SOLUBILITY AND DYE-BINDING PROPERTIES OF QUATERNIZED AND PEROXIDASE-POLYMERIZED KRAFT LIGNIN

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ABSTRACT

The ability of cationic Kraft lignin to function as a flocculant for decolorization of textile wastewaters was examined. Kraft lignin was quaternized with high efficiency in alkaline solution with 3-chloro-2-hydroxypropyl-trimethylammonium chloride. The product formed was separated into two fractions arbitrarily based on their aqueous solubility at pH 7. The pH 7-soluble fraction was soluble over the pH 2 to 13 range. The pH 7-insoluble fraction was soluble at low (< pH 4) and high (pH > pH 12) pH values. Treatment of the pH 7-soluble quaternized lignin fraction with soybean peroxidase and hydrogen peroxide under nitrogen produced a soluble, high molecular weight polymer, as determined by ultrafiltration analysis. Peroxidase treatment of the pH 7-insoluble fraction at pH 3 produced a material not completely soluble at any pH, probably by modifying the lignin structure but not necessarily by increasing its molecular weight. All quaternized lignin fractions were demonstrated to bind Orange II and hydrolyzed Reactive Red 180, but only peroxidase-polymerized lignin produced rapidly settling flocs. Peroxidase-treated, quaternized Kraft lignin can be used in place of synthetic cationic polymers for decolorization of textile wastewaters.

Keywords: Cationic lignin, peroxidase, wastewater treatment.

INTRODUCTION

Lignin, a highly abundant biopolymer, is generated as a by-product by the pulp and paper industry. The properties and industrial applications of lignin have been recently reviewed [1]. Lignosulfonates, derived from the sulfite pulping process, are strongly anionic, high molecular weight polymers commonly used as dispersants and complexing agents. Kraft lignins, generated by sodium hydroxide and sulfide pulping, are weakly anionic, low molecular weight polymers that have limited utility without further modification (e.g., hydroxymethylation, epoxidation, and isocyanatation). So-functionalized Kraft lignins can be used in adhesive formulations [2]. Sulfonation allows Kraft lignin to be used in many of the same applications where lignosulfonates are employed [1]. The superior utility of lignosulfonates helps explain why the vast majority of the Kraft lignin generated is used simply as fuel for steam generation in the mill rather than in value-added products.

Cationic Kraft lignins show some promise for being useful as flocculating agents in applications that currently employ synthetic cationic polymers, such as in wastewater treatment and sludge dewatering [3, 4]. Cationic lignins can be prepared by a variety of methods, including: Mannich reaction with amine monomers [5], graft copolymerization polymer in an effort to remove residual color, which, instead, destabilizes the floc and increases soluble dye levels. An ideal with acrylamide and cationic monomers [6], and etherification with reactive amines [3, 7]. Graft copolymerization and aminomethylation can lead to an increase in molecular weight, which can improve the performance of the product. An alternative approach to functionalizing and increasing the molecular weight of Kraft lignin is through enzymatic copolymerization of lignin with low molecular weight monomers [8-11]. However, no cationic lignins have been reported to be produced by this method. Because of the low solubility of Kraft lignin in aqueous solution at pH values at which the enzymes (peroxidases and laccases) can function, polymerizations have been conducted in aqueous/organic solvent (dimethylsulfoxide or dioxane) systems. The cost associated with removing all traces of the organic solvent from the product may limit the commercial viability of enzymatic lignin polymerization in non-aqueous media. The present work demonstrates that peroxidase-catalyzed polymerization in aqueous solution proceeds readily with cationic Kraft lignin.

Synthetic cationic polymers have been suggested as effective flocculants for textile wastewater decolorization [12-15]. This approach is attractive because it is relatively simple to implement; no special equipment nor specialized knowledge is required. A problem particular to the use of cationic polymers results from the tendency to overdose polymer for effluent treatment would be highly water soluble for easy dosing, would form rapid settling precipitates, upon
mixing with dye, and would not be susceptible to overdosing problems. The present work examines the ability of cationic (quaternized) Kraft lignin to meet these performance criteria.

MATERIALS AND METHODS

Kraft lignin (Indulin AT) was obtained from Westvaco Corp. and used as received\(^{10}\). The quaternizing reagent 3-chloro-2-hydroxypropyl-trimethylammonium chloride (CHMAC) was a gift from Dow Chemical Co. (tradename: Quat-188). Soybean peroxidase was purchased from Sigma. The enzyme had a specific activity of 240 purpurogallin units mg\(^{-1}\) (one unit of activity equals 1.0 mg of purpurogallin produced in 20 s at pH 6.0 and 20 °C). Orange II (Acid Orange 7), a monovalent anion, was purchased from Aldrich. The trivalent anionic dye F3B (hydrolyzed Reactive Red 180) was prepared as described previously\(^{16}\).

To prepare quaternized lignin, Kraft lignin (10 g) was dissolved in 50 ml of 1 mol l\(^{-1}\) NaOH and heated to 60 °C in a water bath. CHMAC (2.0 mmol g\(^{-1}\) of lignin) was added to the solution, which was then stirred for 2 h at 60 °C. The solution was cooled, brought to pH 7 with 2 mol l\(^{-1}\) HCl, and diluted with water to bring the lignin concentration to approximately 40 g l\(^{-1}\). The pH 7-soluble and insoluble fractions were isolated from the quaternized lignin by centrifugation for 30 min at 24000 g. The pellet (i.e., the pH 7-insoluble fraction) was dried under vacuum at room temperature. The supernatant (pH 7-soluble fraction) was refrigerated until used.

The concentration of 2,3-dihydroxypropyltrimethylammonium chloride (Diol) present in the neutralized, quaternized lignin reaction solution was quantitated by high-performance liquid chromatography (HPLC) using a Spectra-Physics SP8800 liquid chromatograph system interfaced to a Waters 410 differential refractometer. The Microsorb MV (Rainin) column (4.6 mm i.d. x 15 cm) contained a C-18 reverse phase packing with a 5-μm particle size. The sample injection volume was 25 μl. The column was developed at a flow rate of 0.7 ml min\(^{-1}\) with an eluent consisting of 950 g of water, 50 g of methanol, 20 g of NaClO\(_4\), and 1 g of 1-octanesulfonic acid monohydrate (Sigma-Aldrich). Standardizing concentrations of Diol (1.0 to 10 mmol l\(^{-1}\)) were prepared from CHMAC hydrolyzed with 5.0 mol l\(^{-1}\) NaOH.

Peroxidase polymerization of lignin was performed with samples dissolved or dispersed at a concentration of 10 g l\(^{-1}\) in 10 mmol l\(^{-1}\) CaCl\(_2\), pH 6.0, containing 5 mg of peroxidase per g of lignin. The loosely sealed container was stirred continuously under a bed of flowing, water-saturated N\(_2\), for 2 h, which reduced the dissolved oxygen concentration of the solution to less than 1.0 mg l\(^{-1}\) (measured polarographically) before the addition of H\(_2\)O\(_2\). Portions of 1.0 mol l\(^{-1}\) H\(_2\)O\(_2\), were added at 1 h intervals to the sample; each addition brought the peroxide concentration to 2.5 mmol l\(^{-1}\) (assuming there was no remaining peroxide from previous additions). Following the final treatment period, catalase (5 mg g\(^{-1}\) of lignin) was stirred into the sample to remove residual H\(_2\)O\(_2\).

Ultrafiltration analysis of lignin molecular weight distributions was performed following the procedure of Li et al.\(^{17}\). A 50 ml Amicon stirred cell was used with a series of regenerated cellulose membranes having molecular weight cutoff values determined by Li et al. of 660 (YM1), 2,200 (YM10), 4500 (YM30) and 34000 (YM100) Da evaluated using a series of linear sulfonated polystyrenes and several globular proteins. The cell was pressurized with N\(_2\) at 140 and 100 kPa for the YM1 and YM10 membranes, respectively, and 60 kPa was applied for the YM30 and YM100 membranes. Stirring was maintained at 460 revolutions per min. Lignin was dissolved in 0.1 mol l\(^{-1}\) NaOH at a concentration of 1.0 g l\(^{-1}\). Lignin concentration in permeates and retentates was determined from the absorbance at 280 nm in 0.1 mol l\(^{-1}\) NaOH assuming an absorption coefficient of 24 1 (cm\(^{-1}\) g\(^{-1}\)) for all fractions\(^{1}\). The ultrafiltration cell was filled with 50 ml of solution and pressurized. The first 2.5 ml of permeate were discarded; the subsequent 2.5 ml of permeate were collected and analyzed for lignin concentration using duplicate dilutions into 0.1 mol l\(^{-1}\) NaOH. The cell was depressurized and the retentate lignin concentration was determined. This procedure was repeated with each membrane, using fresh lignin solution each time. The permeate lignin concentration was expressed as a fraction of the retentate concentration. The amount of lignin in each molecular weight range was calculated by subtracting the measured permeate fraction of one membrane from the permeate fraction of the next higher membrane (e.g., the 660 to 2200 Da range equals the YM10 permeate minus the YM1 permeate).

The solubility of various lignins was assessed by dissolving or diluting the sample to a concentration of 2 g l\(^{-1}\) in dilute NaOH or HCl at room temperature and adjusting pH with 2.0 mol l\(^{-1}\) NaOH or HCl. The sample was centrifuged for 30 min at 20,000 g and the supernatant was assayed for lignin following dilution into 1.0 mol l\(^{-1}\) NaOH.

The amount of Orange II or F3B bound to lignin derivatives was determined following procedures described previously\(^{16, 18}\). Dye and lignin were combined in 2.0 mmol l\(^{-1}\) MOPS (3-N-morpholinopropanesulfonic acid)/NaOH, pH 7.0. Free dye was separated from bound dye complexes by filtration through an Anotop 25 (Whatman International) 0.2-μm pore, inorganic membrane. Filtrates were diluted with 2.0 mmol l\(^{-1}\) MOPS/NaOH, pH 7.0, and dye concentrations were then determined spectrophotometrically (484 nm for Orange II, 540 nm for F3B).

Alternatively, dye binding to soluble, peroxidase-polymerized quaternized lignin was determined by equilibrium dialysis. Following polymerization as described above, the lignin sample (50 ml) was dialyzed in Spectra/Per 7 tubing (nominal 2,000 MWCO; Spectrum Medical
Industries) for two weeks against a stirred solution of 21 of 2.0 mmol l⁻¹ MOPS/NaOH, pH 7.0, at room temperature, with replacement of the buffer solution after one week. The dialyzed sample was equilibrated for two weeks with 1 l of 2.0 mmol l⁻¹ MOPS/NaOH, pH 7.0, containing 0.2 mmol l⁻¹ Orange II. The Orange II concentration in the solution outside the dialysis tubing was determined spectrophotometrically. The difference between the initial and final dye concentrations was used to calculate the amount of Orange II bound to lignin, after correcting for the dilution of dye into the tubing compartment. The tubing bound a negligible amount of dye. F3B did not equilibrate across the membrane under these conditions.

RESULTS

Reaction efficiency

The quatemizing agent CHMAC is converted to its reactive epoxide form under alkaline conditions (i), which can then react with lignin hydroxyl groups to form cationic lignin (ii), or with water to form inactive Diol (iii).

\[
\begin{align*}
\text{(i)} & \quad \text{CICH}_2\text{CH(OH)CH}_2\text{N(CH}_3)_3\text{Cl} + \text{NaOH} \rightarrow \text{CICH}_2\text{CHCH}_2\text{N(CH}_3)_3\text{Cl} + \text{NaCl} \\
\text{(ii)} & \quad \text{CH}_2\text{CHCH}_2\text{N(CH}_3)_3\text{Cl} + \text{Lignin-OH} \rightarrow \text{Lignin-OCH}_2\text{CH(OH)CH}_2\text{N(CH}_3)_3\text{Cl} \\
\text{(iii)} & \quad \text{CH}_2\text{CHCH}_2\text{N(CH}_3)_3\text{Cl} + \text{H}_2\text{O} \rightarrow \text{HOCH}_2\text{CH(OH)CH}_2\text{N(CH}_3)_3\text{Cl}
\end{align*}
\]

Incorporation of quaternary ammonium groups into Kraft lignin under the conditions detailed in the experimental section proceeds with an apparent high efficiency. Residual Diol in the reaction solution was found to be 10% of the added CHMAC. When CHMAC was hydrolyzed with base prior to being combined with lignin, complete recovery of Diol was achieved. This indicates that treatment of Kraft lignin with CHMAC resulted in a 90% incorporation of quaternary ammonium groups into the lignin substrate, which is substantially greater than the 50% reaction efficiency of CHMAC with Kraft lignin (Indulin AT) reported by Pulkkinen et al. [3].

However, it was not possible to verify that 90% of the quaternizing agent was incorporated into lignin polymer (as opposed to low molecular weight molecules). With 2 mmol of CHMAC applied per g of lignin, the product's theoretical maximum quaternary ammonium content is 1.53 mmol g⁻¹ (2 mmol incorporated into 1.305 g of HCl-neutralized product). The neutral-pH precipitable portion of the reaction product, amounting to approximately half of the total sample lignin (see below), had a nitrogen content of 1.66 mmol N g⁻¹ (2.33 %N). Unmodified lignin contained 0.83 mmol N g⁻¹ (0.16 %N). Assuming that none of the initially present nitrogen was lost during quaternization, then the quaternized, neutral-pH insoluble lignin contained 0.83 mmol of quaternary ammonium groups per g. This suggests that, at least for this fraction, the quaternization efficiency was well below 90%. Efforts to separate all of the quaternized lignin (not just the neutral-pH insoluble fraction) from Diol, quantitatively by either precipitation from ethanol or diafiltration as recommended by Pulkkinen et al. [3], were not successful. Thus, there is inconclusive evidence that the quaternization efficiency was lower than the 90% determined from HPLC analysis of the CHMAC hydrolysis product present in the reaction mixture.

Molecular weight distribution and solubility

Quaternization did not alter the molecular weight distribution of Kraft lignin as determined by ultrafiltration (Table 1). Only the lowest molecular weight fraction (< 660 Da) increased slightly (p < 0.05) upon quaternization. This indicates that introduction of cationic charges into the polymer did not alter its membrane rejection characteristics. Therefore, the membrane molecular weight cutoff values determined by Li et al. [17] should be valid for determining the molecular weight distribution of the modified lignin. Quaternization dramatically altered the aqueous solubility of lignin (Figure 1). Unmodified Kraft lignin solubility diminished greatly as the solution was lowered below pH 12, reflecting the progressive protonation of weakly anionic groups and loss of charge on the polymer.

Table 1. Molecular weight distributions of Kraft lignin and quaternized Kraft lignin.

<table>
<thead>
<tr>
<th>Weight range (Da)</th>
<th>% of total lignin (mean ± rel std dev, n=3)</th>
<th>Quaternized Kraft lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 660</td>
<td>3.3 ± 0.1</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>660-2200</td>
<td>10.6 ± 0.6</td>
<td>11.9 ± 0.7</td>
</tr>
<tr>
<td>2200-4500</td>
<td>19.1 ± 0.3</td>
<td>18.6 ± 2.4</td>
</tr>
<tr>
<td>4500-34000</td>
<td>64.5 ± 2.1</td>
<td>61.8 ± 0.6</td>
</tr>
<tr>
<td>&gt; 34000</td>
<td>2.5 ± 1.3</td>
<td>2.7 ± 1.2</td>
</tr>
</tbody>
</table>
Quaternized lignin was minimally soluble at pH 9, and completely soluble at high pH (> 12) and low pH (< 4). The complete solubility of the quaternized lignin at low pH indicates that all of the lignin is quaternized to some extent. Using much higher quaternizing agent to lignin ratios than employed here, Pulkkinen et al. [31] produced quaternized lignins that were completely soluble at all pH values. The use of less quaternizing agent thus permits the production of two modified lignin fractions, one soluble and the other insoluble at neutral pH.

The soluble and insoluble quaternized lignin fractions differed significantly in their molecular weight distributions (Figure 2). The soluble fraction was comprised of more low molecular weight (i.e. < 4500 Da) material compared to the insoluble fraction. This indicates that solubility of the derivatized lignin is influenced by polymer size, or that lower molecular weight fractions were more extensively quaternized than higher molecular weight fractions.

Peroxidase polymerization

The ability of soybean peroxidase to increase the molecular size of the pH 7-soluble quaternized lignin fraction via free radical polymerization was examined.
Unbuffered lignin solution was combined with peroxidase and adjusted to pH 6, the pH optimum of the enzyme [19]. After stirring for 2 h, H₂O₂ was added incrementally to the reaction solution. The first addition of H₂O₂ caused a darkening of the lignin solution that was complete after a few minutes, suggesting that there was a rapid condensation or oxidation of chromophoric molecules. In the absence of peroxidase, H₂O₂ did not darken the lignin solution. A slight rise of the solution pH (to approximately pH 6.5) was noted at the end of the reaction period, which also indicates that the structure of the lignin was being modified by peroxidase/H₂O₂ treatment. The product remained soluble over the pH range 2 to 13.

Peroxidase treatment, with incremental addition of H₂O₂, of pH 7-soluble quaternized lignin progressively increased the molecular weight of the lignin (Figure 3). The three low molecular weight fractions (< 660, 660-2200, and 2200-4500 Da) decreased in amount with each subsequent H₂O₂ addition. The lowest molecular weight fraction (< 660 Da) was the least reactive of all the fractions, decreasing only from 9% to 6% of the total lignin. Peroxide additions up to 0.5 mmol g⁻¹ of lignin increased the amount of middle molecular weight lignin (4500-34000 Da), after which additional H₂O₂ additions decreased the amount of this fraction. The decrease in the amount of middle molecular weight fraction with H₂O₂ additions greater than 0.5 mmol g⁻¹ of lignin coincided with a tremendous increase in the high molecular weight fraction (> 34000 Da), which reached a maximum of 70% of the total lignin at 1.25 to 1.5 mmol of peroxide per g of lignin. This pattern of progressive change in the molecular weight distribution of the quaternized lignin with peroxidase/H₂O₂ treatment indicates that low molecular weight lignin, except for the very smallest monomeric or oligomeric components, is preferentially polymerized. That the produced high-molecular weight polymer remains soluble indicates that the observed solubility difference of the pH 7-soluble and insoluble fractions is not due to differences in molecular weight.

Soybean peroxidase retains some catalytic activity even at pH values far below optimum [20]. Therefore, it was feasible to test whether the neutral-pH insoluble quaternized lignin fraction could be polymerized by peroxidase at a pH low enough to solubilize entirely this fraction. Treatment was performed at pH 3.0 (in 10 mmol l⁻¹ CaCl₂ under N₂) for 20 h with 20 mg of peroxidase and 4.0 mmol H₂O₂ per g of lignin. A large portion (40 to 50%) of the product was insoluble at any pH, including the pH 13 conditions used for determination of molecular weight distributions. Thus it is unknown whether the peroxidase treatment increased the molecular weight of this fraction. The portion that remained soluble in 0.1 mol l⁻¹ NaOH did not differ from the starting material in its molecular weight distribution (data not shown). This indicates that soybean peroxidase treatment of quaternized lignin at low pH alters the lignin structure, but does not necessarily lead to polymerization.

Treatment of quaternized lignin (entire preparation) with peroxidase/H₂O₂ at pH 6.0 produced a material with a pH solubility profile (Figure 4) similar to that of the peroxidase-treated, quaternized lignin pH 7-insoluble fraction. This product had a solubility minimum at pH 11 and a higher solubility than untreated quaternized lignin at pH 7, but was not completely soluble at either low or very high pH.

![Figure 3](image-url) Figure 3. Peroxidase catalyzed change in the molecular weight distribution of pH 7-soluble quaternized lignin with incremental additions of H₂O₂.
Dye binding

The pH 7-insoluble quaternized lignin was examined for its ability to bind dye and precipitate. The lignin was solubilized at pH 3, combined with dye (either Orange II or F3B) and then adjusted to pH 7 with a small amount of NaOH. The amount of dye remaining in the filtered solution was used to estimate the extent of dye binding. For six different preparations of the pH 7-insoluble quaternized lignin, the binding capacity for either dye was 0.69 ± 0.06 meq g⁻¹ (mean ± one standard deviation). The dye binding capacity of this fraction was lower than its estimated 0.83 meq g⁻¹ quaternary amine content, which indicates that a portion of the quaternized lignin does not bind dye or forms a soluble dye-lignin complex. At pH 3, in the presence of excess dye, the lignin formed a slowly precipitating floc, but at pH 7 a non-settling suspension was formed. This observation may be explained by changes in the lignin net charge (zeta potential) as a function of pH and bound dye. The pH 7-insoluble quaternized lignin contains positively charged amine groups and negatively charged carboxyl groups. At neutral pH the number of dissociated carboxyl groups approximately equals the number of amine groups, so the net charge on the polymer is low and a colloidal suspension is formed. At pH 3 the carboxyl groups are protonated and the polymer takes on a net positive charge and becomes soluble. Dye added to the lignin solution at pH 3 neutralizes all the positive amine groups present on the polymer, which lets the polymer slowly precipitate out of solution. As the solution is raised to pH 7, the carboxyl groups dissociate and charge repulsion forces the polymer to remain in suspension, even though the amount of dye bound is the same as at pH 3. Thus, the unpolymerized, pH-7 insoluble quaternized lignin does not have the desired property of forming a readily flocculating precipitate at pH 7 upon binding dye.

Peroxidase polymerized, pH 7-soluble quaternized lignin demonstrated the ability to bind dye and precipitate. The ability of this fraction to bind Orange II was tested by equilibrium dialysis at pH 7. Over a period of two weeks the lignin gradually formed a precipitate in the bottom of the dialysis tube as the extent of dye bound to the polymer increased. For two separate preparations of this material, the maximum Orange II bound was 0.90 and 0.65 mmol g⁻¹ of lignin, which indicates that the pH 7-soluble and insoluble lignin fractions did not differ substantially in their extent of quaternization. Peroxidase polymerized, soluble quaternized lignin mixed at various ratios with dye (F3B) was effective at solution decolorization (Figure 5). Maximum dye removal (92%) was achieved with a dye to lignin ratio of 0.2 mmol g⁻¹. At F3B:lignin ratios above 0.1 mmol g⁻¹, rapidly settling precipitates were formed. However, when the amount of polymer was present in excess over the amount of available dye (i.e., at 0.1 mmol of F3B per g of lignin) there was no precipitation. The lack of polymer precipitation in this 'overdose' situation was remedied by the addition of ferric chloride, upon which rapid precipitation of polymer and complete decolorization of the solution ensued.

Figure 4. Influence of solution pH on the solubility of peroxidase polymerized (whole) quaternized lignin.

Figure 5. Effect of varying amounts of peroxidase polymerized, pH 7-soluble quaternized lignin on the extent of F3B removed from solution. The F3B concentration was held constant at 0.1 mmol l⁻¹ while the lignin concentration varied between 0.01 to 0.1 g l⁻¹. The solution was buffered with 2.0 mmol l⁻¹ MOPS/NaOH, pH 7.0. Dye and lignin were combined and allowed to settle for 30 min. The supernatant was filtered and then diluted with an equal volume of buffer to determine the amount of dye remaining in solution. For the datum labeled ‘FeCl₃’, FeCl₃·6H₂O was added (after the lignin) to a concentration of 2.5 g l⁻¹ and the solution was adjusted back to pH 7.0 with dilute NaOH.
Thus, as with conventional cationic polymers, polymerized, quaternized lignin is susceptible to overdosing problems.

Both non-polymerized and polymerized, (whole) quaternized lignins demonstrated the ability to bind dye and precipitate at pH 7 (Figure 6). The polymerized material required less polymer than the non-polymerized lignin to achieve the same extent of decolorization. The polymerized lignin settled rapidly from the dye-containing solution, while the settling rate of the non-polymerized lignin was much slower and to a lesser extent. These observations indicate that polymerization of the entire quaternized lignin preparation increased its dye-removal performance. However, the data in Figure 6 also indicate that the polymerized material was sensitive to overdosing.

DISCUSSION AND CONCLUSIONS

The apparently high quaternization efficiency (90%) of CHMAC with Kraft lignin suggests that this substrate reacts more readily than cotton or lignocellulose [18, 21], which may result from being able to form a true solution with the lignin under the high pH reaction conditions.

This finding is important because the cost of CHMAC largely determines the cost of the product. Similar quaternization efficiencies have been achieved with starch by reactive extrusion [22]. Lignin quaternization proceeds well without resorting to the sophisticated processing equipment or time-sensitive operations needed for other substrates. The simplicity and efficiency of Kraft lignin quaternization may help offset for the significantly higher cost of Kraft lignin compared to other industrial biopolymers such as starch and cellulose.

The phenylpropanoid units that comprise native lignin contain primary, secondary and phenolic hydroxyl groups with which CHMAC may react to form alkali-stable ether linkages. Pine Kraft lignin has 0.58 phenolic and 0.77 aliphatic hydroxyls per C9 unit (therefore, approx. 7.5 mmol of total hydroxyl groups per g of lignin) [1]. Kraft pulping introduces carboxyl groups (0.8 mmol g⁻¹) [1], which can react with CHMAC but not form alkali-stable bonds. The distribution of quaternary ammonium substituents in the derivatized Kraft lignin described herein is not known, but the most likely sites are the phenolic and primary aliphatic (γ position) hydroxyls.

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The solubility characteristics of Kraft lignin are altered substantially by quaternization and peroxidase-catalyzed polymerization. Introduction of cationic groups into the lignin allows the polymer to be soluble at neutral or low pH values (Figure 1). Limited quaternization can be used to produce fractions with novel solubility properties (Figure 1), but more extensive quaternization generates a product that is completely soluble at all pH values [3]. Thus the solubility behavior of 'partially' quaternized lignin fractions can be rationalized by considering the balance of anionic sites (ionized carboxylates) and cationic (quaternary amine) sites in the polymer. The pH 7-insoluble lignin fraction likely contains an equal number of negative and positive charges while the pH 7-soluble fraction has an excess of positive charges. The decreased solubility of (the entire) quaternized Kraft lignin at high pH induced by peroxidase treatment (Figure 4) may result from a partial decarboxylation or loss of phenolate groups, which would raise the isoelectric point of the polymer. Thus, by controlling the extent of quaternization and polymerization, it should be possible to design lignins with solubility characteristics suitable for particular applications.

Blinkovsky and Dordick [10] described the use of horseradish peroxidase to polymerize Kraft lignin in 50% aqueous dioxane. They reported that enzyme treatment of the lignin (in the absence of p-cresol) produced a material insoluble in dioxane or dimethylformamide, indicating that the lignin had been modified. However, they were not able to demonstrate that the molecular weight distribution of the polymer had been altered. The present work demonstrates that peroxidase (soybean, in this case) polymerizes Kraft lignin to produce high molecular weight polymer, without resorting to organic solvents and copolymerants. It is notable that peroxidase treatment of the quaternized lignin fraction that was insoluble at neutral pH clearly modified the polymer, but did not alter its molecular weight distribution. This behavior is
similar to that observed by Blinkovsky and Dordick with peroxidase treatment in aqueous-organic solvents.

The use of purified peroxidase for the polymerization of lignin is an expensive proposition, particularly as there is no evidence that the enzyme can be recovered for reuse [10, 23]. Fortunately, soybean hulls are an abundant source of peroxidase from which a crude isolate of the enzyme can be prepared with ease [24]. The use of a crude soybean hull extract for lignin polymerization should minimize the cost of this process.

It is evident that polymerized, quaternized Kraft lignin can be used to remove anionic dyes from solution (Figure 5 and 6). The soluble polymer forms a rapidly settling floc as it becomes saturated with dye. However, as with synthetic polymers, quaternized lignin is susceptible to overdosing, which leads to decreased color removal and inefficient polymer usage. The overdosing problem observed with cationic lignin can be corrected in the conventional manner with addition of iron salts. A more elegant solution may be to adjust the extents of lignin quaternization and polymerization to produce a product that will precipitate at neutral pH when less than fully saturated with dye. However, the complex interplay between quaternization levels and polymerization effects on polymer solubility will need to be more fully delineated for this approach to be successful.

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