Styrene-Spaced Copolymers Including Anthraquinone and β-O-4 Lignin Model Units: Synthesis, Characterization and Reactivity Under Alkaline Pulping Conditions

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ABSTRACT: A series of random copoly(styrene)s has been synthesized via radical polymerization of functionalized anthraquinone (AQ) and β-O-4 lignin model monomers. The copolymers were designed to have a different number of styrene spacer groups between the AQ and β-O-4 lignin side chains aiming at investigating the distance effects on AQ/β-O-4 electron transfer mechanisms. A detailed molecular characterization, including techniques such as size exclusion chromatography, MALDI-TOF mass spectrometry, and 1H, 13C, 31P NMR and UV–vis spectroscopies, afforded quantitative information about the composition of the copolymers as well as the average distribution of the AQ and β-O-4 groups in the macromolecular structures. TGA and DSC thermal analysis have indicated that the copolymers were thermally stable under regular pulping conditions, revealing the inertness of the styrene polymer backbone in the investigation of electron transfer mechanisms. Alkaline pulping experiments showed that close contact between the redox active side chains in the copolymers was fundamental for an efficient degradation of the β-O-4 lignin model units, highlighting the importance of electron transfer reactions in the lignin degradation mechanisms catalyzed by AQ. In the absence of glucose, AQ units oxidized phenolic β-O-4 lignin model parts, mainly by electron transfer leading to vanillin as major product. By contrast, in presence of glucose, anthrahydroquinone units (formed by reduction of AQ) reduced the quinone-methide units (issued by dehydration of phenolic β-O-4 lignin side chains) mainly by electron transfer leading to guaiacol as major product. Both processes were distance dependent.

INTRODUCTION

Lignins are amorphous hydrophobic branched heteropolymers composed of phenylpropanoid units mostly linked to each other through glyceroyl ether linkages. In the concept of biorefinery, lignins are regarded as major renewable sources for fuel production as well as for bulky and fine chemicals, particularly aromatics. Therefore, depolymerization of lignin (delignification) is considered a key process that needs to be mastered in order to reach the full potential of a biobased economy. Current large-scale delignification technology relies on pulping techniques. In these processes, biomass materials (mostly wood) are cooked under strong alkaline conditions in order to break the lignin macromolecule into smaller water-soluble fragments, which are then separated from the insoluble carbohydrate fibers (pulp). Although widespread, pulping processes are inefficient, resulting in incomplete lignin removal and significant degradation of the carbohydrate fibers.

To improve the pulping process, many different additives have been proposed. Among them, anthraquinone (AQ) has shown good performance and selectivity. Marked catalytic effect on delignification rate and reduction of carbohydrate degradation has been observed in the pulping process at only 0.1% AQ level (w/w). Several fundamental studies have examined the AQ catalytic effects and have concluded that AQ acts as a redox mediator between the insoluble carbohydrate and lignin biopolymers. Oxidation of carbohydrate end groups by AQ stabilizes the fibers against degradation, while the generated anthrahydroquinone (AHQ) reacts with the lignin macromolecule, resulting in oxidation of AHQ back to AQ and reductive lignin depolymerization.

Despite many efforts, the redox mechanisms by which the AQ catalytic cycle operates are still controversial. The main barrier to research progress was that delignification mechanisms were difficult to be studied directly. The structural complexity of lignins associated with its structural heterogeneity make the
mechanism investigations rather difficult.1,9 Consequently, major advances on lignin reactivity have been performed on well-defined lignin models,10–23 which are designed to mimic the chemical and physical properties of natural lignins. A large variety of lignin model compounds has been reported, particularly the so-called dimeric β-O-4 derivatives, which mimic the major subunits of the native lignin macromolecular structure.24–26 Nevertheless, lignin model compounds may not completely accurately represent the interactions in native lignin because the inherent complexity of the biopolymer.

Investigations of lignin model compound reactivity have underlined some mechanistic aspects of AQ reactions under pulping conditions. For example, studies using β-O-4 models carried out by Dimmel et al.24–26 highlighted the importance of single electron transfer (SET) reactions in the AQ catalytic cycle. According to the proposed SET mechanism, carbohydrate stabilization is explained by the formation of stable single electron transfer (SET) reactions in the AQ catalytic cycle. These AQ/carbohydrate redox reactions produce because the inherent complexity of the biopolymer. These investigations have suggested that strong interactions with sulfur species can reduce AQ to AHQ2−, which would assist lignin fragmentation.27 However, the role of sulfur compounds in the electron transfer mechanism is not evidenced yet.

We have investigated28–30 the electron transfer mechanisms involved in the AQ/lignin interactions as well as the synergistic effects with sulfide-based additives under pulping conditions. These investigations have suggested that strong interactions between AQ and the insoluble lignin and carbohydrate biopolymers is critical to the efficiency of the electron transfer reactions. Therefore, a better understanding of the distance effects in the electron transfer mechanisms is judicial to improve the delignification rate and the efficiency of the carbohydrate stabilization process promoted by the AQ/AHQ catalytic cycle.

In the present manuscript, we report a step toward this goal by describing the synthesis of a series of styrene copolymers composed of AQ and β-O-4 lignin model side chains. The copolymers were designed to have variable proportions of styrene spacer groups in the polymer backbone in order to improve the delignification rate and the efficiency of the carbohydrate stabilization process promoted by the AQ/AHQ catalytic cycle.

In the present manuscript, we report a step toward this goal by describing the synthesis of a series of styrene copolymers composed of AQ and β-O-4 lignin model side chains. The copolymers were designed to have variable proportions of styrene spacer groups in the polymer backbone in order to increase the intramolecular distance between the pendant redox active units. Accordingly, the β-O-4 model units in the copolymers containing larger amounts of styrene in their backbone would be less available for interactions with the AQ moieties, which in principle should reduce the efficiency of electron transfer reactions.31,32 Therefore, comparative analysis of the degradation products released from the β-O-4 lignin model units in the copolymers under pulping conditions might provide insight into the distance effects in the SET mechanisms and indeed in the AQ/lignin interactions. For the success of this approach, quantitative information about the composition of the copolymers as well as the statistical distribution of the AQ and β-O-4 units in the polymer structure are crucial. Therefore, UV–vis33 and 31P NMR spectroscopies34–40 were used in the present investigation to accurately determine the content of AQ and β-O-4 groups in the copolymers, while the average distribution of these groups in the polymer structures were afforded by MALDI-TOF analysis. Physical transitions and possible thermal cleavage of the different chemical bonds in the copolymers were investigated by TGA and DSC techniques in order to verify the integrity of the polymer backbone at pulping temperatures. Alkaline pulping experiments were then carried out to investigate the major degradation mechanisms of the β-O-4 lignin model units within the copolymer structures.

### MATERIALS AND METHODS

**Materials.** All chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) or ACROS (Halluin, France) and used without further purification, unless otherwise noted. Commercially available azobisisobutyronitrile (AIBN) was recrystallized from hot methanol prior to use. Tetrahydrofuran and toluene were freshly distilled from sodium/benzophenone. Chromatography was performed on Merck 60 silica gel (0.04–0.063 mm). Analytical TLC was performed using 0.2 mm Fluka silica gel plates and visualized by 254 nm UV light. Monomer 2-[4-(4-vinyl-phenyl)-but-3-enyl]-anthraquinone 4 and compound 1-(4-benzyloxy-3-methoxy-phenyl)-2-(2-methoxy-phenoxo)-3-(4-phenyl-phenyl)-1-propanol 5 were synthesized according to our previous work (Scheme 1).

**Scheme 1. Formulae of Anthraquinone Monomer 4 and Lignin Model Monomer 5 Previously Synthesized and Used in the Study**

**Methods.** 1H and 13C NMR spectra were recorded at 298 K on a Bruker Avance 300 FT NMR spectrometer. Chemical shifts were referenced to the solvent residual peak (CHCl3, at δ 7.26 ppm and CDCl3, at δ 77 ppm). Splitting patterns were designated as follows: s, singlet; br, broad; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet. For the NMR assignments, phenyl rings were abbreviated as Ar. All signals in the 1H and 13C NMR spectra were attributed by HMOC and HMBC analysis. Quantitative 31P NMR spectra were registered on a Bruker Avance DPX-200 spectrometer operating at 81 MHz. An inverse gated decoupling sequence was used with a pulse-flipping angle of 90° and 25 s of relaxation delay between pulses. To ensure a high signal/noise ratio, 256 transients were acquired. Derivatization of the copolymers with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane was carried out as follows:35,36 copolymer (30 mg) was dissolved in 0.5 mL of a solvent mixture composed of pyridine and deuterated chloroform (1.6:1 v/v ratio) in a 2 mL vial sealed with a Teflon-faced septum. 0.1 mL aliquot of a solution containing the relaxation reagent chromium(III) acetylacetonate in...
Thermogravimetric analysis experiments were carried out with a solution was deposited onto the sample plate and allowed to dry in air. Calibration software 3.07.1 from PerSeptive Biosystems. Mentioned peptides were used to calibrate the mass scale using the two-point calibration modes. The reported mass spectra represent an average of 256 consecutive laser shots (3 Hz repetition rate). Standard peptides were used to calibrate the mass scale using the two-point calibration software 3.07.1 from PerSeptive Biosystems. Mentioned m/z values correspond to monoisotopic masses. The copolymer solutions (10−3 M) were prepared in THF. The suitable matrices, 2,5-dihydroxybenzoic acid (2,5-DHB), 1,8-dihydroxy-9,10-dihydroxybenzoic acid (2,5-DHB), 1,8-dihydroxyanthraquinone, or 2-anthramine, were also dissolved in THF (10 g/L). Samples of about 7 mg at 10°C/min under a N2 atmosphere (20 mL/min). DSC measurements were carried out on a Thermo Separation Product setup equipped with a SP 1000 pump, an AS 3000 autoinjector, and an AS 2000 UV diode array detector, set at 280 nm. Separation was realized on a Hyperclone ODS (250 × 4.6 mm; 120 Å; packing size = 5 μm) column with gradient elution (flow rate 0.6 mL/min) using H2O/methanol mixtures as follows: 0–10 min, H2O/methanol (85/15 v/v) and 10–40 min, methanol (100%). GC-MS analyses of reaction products were performed on a GC Finnigan Trace Ultra Gas equipped with a PTV Injector (splitless), using a RTX-SMS column (15 m; 0.25 mm; stationary phase 5% phenyl-silicone and 0.25 mm film thickness) and an isothermal temperature profile of 40°C for the first minute, followed by a 15°C/min temperature gradient to 320°C and, finally, an isothermal period at 320°C for 15 min. The injector temperature was kept at 250°C and GC-MS grade helium was used as the carrier gas. Electron impact mass spectra were acquired at 70 eV. Positive identification of vanillin and guaiacol were afforded by comparison with authentic samples.

**Polymer Syntheses. General Procedure for Radical Polymerization.** The suitable amount of styrene, AQ, and β-O-4 monomers (4 and 5) were dissolved in freshly distilled, oxygen-free toluene and heated at 80°C. Azobisisobutyronitrile (AIBN; 8 mol % of total styrene monomers) was added under a nitrogen atmosphere and the reaction mixture was stirred at 80°C for 24 h. Toluene was removed under reduced pressure and the crude polymer was dissolved in CH2Cl2. The resulting yellow solution was added dropwise to a 40-fold excess of methanol to precipitate the copolymer. The suspension was filtered through a Celite pad and washed with cold methanol.

Another purification step was performed by dissolving the copolymer in CH2Cl2 and precipitated in n-pentane. The remaining yellow solid was washed with pentane, recovered, and dried under vacuum at room temperature for 18 h.

**Copolymer 1. Lignin model 5 (1 g, 2.02 mmol) and anthraquinone 4 (0.745 g, 2.02 mmol) monomers were copolymerized using AIBN (53 mg, 0.32 mmol) as the initiator accordingly to the conditions described in the polymerization general procedure. Copolymer 1 was isolated as a yellow solid (yield 68%). 1H NMR (CDCl3) δ ppm: 2.00–0.45 (CH2 and CH of styrene polymer chain); 3.20–2.50 (CH2, γ-position β-O-4 units); and CH2, α-oriented AQ-aliphatic chain); 3.30–4.00 (OCH2β-O-4 units); 4.30 (CH, α-position β-O-4 units); 4.80 (CH, β-position β-O-4 units); 5.15 (CH2 benzyl β-O-4 units); 5.20–5.50 (CH=CH, AQ-aliphatic side chain); 6.00–7.65 (Ar-H, styrene and benzyl and guaiacol moiety β-O-4 units), 7.70–8.50 (Ar-H, AQ units). 13C NMR (CDCl3) δ ppm: 28.9 (CH2-CH2, polymer chain); 30.0 (Ar-CH3.CH2 of AQ-aliphatic side chain); 32.4 (CH-CH2, polymer chain); 36.4 (Ar-CH2-CH2 AQ-aliphatic side chain); 40.1 (CH2 γ-position); 55.1 and 56.2 (OCH3); 70.9 (CH2 benzyl); 74.4 (CH- OH, α-position); 83.7 (CH2 β-position); 111.7–123.4 (Ar of guaiacol units); 126.2–136.5 (Ar of AQ unit); 174.7–152.8 (quaternary Ar-C of AQ and β-O-4 units); 183.1 (C of AQ unit). 31P NMR (CDCl3/pyridine) δ ppm: 148.1 and 147.7 (aliphatic OH groups, erythro form of the β-O-4 units). SEC (THF, polystyrene) Mn = 3260 g mol−1; Mw = 5220 g mol−1; PD = 1.6. TGA analysis: 350°C (95%), 390°C (85%), 430°C (70%), 580°C (50%). Tg = 84°C.

**Copolymer 2. Lignin model 5 (1 g, 2.02 mmol), anthraquinone 4 (0.745 g, 2.02 mmol), and styrene (0.420 g, 4.04 mmol) were copolymerized using AIBN (0.105 g, 0.643 mmol) as the initiator accordingly to the conditions described in the polymerization general procedure. Copolymer 2 was isolated as a yellow solid (yield 75%). 1H NMR (CDCl3) δ ppm: 2.00–0.45 (CH2 and CH of styrene polymer chain); 3.20–2.50 (CH2, γ-position β-O-4 unit and CH2, AQ-aliphatic chain); 3.35–4.00 (OCH2β-O-4 unit); 4.30 (CH α-position β-O-4 unit); 4.80 (CH β-position β-O-4 unit); 5.15 (CH2 benzyl β-O-4 unit); 5.20–5.50 (CH=CH, AQ-aliphatic chain); 6.00–7.65 (Ar-H, styrene, benzyl, and guaiacol moieties); 7.70–8.50 (Ar-H, AQ unit). 13C NMR (CDCl3) δ ppm: 28.7 (CH2-CH2, polymer chain); 30.0 (Ar-CH2-CH2 of AQ-aliphatic side chain); 32.4 (CH-CH2, polymer chain); 36.4 (Ar-CH2-CH2 AQ-aliphatic side chain); 40.1 (CH2 γ-position); 53.2 and 56.2 (OCH3); 70.6 (CH2 benzyl); 74.4 (CH-OH, α-position); 83.5 (CH, β-position); 111.7–123.4 (Ar of guaiacol units); 126.2–137.1 (Ar of AQ unit); 174.7–152.8 (quaternary Ar-C of AQ and β-O-4 units); 183.1 (C of AQ unit). 31P NMR (CDCl3/pyridine) δ ppm: 148.1 and 147.8 (aliphatic OH group, erythro form of the β-O-4 unit). SEC (THF, polystyrene) Mn = 2820 g mol−1; Mw = 5380 g mol−1; PD = 1.9. TGA analysis: 340°C (95%), 395°C (85%), 430°C (70%), 570°C (50%). Tg = 83°C.

**Copolymer 3. Lignin model 5 (1 g, 2.02 mmol), anthraquinone 4 (0.745 g, 2.02 mmol), and styrene (1 g, 16.16 mmol) were copolymerized using AIBN (0.105 g, 0.643 mmol) as the initiator accordingly to the conditions described in the polymerization general procedure. Copolymer 3 was isolated as a light yellow solid (yield 83%). The 1H, 13C, and 31P NMR spectra of copolymer 3 were very similar to those of copolymer 2. SEC (THF, polystyrene): Mn = 2740 g mol−1; Mw = 4070 g mol−1; PD = 1.5. TGA analysis: 320°C (95%), 390°C (85%), 420°C (70%), 435°C (50%). Tg = 77°C.

**General Procedure for Debenzylation of Copolymers.** In a Schlenk flask, benzylated copolymer (0.5 g) was dissolved in 50 mL of a solvent mixture composed of THF and ethanol (90:10 v/v). The resulting solution was stirred for 10 min at room temperature, followed by the addition of 0.05 g of commercial 10% palladium over carbon and a catalytic amount (5 drops) of acetic acid aqueous solution (10%, v/v). The resulting suspension was purged with H2 for 10 min and then stirred under H2 pressure (1 atm) for 6 h. The
reaction mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure. The remaining yellow solid was dissolved in CH$_2$Cl$_2$ and precipitated in methanol. After filtration and dissolution of the yellow solid in CH$_2$Cl$_2$, the debenzylated copolymer was precipitated in n-pentane. The remaining solid was recovered by filtration and dried under vacuum at room temperature for 12 h. The yield of debenzylation was obtained by quantitative $^{31}$P NMR analysis of the recovered yellow solid indicated 25% debenzylation yield. 1H NMR (CDCl$_3$) δ ppm: 2.00–0.45 (CH$_2$ and CH of styrene polymer chain); 3.20–2.50 (CH$_3$, β-position β-O-4 unit and CH$_2$, AQ-aliphatic chain); 3.30–4.00 (OCH$_3$, β-O-4 unit); 4.30 (CH, α-position β-O-4 unit); 4.80 (CH, β-position β-O-4 unit); 6.00–7.65 (Ar-H, styrene and guaiacyl moieties); 7.70–8.50 (Ar-H, AQ unit). 13C NMR (CDCl$_3$) δ ppm: 28.9 (CH$_2$); 30.0 (Ar–CH$_2$CH$_3$ of AQ-aliphatic side chain); 32.4 (CH–CH$_2$, polymer chain); 40.1 (CH$_2$, γ-position); 55.1 and 56.2 (OCH$_3$); 74.4 (CH–OH, α-position); 83.5 (CH, β-position); 111.7–123.4 (Ar-C of guaiacyl units); 126.2–137.1 (Ar-C of AQ unit, and Ar-C of styrene group); 134.9–144.4 (Ar-C=O of debenzyalted β-O-4 unit); 147.4–152.8 (quaternary Ar-C of AQ and β-O-4 units); 183.1 (C=O of AQ unit). $^{31}$P NMR (CDCl$_3$/pyridine) δ ppm: 148.1 and 147.8 (aliphatic OH groups, erythro form of the β-O-4 units); 145.8 (phenolic OH group), SEC (THF, polystyrene): $M_w = 5240$; PD = 1.8. TGA analysis: 340 °C (95%), 395 °C (85%), 420 °C (70%), 570 °C (50%), $T_g = 85 °C$.

Copolymer 2 (Yellow Solid). Quantitative $^{31}$P NMR analysis of the recovered yellow solid indicated 24% debenzylation yield. 1H NMR (CDCl$_3$) δ ppm: 2.00–0.45 (CH$_3$ and CH of polymer chain); 3.20–2.50 (CH$_3$, γ-position β-O-4 unit and CH$_2$, AQ-aliphatic chain); 3.30–4.00 (OCH$_3$, β-O-4 unit); 4.30 (CH, α-position β-O-4 unit); 4.85 (CH, β-position β-O-4 unit); 6.00–7.65 (Ar-H, styrene and guaiacyl moieties); 7.70–8.50 (Ar-H, AQ unit). 13C NMR (CDCl$_3$) δ ppm: 28.7 (CH–CH$_2$, polymer chain); 30.0 (Ar–CH$_2$CH$_3$ of AQ-aliphatic side chain); 32.4 (CH–CH$_2$, polymer chain); 36.4 (Ar–CH$_2$CH$_3$, AQ-aliphatic side chain); 40.1 (CH$_2$, γ-position); 55.2 and 56.2 (OCH$_3$); 74.4 (CH–OH, α-position); 83.5 (CH, β-position); 111.7–123.4 (Ar-C of guaiacyl units); 126.2–137.1 (Ar-C of AQ unit, and Ar-C of styrene group); 143.9–144.4 (Ar-C=O of debenzyalted β-O-4 unit); 147.4–152.8 (quaternary Ar-C of AQ and β-O-4 units); 183.1 (C=O of AQ unit). $^{31}$P NMR (CDCl$_3$/pyridine) δ ppm: 148.1 and 147.8 (aliphatic OH groups, erythro form of the β-O-4 units); 139.8 (phenolic OH group), SEC (THF, polystyrene): $M_w = 5240$; PD = 1.8. TGA analysis: 340 °C (95%), 395 °C (85%), 420 °C (70%), 570 °C (50%), $T_g = 85 °C$.

Copolymer 3 (Light Yellow Solid). Quantitative $^{31}$P NMR analysis of the recovered yellow solid indicated 25% debenzylation yield. The $^1$H, $^13$C, and $^{31}$P NMR spectra of debenzylated copolymer 3 are very similar to those afforded for debenzylated copolymer 2. SEC (THF, polystyrene): $M_w = 2750$; $M_n = 4050$; PD = 1.5. TGA analysis: 320 °C (95%), 390 °C (85%), 430 °C (70%), 435 °C (50%), $T_g = 75 °C$.

Pulping Studies. Reactions of copolymers 1–3 were carried out in stainless steel pressurized vessels (capacity: 15 mL). The reactors were...
loaded with 5.0 × 10⁻⁵ mol of β-O-4 units (1 equiv), which were calculated from the data reported in Table 1. Accordingly, 50 mg of copolymer 1, 66 mg of copolymer 2, and 82 mg of copolymer 3 were used in each trial. The copolymers were suspended in 8 mL of a 0.15 M sodium hydroxide aqueous solution (1.2 × 10⁻¹ M sodium hydroxide aqueous solution (1.2 equiv) and, for the experiments containing glucose, 2 equiv (0.018 g, 1.0 × 10⁻¹ mol) were added to the pulping liquor. The reactors were sealed, placed in a 150 °C preheated oil bath, and electrically rotated for 90 min before being quickly cooled in an ice bath. The reaction liquors were brought to pH 5 with hydrochloric acid aqueous solution (0.15 M). A fraction of the reaction liquor (0.4 mL) was collected, and a small aliquot was taken for HPLC analysis. The degradation products isolated by HPLC were then identified by GC-MS. The reported pulping data for all copolymers were averages of five cooking experiments.

Results and Discussion

Copolymer Synthesis and Characterization. To study spacer effects in the electron transfer mechanisms between AQ and β-O-4 lignin model, three different random copolymers were synthesized using different amounts of styrene (spacer) in their polymerization reactions (Scheme 2). Copolymer 1 was prepared using an equimolar ratio of monomers 4 and 5 (no styrene added), while copolymers 2 and 3 were prepared using AQ/styrene/β-O-4 monomer ratios equal to 1:2:1 and 1:8:1, respectively. Copolymerization reaction was achieved by classic radical polymerization in anhydrous oxygen-free toluene using azobisisobutyronitrile (AIBN) as the initiator. Copolymers 1, 2, and 3 were obtained as yellow solids in 68, 75, and 83% yield, respectively. They were readily soluble in common organic solvents at room temperature, which allowed their characterization by SEC chromatography and 1H and 13C NMR spectroscopies.

Similar 1H NMR spectra were obtained for all copolymers. A complete assignment of the typical broad resonances of polymers, afforded from HMBC and HMQC 2D NMR experiments, confirmed the expected copolymer structures (see Figure 1 for 1H NMR spectrum of copolymer 2 as an example). Signals centered at 8.20 and 7.70 ppm corresponded to the aromatic protons of the AQ groups, while the large signal at the region between 7.65 and 6.00 ppm was due to overlapping resonances of the aromatic protons on the styrene backbone as well as the two guaiacyl moieties (assigned as A and B in Scheme 2) and the protecting benzyl groups (Bz rings in Scheme 2) of the β-O-4 units. The peak at 5.65 ppm was assigned to the olefin protons of the AQ aliphatic chain, while the resonance at 5.17 ppm was attributed to the methylene protons of the benzyl protecting groups. The propanoid moiety of the lignin model units could be unambiguously identified by the presence of the signals centered at 4.85, 4.32, and 2.80 ppm, corresponding to the protons attached to the carbons at α, β, and γ positions, respectively. The styrene polymer backbone appeared in the usual region between 3.20 and 2.48 ppm and 2.30–0.60 ppm, while the sharp signal at 1.46 ppm was due to CH₃ groups of AIBN initiator fragments.

Confirmation of the copolymer structures came from 13C NMR analysis (see SI). Presence of AQ groups was confirmed by signals of carbonyl functionalities and aliphatic side chains (183.1, 36.4, and 30.0 ppm, respectively), while signals due to Cβ, Cα, and Cγ carbons (83.7, 74.4, and 40.1 ppm, respectively) attested for the presence of β-O-4 units. SEC analysis of copolymers revealed a monomodal molecular weight distribution with values (Table 1) analogous to other styrene polymers having large size groups.

Quantitative information about the composition of the copolymers was obtained using UV–vis spectroscopy (for AQ units) and 31P NMR (for β-O-4 units). UV–vis absorption spectra of all copolymers were similar, displaying the characteristic absorption band of anthraquinone derivatives at 328 nm (Figure 2), which allowed the determination of the AQ content in each copolymer (Table 1). 31P NMR spectroscopy has proved to be a powerful technique to quantify hydroxyl groups in arylglycerol β-O-4 ether units in lignins and model compounds. The method is based on a selective and quantitative phosphitylation reaction of the hydroxyl groups of the substrate and differentiation of the chemical environments of the phosphorus atoms by NMR spectroscopy. In the studied copolymer structures, hydroxyl groups are present only in the β-O-4 subunits (Scheme 2), therefore, quantitative 31P NMR spectroscopy could be used to selectively determine the content of these subunits in the macromolecules. Figure 3 shows the 31P NMR spectrum afforded from phosphorylated copolymer 2. Similar completely resolved 31P NMR spectra were also obtained for copolymers 1 and 3 (for details, see SI).

The 31P NMR spectrum of phosphorylated copolymer 2 (Figure 3) exhibited signals within the region of aliphatic alcohols (148.8–145.1 ppm). Previous 31P NMR studies revealed that the erythro stereoisomer in phosphory derivative...
alpha hydroxyl groups present in β-O-4 lignin model compounds gave signals centered at 148 ppm, while the threo stereoisomer was centered at higher field at 147 ppm. Congruently, the 31P NMR data of the present copolymers revealed that only the erythro form was present; it is likely that the threo form is less reactive. The total content of β-O-4 subunits (Table 1) could be easily determined by integrating the region between 148.7 and 147.5 ppm and comparing the resulting number with the internal standard (IS) integration. The data showed that the three copolymers have similar AQ/β-O-4 ratio (about 1.5), indicating a slightly preferred radical reaction between AQ monomers. The reduced reactivity of 5 might be explained by steric reasons. The relatively larger size of the guaiacyl rings in the β-O-4 monomer structure can act as a steric shield to propagating vinyl radical, rendering the addition of β-O-4 monomers to the growing polymer chains difficult, thereby reducing the formation of copolymers relatively richer in β-O-4 units.

MALDI-TOF mass spectrometry was used to determine the distribution of AQ, styrene, and β-O-4 subunits in the copolymer structures (for details, see SI). For all copolymers, the observed m/z values corresponded to the sum of AQ unit + β-O-4 unit + styrene unit + two AIBN initiator fragments + one sodium ion, suggesting that the dominant termination mode of the radical polymerization reactions in the present case was a recombination of two polymer chains, as it is already known for pristine polystyrene. The detected ion peaks were in excellent agreement with the theoretical m/z values calculated for the main isotope of each species.

The random copolymer structure proposed for 1 (Figure 4) could be unambiguously verified by the detection of species containing a statistical distribution of both AQ and β-O-4 subunits. For example, the species marked with X were mostly composed of AQ units, while those marked with ● have a larger number of β-O-4 than AQ moieties. The species assigned with ● have an equal number of these units in their structure. These findings suggested that at least in some species one β-O-4 subunit is next to an AQ group in the random structure of copolymer 1. However, the species marked with an upside down black spade symbol in Figure 4 indicated the presence of some AQ homo-oligomer species in the composition of 1, which was expected due to the higher reactivity of the AQ monomer 4 toward radical polymerization.
MALDI-TOF analysis of copolymer 2 showed the presence of species containing both AQ and β-O-4 units with at least one unit of styrene in their backbone, as revealed by the series marked with Ø in Figure 5. Copolymer 3 gave similar results with species composed of AQ/(stylene)_n/β-O-4 units with n > 1. Nonetheless, some styrene homo-oligomer was also observed in the MALDI-TOF spectrum of 3 (SI). Despite of the random character of the synthesis of the copolymers, MALDI-TOF data indicated that the AQ and β-O-4 units were next to each other in copolymer 1, while they were separated by styrene units in 2 and 3. Thus, interactions between the AQ and the lignin model components might readily take place in 1, resulting in more efficient degradation of the β-O-4 groups through electron transfer reactions when compared to 2 and 3.

**Debenzylation Reaction of Copolymers.** To prepare β-O-4 lignin model monomer 5 (Scheme 2), the phenolic function should be protected using a benzyl group. However, the proposed electron transfer mechanisms in pulping system involves transferring of electrons from reduced AQ species to an intermediate quinone methide (QM), which is generated from free phenolic hydroxyl groups in the lignin structure. Consequently, the benzyl protecting group of the β-O-4 subunits should be partially removed in the present copolymers (Scheme 3).

In softwood lignins, which are mostly composed of similar guaiacyl-propane units of the present copolymers, about 30% of the guaiacyl units are involved in terminal β-O-4 linkages, that is, the guaiacyl unit is at its free phenolic form, while the rest are engaged in ether bonds with other units in the lignin macromolecular structure. From a modeling point of view, the guaiacyl β-O-4 lignin model units in the present copolymers should mimic the basic chemical reactions of softwood lignins. Accordingly, the protecting benzyl groups should be partially removed from the copolymers in order to better reproduce the reactivity of natural softwood lignins. Therefore, the debenzylation reaction of the copolymers was carefully conducted, aiming yields of about 30%.

Debenzylation yield of β-O-4 units could be easily determined by 31P NMR spectroscopy. The 31P NMR spectra of the debenzylated copolymers (see Figure 6 for 2 as an example) exhibited a typical signal at 139.6 ppm attributed to hydroxy phenol group in the β-O-4 lignin model compounds. After several laboratorial tests, where many parameters involved in the debenzylation reaction were scrutinized, quantitative analysis of the 31P NMR spectra of the three copolymers indicated a close content of free-phenolic units (about 25%, Table 1) to that of a typical softwood lignin, confirming that the debenzylation conditions reported in the experimental section were appropriate. H NMR analysis of the debenzylated copolymers showed that the olefin group in the alkyl side chain of the AQ units was fully saturated under the conditions used for debenzylation of the copolymers.

**Thermal Analysis of Debenzylated Copolymers.** Due to the high temperature involved in the pulping process (150–170 °C), thermal cleavage of the different bonds in the copolymers should be taken into account when analyzing the chemical pulping data. Thus, the thermal stability of the debenzylated copolymers was examined by TGA and DSC analysis.

TGA curve of the debenzylated copolymer 2 (Figure 7) showed that the styrene backbone was thermally stable, presenting no weight loss at the typical temperature range used in pulping processes (150–170 °C). The first derivative curve revealed that the thermal degradation of the debenzylated copolymer 2 occurred in two stages. The first one took place at about 300 °C, with weight loss of 30%; the second stage, with higher weight loss rate, initiated at 380 °C and extended to 500 °C. At this temperature, the weight loss corresponded to

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**Figure 6.** 31P NMR spectrum of debenzylated copolymer 2. IS = internal standard (benzoic acid).

**Figure 7.** TGA (---) curve and first derivative TGA curve (-----) of debenzylated copolymer 2 (N₂ atmosphere, 20 mL/min, and 10 °C/min).

**Figure 8.** HPLC chromatogram of reaction liquor after alkaline pulping of copolymer 3 (90 min, 150 °C).
around 55%, remaining fairly constant until 800 °C. The DSC data of debenzylated 2 (see Figure S6 in SI) supported the TGA analysis. The first degradation exothermic peak was only detected at about 200 °C, confirming the thermal stability of the copolymers. Similar thermal properties were observed for the analogous debenzylated copolymers 1 and 3.

Studies of Copolymer Degradation Mechanisms under Pulping Conditions. AQ catalytic effects on the β-O-4 subunits were investigated by heating the copolymers at 150 °C for 90 min followed by analysis of the degradation products by HPLC and GC-MS techniques. Three pulping experiments were performed in order to highlight the effects of electron transfer reactions in the degradation mechanisms of the copolymers. In the first control experiment, the copolymers were just heated in pure water to evaluate any degradation by thermal hydrolysis. In the second experiment, the copolymers were reacted under usual alkaline pulping conditions, while in the last trial, glucose was added to the alkaline pulping liquor in order to mimic the reducing end groups on the carbohydrate fibers.

In the control experiments using water, no products were detected by HPLC in the reaction mixtures of the three copolymers. 31P NMR and UV–vis analyses of the recovered copolymers revealed less than 1% loss of hydroxyl groups from the β-O-4 component and no loss of AQ units. 1H NMR spectrum and SEC analysis of the recovered materials also indicated no damage to the copolymer structures. These findings clearly demonstrated that the copolymers were thermally stable in water at 150 °C, in complete agreement with TGA and DSC data.

On the other hand, HPLC chromatograms of the copolymers after alkaline pulping process (see Figure 8 for copolymer 3 as an example) exhibited two major peaks with retention times at 5.30 and 6.10 min, which were unambiguously identified by GC-MS as vanillin and guaiacol, respectively. Other two minor peaks at longer retention times were also observed but not identified. HPLC quantitative analysis showed that under alkaline pulping conditions, degradation of the β-O-4 in the copolymers yielded mostly vanillin (Figure 9), indicating that the major operating mechanism was alkyl side-chain oxidation.
Vanillin yields were dependent on the distance between the AQ and β-O-4 side-chains, decreasing in the order copolymer 1 > copolymer 2 > copolymer 3, which suggested that the oxidation mechanism involved electron transfer reactions between AQ and β-O-4 units. This oxidation mechanism (Figure 10) is believed to occur through a single-electron transfer from the phenolic β-O-4 lignin model units, as electron donor, to AQ, as electron acceptor, resulting in AHQ radical anion and aromatic radical cation. The latter decomposes via Cα–Cβ bond cleavage, yielding vanillin as final product.

Reasonable amounts of guaiacol were also detected after alkaline pulping of copolymers (Figure 9), suggesting non-negligible cleavage of β-O-4 bonds. Guaiacol yields were not dependent on the statistical distance between the active subunits, as virtually the same amount of guaiacol was detected for the three copolymers. Therefore, no electron transfer reactions between AQ and lignin model units seemed to be involved in the cleavage of the β-O-4 bonds under alkaline conditions. Instead, we propose that formation of guaiacol occurred through oxidation of the benzylic hydroxyl group of the lignin model to α-carbonyl units, which is a process known to weaken the β-O-4 bonds, thereby leading to their cleavage under alkaline conditions (Figure 11). The side-chain oxidation mechanism likely involved single electron transfer from the phenolic β-O-4 units to residual mobile oxygen species. Congruently, this process was independent of the AQ/β-O-4 distance because oxygen species were free to interact with the copolymer active groups during the pulping reactions.

In the alkaline pulping experiments containing glucose as carbohydrate-mimicking group, the degradation of the β-O-4 units of the copolymers occurred with opposite quantitative composition of the reaction products (Figure 9). Addition of glucose led to higher guaiacol yields and substantially reduced formation of vanillin. Therefore, presence of glucose in the alkaline pulping liquor dramatically inhibited the side-chain oxidation processes and improved the cleavage of the β-O-4 bonds. More interesting, the yield of guaiacol increased in the order copolymer 1 > copolymer 2 ∼ copolymer 3, while vanillin formation was not dependent on the statistical distance between the AQ and β-O-4 side chains.

These findings strongly suggested that the classical SET mechanism (Figure 12) proposed by Dimmel was operative during the alkaline pulping of the copolymers in presence of glucose. Interactions between the AQ subunits and glucose resulted in AHQ^− and AHQ2^− anions, which reacted through electron transfer reactions with the QM intermediates formed in situ from the phenolic β-O-4 units under alkaline conditions. The unstable QM^− radical anions decayed through homolytic cleavage of the β-O-4 bonds to yield guaiacol. As the AQ units were much more available for electronic interactions with the β-O-4 units in copolymer 1 than in copolymers 2 and 3, the electron transfer reactions that ultimately led to cleavage of the β-O-4 bonds occurred with higher efficiency in 1 than in 2 and 3. The distance independent amount of vanillin detected in the pulping liquor containing glucose suggested oxidation of the β-O-4 side chain, which most likely involved residual mobile oxygen species as the oxidant agent (Figure 13).

**CONCLUSIONS**

Three styrene copolymers composed of AQ and β-O-4 units, containing different amounts of styrene as spacer in their backbone, were synthesized and fully characterized. Quantitative information and statistical distribution of the active side chains were afforded through spectroscopic techniques, which unambiguously revealed that the AQ and β-O-4 side chains were at distinct distances from each other in the three copolymers studied. The choice for a styrene type polymer was fundamental because the main chain proved to be thermally stable under pulping temperature. Investigation of the pulping...
products released from β-O-4 units in the copolymers allowed the diagnostic of the major operating mechanisms of degradation, which clearly involved electron transfer reactions. Comparative analysis of the pulping data provided pristine evidence for distance effects in the efficiency of the electron transfer reactions, despite the randomness of the copolymer structures. These findings supported the hypothesis that close contact between AQ and β-O-4 moieties in the copolymer structures was crucial for efficient degradation of the lignin model through electron transfer processes. The mechanisms involved in the degradation pathway of the copolymers might allow a better understanding of the mechanism of action when anthraquinone derivatives are used as catalyst in alkaline pulping processes despite other reactions may be observed in native lignin polymer of lignocellulosic biomass.

■ ASSOCIATED CONTENT

1 Supporting Information

The 1H NMR spectra of copolymers 1 and 3; 13C NMR spectrum and assignments of copolymer 1; MALDI-TOF spectrum of copolymer 3 and assignments of MALDI-TOF spectra of copolymers 1–3; 31P NMR spectra of debenzylated copolymers 1 and 3; DSC curves of debenzylated copolymers 1–3; TGA curves of debenzylated copolymers and 1; 31P NMR spectra of debenzylated copolymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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(49) Gierer, J. Svensk Papperstid. 1980, 73, 75–82.