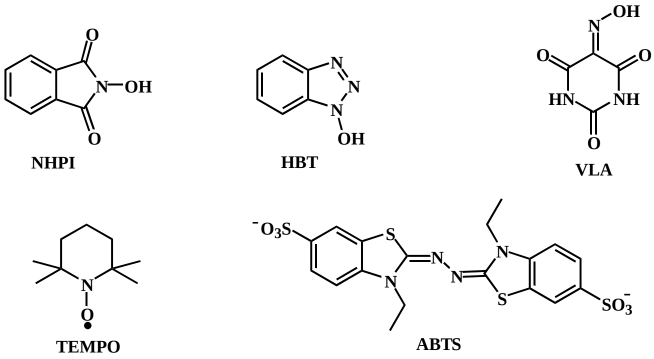


Figure 2 Laccase mediators

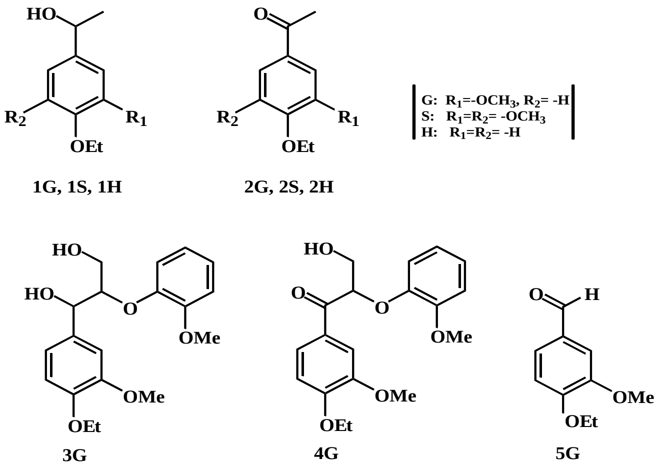


Figure 3 Non-phenolic lignin model compounds (substrates and authentic samples) used in the current study

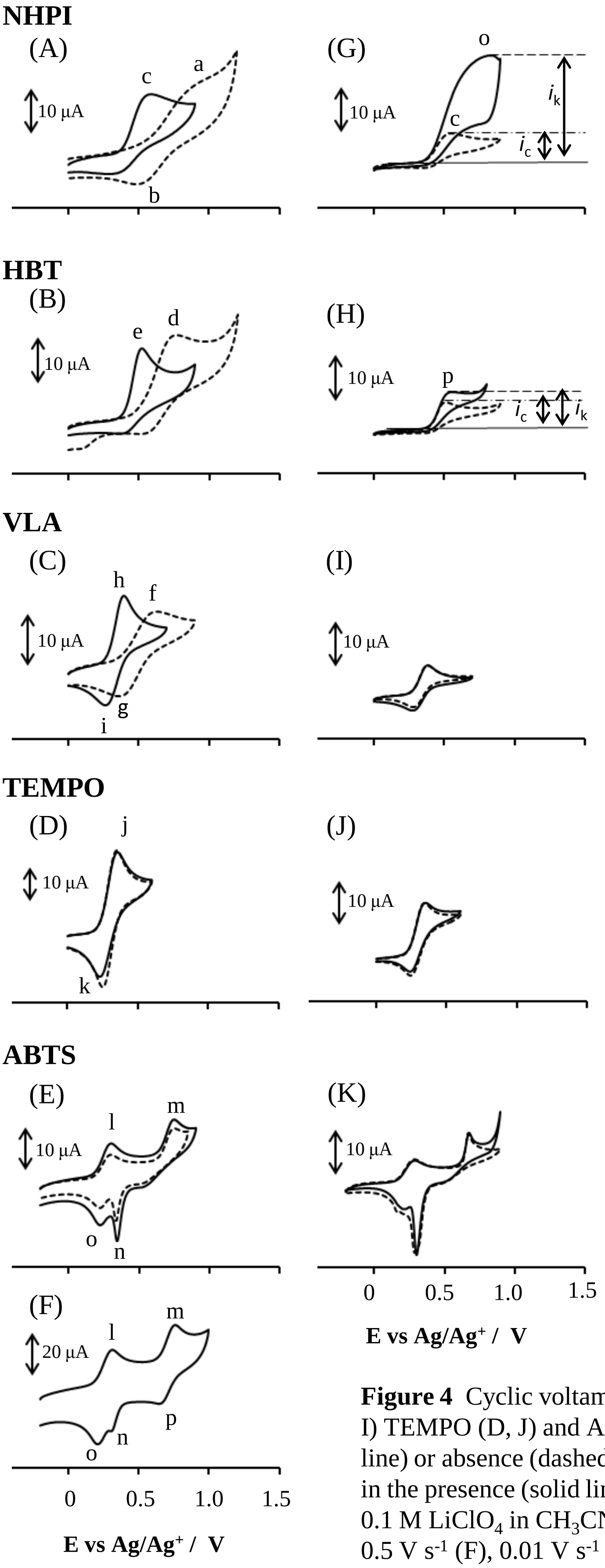


Figure 4 Cyclic voltammograms of NHPI (A, G), HBT (B, H), VLA (C, I) TEMPO (D, J) and ABTS (E, F, K) reactions in the presence (solid line) or absence (dashed line, pH 6) of 2,6-lutidine (A-F, pH 8), as well as in the presence (solid line) or absence (dashed line) of compound 1 (G-K); 0.1 M $LiClO_4$ in CH_3CN/H_2O = 7/3 by (v/v); scan rate 0.05 V s^{-1} (A-E), 0.5 V s^{-1} (F), 0.01 V s^{-1} (G-K) at 28°C

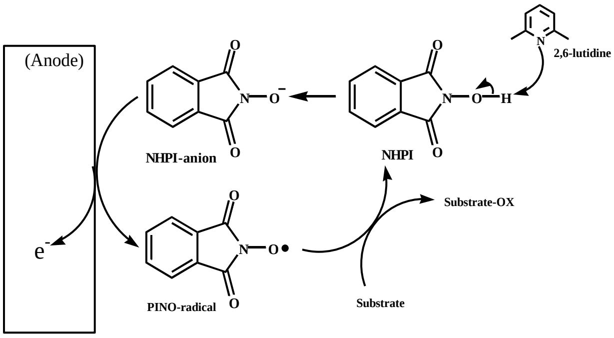


Figure 5 Reaction mechanism for the NHPI-mediated electro-oxidation

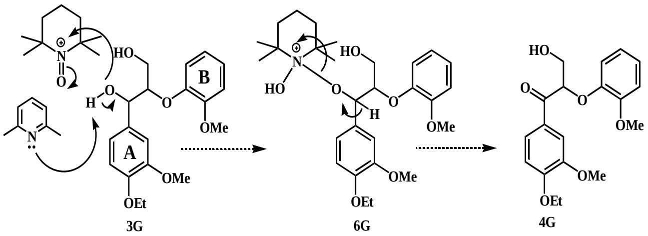


Figure 7 Proposed reaction mechanism of the TEMPO-mediated electro-oxidation of compound 3G

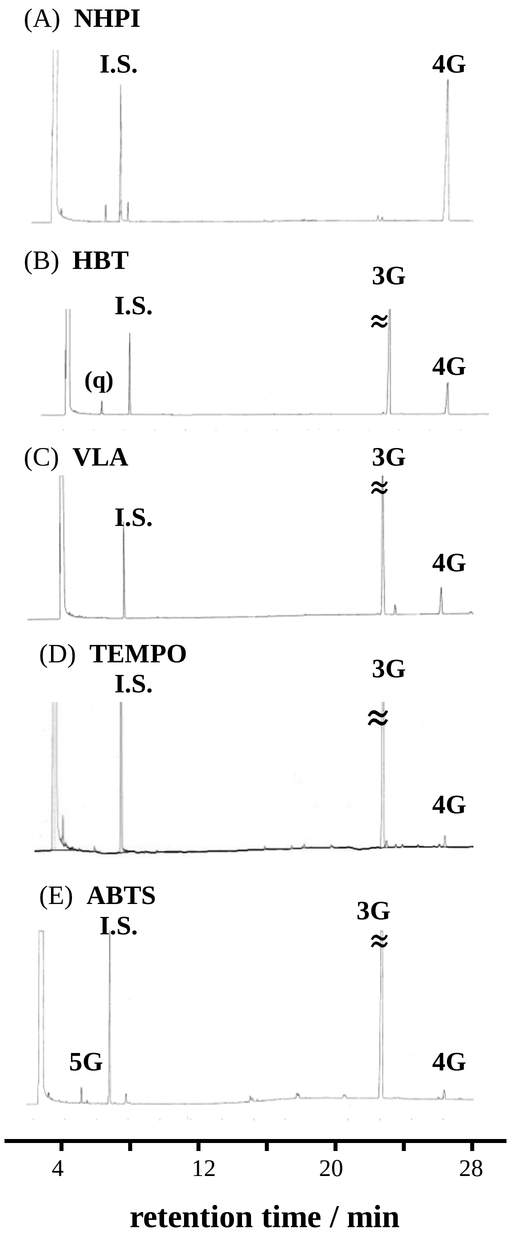


Figure 6 Gas chromatograms of the products observed in the electro-oxidation reactions conducted in the presence of NHPI (A), HBT (B), VLA (C) , TEMPO (D) and ABTS (E).