The metal binding properties of kraft lignin

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Abstract

There is a strong driving force to increase the competitiveness of the pulping industry by finding new business opportunities. In this context full utilisation of the wood raw material used in conventional pulping mills is of vital importance. One focus area is to increase the utilisation areas of lignin. LignoBoost is a new method to obtain kraft lignin of high purity.

The aim of the project was to investigate and increase the ability of LignoBoost kraft lignins to bind metals in aqueous solutions.

The metal binding property of kraft lignins was evaluated using copper (II) ions. The metal binding capacities were 1.76 mg Cu^{2+}/g LignoBoost softwood kraft lignin, 0.96 mg Cu^{2+}/g LignoBoost hardwood kraft lignin and 1.12 mg Cu^{2+}/g condensed softwood kraft lignin. The metal binding capacities of the softwood and hardwood kraft lignins from LignoBoost were lower than expected, when compared to the metal binding capacities of other lignins found in literature. The highest copper binding capacity of a kraft lignin found in literature was almost 50 times greater than that of the LignoBoost softwood kraft lignin.

The Mannich reaction was used to increase the nitrogen content in softwood lignin, and thereby increase its electron-donating capacity. An increase in electron-donating capacity should promote its metal binding capacity.

The Mannich reaction occurs in the vacant ortho position of the phenolic groups of lignin, introducing an aminomethyl group at that position. The only vacant ortho position in the phenol unit for reaction is in the guaicyl unit. Softwood lignin underwent a Mannich reaction, since softwood contains a greater number of guaiacyl units than hardwoods.

A screening of the products from this Mannich reaction on LignoBoost softwood kraft lignin was performed to optimise the reaction conditions. The reaction time, temperature, amount of formaldehyde and dimethylamine were varied. The Mannich products were evaluated by elemental analysis. The conditions giving the highest nitrogen content in the product were used further in a Mannich reaction of condensed softwood kraft lignin. The condensed softwood kraft lignin (7 g) was treated with dimethylamine (0.35 mol) and formaldehyde (0.35 mol) at 80°C for 24 hours.

The metal binding experiment could not be carried out with Mannich-modified condensed softwood kraft lignin since the lignin dissolved in the copper solution.

When introducing nitrogen functionalities into lignin the electron-donating capacity should increase. An increase in donor groups should promote the metal binding capacity of the lignin. A problem that occurred when introducing aminomethyl groups was an increase in solubility of the lignin. Water solubility of the lignin derivative is undesirable in the envisaged utilization area, metal binding in, for example mine deposits, from where contaminated water may be a concentrated source of heavy metals.

Contents

1 Introduction

The Kraft process is the main process for paper pulp production. About half of the organic part of wood consists of lignin and is dissolved in the pulping liquor. Kraft lignin, or sulphate lignin, derives from the spent liquor, called the black liquor, of the Kraft process. In conventional kraft pulping, "white liquor"- containing mainly the active cooking chemicals, sodium hydroxide and sodium sulphide- is used for cooking and to delignify the wood chips **(Stenius, 2000).** Kraft lignin is today used as an energy source in heat production whereas lignosulfonate, obtained from the sulphite process, is commercially used as a raw material for production of chemicals. The driving force to finding new utilization areas for the Kraft by-product lignin is the need to increase the profitability of the Kraft process. Lignin is a renewable organic resource that is relatively cheap as a raw material which has increased interest in finding more value-added applications exceeding its current fuel value.

A way to remove lignin from black liquors is to precipitate kraft lignin. The precipitation is achieved by lowering the pH to about 9-10 with carbon dioxide. The lignin is then separated and washed with water. This procedure gives considerable process problems with plugging of the filter cake as well as low yields of lignin.

LignoBoost is a process to obtain a pure kraft lignin, based on precipitation and separation. The lignin is precipitated by acidification with carbon dioxide and filtered, but thereafter an improved washing procedure is applied compared to earlier published methods. The filter cake is re-dispersed at controlled pH and temperature to even out the ionic strength, instead of washing the lignin directly after filtration. The lignin is precipitated from the re-slurried suspension, filtered and finally washed by displacement washing. This procedure facilitates the filtration of the lignin and a higher recovery of kraft lignin can thus be realized **(Öhman, 2006)**.
From the LignoBoost process two types of lignin products can be produced, an intermediate lignin product and washed lignin. The intermediate lignin product is collected before the re-dispersion and the washed lignin is the product from the LignoBoost process.

The Mannich reaction was performed on softwood lignin as this lignin contains predominantly guaiacyl propane units. In guaiacyl propane units, the ortho position of the phenol is available for reaction, and it is this ortho position that is the reaction site for the Mannich reaction. Hardwood lignin contains both guaiacyl propane units and syringyl propane units. In the syringyl propane units the ortho positions of the phenol are occupied by methoxy groups, and thus are not available for reaction **(Henriksson, 2005).** Thus, softwood lignin is more suitable than hardwood lignin for modification through the Mannich reaction.

Experiments to test LignoBoost softwood and hardwood kraft lignins ability to bind copper ions from a copper solution were carried out at room temperature.
Acid-condensed LignoBoost softwood kraft lignins metal binding capacity was tested in the same way as for the LignoBoost softwood and hardwood kraft lignin.

2 Background

2.1 Structure and chemical composition of wood

Wood is a heterogeneous, hygroscopic, cellular and anisotropic material, and has been used as raw material for many applications such as pulp and paper production, construction materials and fuel for a long time.

Wood cells are built up of a polymeric matrix consisting mainly of the carbohydrates, cellulose (40-50%) and hemicellulose (15-25%), and lignin (15-30%) **(Stenius, 2000).** In the living tree, lignin acts like glue holding the fibers together, and brings stiffness to the tree.

2.1.1 Lignin

Lignin is one of the most abundant macromolecules on earth. It is found in all vascular plants. The lignin content differs between species and is also unevenly distributed within the tree; normal softwood contains 26 – 32 % lignin, while normal hardwood contains 20 – 25 % lignin **(Sjöström, 1993).**
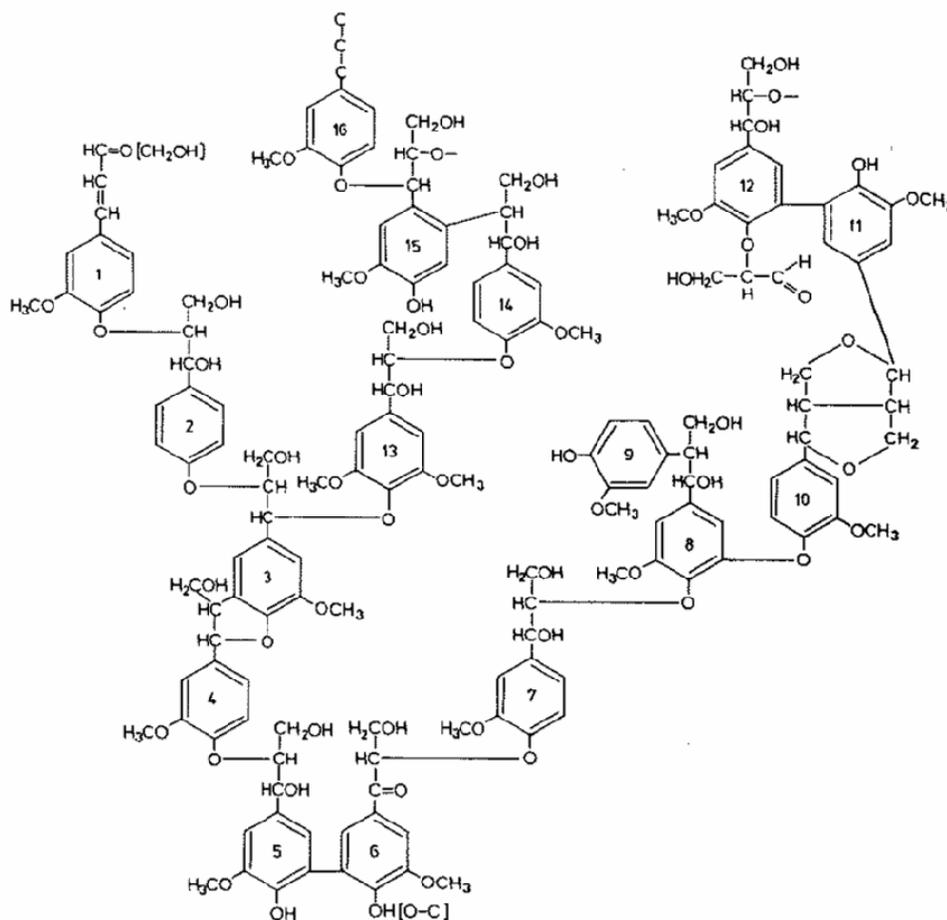

Figure 1 – *A scheme proposed by Adler showing a structural segment of softwood lignin.* **(Sjöström, 1993)**

Technical lignin is a by-product from the paper pulping industry. Besides lignosulfonates obtained from the sulphite process, it has not been utilized sufficiently (**Yanhua, 2004**), even though it is a renewable organic resource.

Lignin is an amorphous macromolecule with a chemical structure that distinctly differs from the other polymers found in wood. Unlike wood carbohydrates, the chemical structure of lignin is irregular in the sense that it consists of different structural units linked to each other by different types of bonds, see Figure 1. Lignins are roughly classified into three major groups: softwood, hardwood and grass lignins (**Stenius, 2000**), depending on their monomer composition.

Lignins are polymerized from primarily three monomers called monolignols. These monolignols are *p*-hydroxyphenyl propanol, guaiacyl propanol and syringyl propanol (see Figure 2), which are propylphenol derivatives with the differences in the number of methoxy groups attached to the ring. (**Henriksson, 2005**)
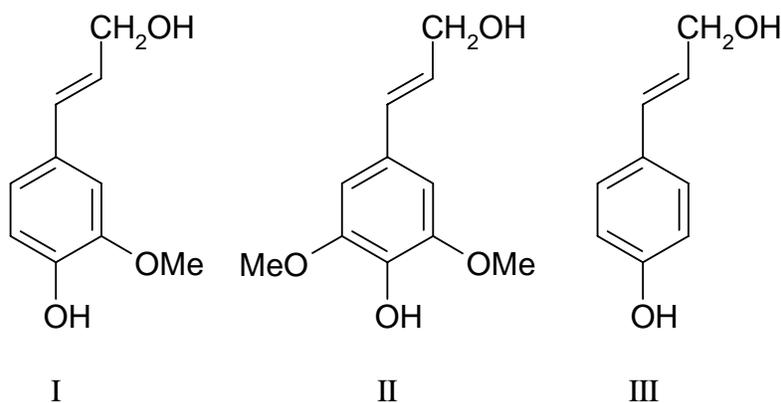
Figure 2 – *The building units of native lignin: guaiacyl propanol (I), syringyl propanol (II) and p-hydroxyphenyl propanol (III).*

Three main types of lignin are recognized according to their content of these three monolignols, see Table 1 (**Henriksson, 2005**).

Table 1 – *Composition of the monolignols in different plants (**Henriksson, 2005**).*

Plant	Guaiacyl propane %	Syringyl propane %	*p*-hydroxyphenyl propane %
Softwood	>95	None or trace	<5
Hardwood	25-50	46-75	0-8
Grass	33-80	20-54	5-33

The structure of lignin does not only differ between different kinds of plants. The structure of lignin can also vary between different plant tissues, different types of cells as well as between different cell wall layers. (**Henriksson, 2005**)

2.1.2 Kraft lignin

Kraft lignin, or sulphate lignin, derives from the spent liquor, called the "black liquor", of the Kraft process. In conventional kraft pulping, "white liquor"- containing mainly the active cooking chemicals, sodium hydroxide and sodium sulphide- is used for cooking the chips. The reactions that occurs during kraft and soda pulping are complex and still not fully understood. The hydrogen sulphide ions (HS^-) primarily react with lignin, whereas carbohydrate reactions are only affected by the alkalinity (HO^-). This also means that the delignifying reactions during kraft pulping proceed faster and provide a higher yield and a stronger pulp compared to soda pulping. About half of the wood substances degrade and dissolve during kraft pulping. The organic matter in the black liquor is composed of the degradation products of lignin and polysaccharides in addition to a minor fraction comprising extractives. Of the charged alkali, about 20 % is consumed to neutralize the acid degradation products of lignin. Dissolution and degradation of lignin during kraft pulping also produces a complex mixture of the breakdown products with a wide molecular mass distribution, ranging from simple low-molecular-mass phenolic compounds to large macromolecules **(Stenius, 2000).**

Most of the kraft lignin can be precipitated from the black liquor by acidification, but the low molecular-mass fragments of the lignin remain in solution. The yield of the precipitation depends first of all on the final pH of the liquor. The use of carbon dioxide is advantageous for technical applications, since no corrosive salts are formed. However, it is not possible to reach below pH 8.5. More lignin can be precipitated if the liquor is acidified by adding strong mineral acid. Carbon dioxide dissociates the weakly acidic phenolic hydroxyl groups of lignin, but not the stronger carboxylic acid groups. **(Sjöström, 1993)**

Kraft lignins can be used in similar applications as lignosulfonates, even though they possess different inherent properties. Lignosulfonates are highly water soluble due to the presence of the sulfonic acid groups, whereas kraft lignin has a limited solubility. When kraft lignin is sulfonated its water solubility increases. Kraft lignin or the modified forms of kraft lignin can thus be used as dispersing agents and as additives in rubber, resins and plastics. When kraft lignin is condensed with formaldehyde and cross-linked with phenols it can yield thermosetting polymers useful as adhesives for different products such as paper laminates and plywood. The lignin polymer can also be degraded into low-molecular-mass chemicals. **(Sjöström, 1993)**

2.2 Mannich reaction

A general introduction of the Mannich reaction is needed, before a more specific description of the Mannich reaction on lignin will be presented.

2.2.1 Mannich reaction in general

The Mannich reaction got its name in 1912 when the German chemist Carl Ulrich Franz Mannich discovered the mechanism for the reaction **(Nationmaster, 2007)**.
A scheme of the Mannich reaction is presented in Figure 3.

The Mannich reaction is initiated through condensation of ammonia, a primary or secondary amine with formaldehyde. The substrate should contain at least one hydrogen atom of sufficient reactivity. The replacement of this active hydrogen atom by an aminomethyl or substituted aminomethyl group is fundamental for the reaction. When a substance contains reactive hydrogen atoms on two or more different carbon atoms the amine can react at different sites of the molecule and lead to different isomers. Dimethylamine is a very reactive amine and generally leads to excellent yields of the Mannich product (**Adams, 1942**).
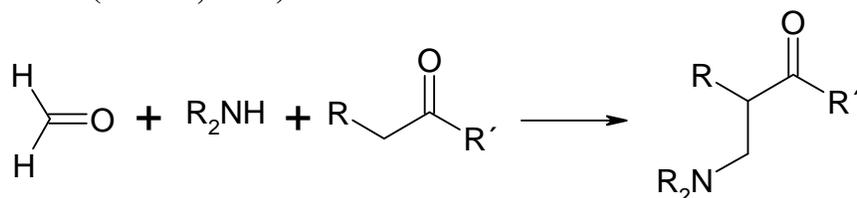

Figure 3 – *Scheme of the Mannich reaction.*

The Mannich reaction is a nucleophilic addition of an amine to a carbonyl group followed by elimination of a hydroxyl anion to the Schiff base. The Schiff base, an electrophile, then reacts in a second nucleophilic addition. The electrophile reacts in the second step with a carbanion generated from a compound containing an acidic proton. A Mannich base is created.

As mentioned above, this reaction is also considered a condensation reaction. In this reaction, ammonia or primary or secondary amines activate formaldehyde. The Mannich reaction requires high reaction temperatures, long reaction times and a protic solvent (**Solomons, 2000**).

2.2.2 Mannich reaction on lignin

Mikawa and co-workers (**Mikawa, 1956**) investigated reactivity of lignins for several amines in the Mannich reaction. They confirmed that the formaldehyde-amine treatment on model compounds affects mainly vacant ortho positions in the phenolic units of lignin. They found that 0.35 nitrogen atoms per methoxyl group could be incorporated into softwood kraft lignin this way. The reactions were made to obtain knowledge of the side chain and nuclear structure of kraft lignin and to investigate the utilization of thiolignin. A scheme of the Mannich reaction on lignin can be seen in Figure 4.
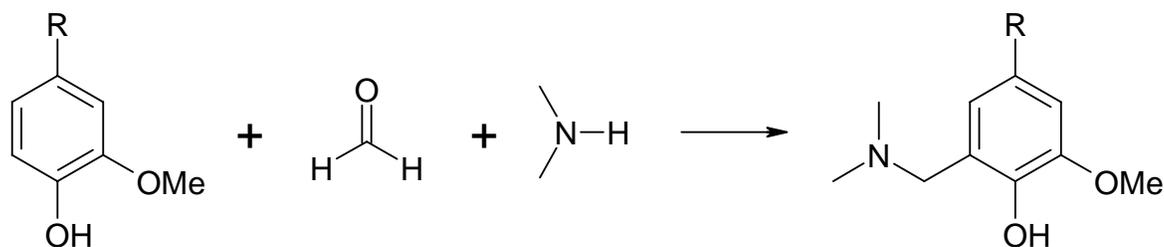

Figure 4 – *Scheme of the Mannich reaction on lignin.*

Brezny and co-workers (**1988**) used three kinds of lignins; spruce organosolv lignin, commercial pine kraft lignin and industrial spruce-pine kraft lignin, and modified them through the Mannich reaction with the amino acids glycine and iminodiacetic acid. The Mannich reactions were carried out to improve the lignins ability to bind metal ions (copper, cadmium and zinc ions).

In the Mannich reaction carried out by Matsushita and co-workers, lignin was dissolved in a solution of 80 % aqueous dioxane and acetic acid. The lignin solution was then reacted at 60°C with dimethylamine and formaldehyde for 24 hours. Preparation of cationic polymers, which acted as the retention aids for normal rosin sizes in neutral papermaking, from sulphuric acid lignin (SAL) was carried out through this experiment. To convert SAL to a cationic polymer, SAL was phenolated and the resulting product was treated by the Mannich reaction to introduce the amino group. The yield of the product ranged from 78 to 90 %. (**Matsushita, 2004**).

According to Matsushita and co-workers (**2003**), addition of a small amount of acetic acid promotes the Mannich reaction. Since the Mannich reaction occurs between a carbon with high electron density and an immonium ion formed from formaldehyde and an amine, they came to the conclusion that an aminomethyl group will be introduced at position five in the guaicyl unit. A condensed type guaicyl lignin model compound was reacted with formaldehyde and dimethylamine to acquire basic knowledge of the reactivity of aromatic nuclei in a Mannich reaction. These experiments were carried out on sulphuric acid lignin (SAL) and phenolized sulphuric acid lignin (P-SAL) and the reaction products were lyophilized to yield the Mannich reaction products (M-SAL and MP-SAL). The Mannich reaction of SAL with dimethylamine did not yield a soluble cationic surfactant, but P-SAL produced a water-soluble cationic surfactant in quantitative yield (**Matsushita, 2003**).

Since the molecular structure of lignin is very complicated, the Mannich modified lignin products could not be easily evaluated by chromatography or spectral analysis. Instead, the evaluation was performed by analysing the change in nitrogen content of the product (**Yanhua, 2004**).

2.2.3 Reaction mechanism for the Mannich reaction on softwood kraft lignin

The reaction mechanism of the Mannich reaction on lignin is shown in two steps in Figures 5 and 6 below.
The reaction mechanism involves the preliminary formation of an imine salt from the amine and formaldehyde.
A nucleophilic attack of dimethylamine on the more electrophilic group, formaldehyde, occurs. No acid is needed for this addition step, but acid-catalysed dehydration of the addition product gives an imine salt (**Clayden, 2001**). For step 1, see Figure 5.
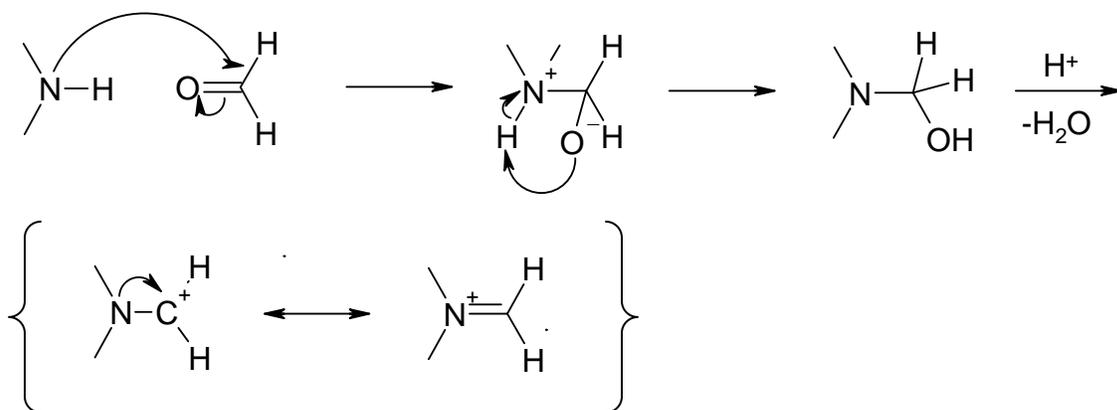
Figure 5 - *Step 1 of the reaction mechanism of the Mannich reaction, where the imine salt is created.*

The electrophilic iminium salt, can then add to the guaiacyl unit at the ortho-position to give the reaction product. For step 2, see Figure 6.
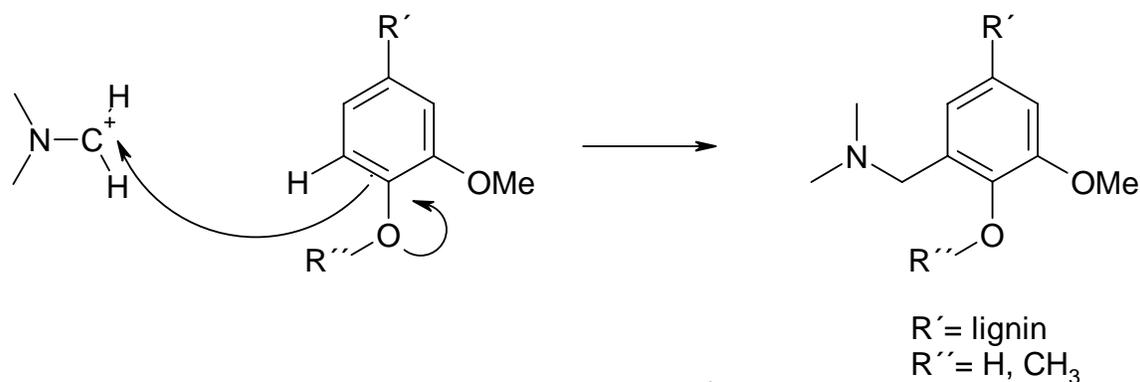
R´= lignin
R´´= H, CH$_3$

Figure 6 – *Step 2 of the reaction mechanism of the Mannich reaction on softwood kraft lignin, where the iminium salt is added to the guaiacyl unit at the ortho-position.*

2.3 Metal binders

Metal binding can occur through different kinds of mechanisms like complex formation, ion-exchange and adsorption.
There are different kinds of metal binders, such as synthetic, organic and inorganic metal binders.
Examples of organic metal binders are pine bark, peat and active carbon. The copper ion binding capacity for pine bark is 4.90 mg/g, for peat 16.1 mg/g and for active carbon 6.99 mg/g (**Täljemark, 2003**).
Other organic metal binders are starch, cellulose, viscose and lignosulfonate (**Filipsson, 1998**).
Some examples of inorganic metal binders are zeolite and leca (**Filipsson, 1998**).

2.4 Metal binding capacity of lignin and Mannich-modified lignin

There are significant differences in the metal binding capacity of different types of lignin. Metal ion adsorption is strongly dependent on pH and ionic strength. It has been reported that lignin (unspecified wood source) from a paper mill in Yunnan Province in south China has affinity with metal ions in the following order: Pb (II) > Cu (II) > Cd (II) > Zn (II) > Ni (II) (**Xueyan, 2007).**

The copper binding capacity of a kraft lignin at 25°C investigated by Mohan and co-workers was 87.05 mg/g. The lignin came from black liquor from pulping of eucalyptus wood (**Mohan, 2006**).

Selective ion-exchanging lignin has been prepared by Brezny and co-workers. Spruce organosolv, commercial pine kraft and industrial spruce-pine kraft lignins were modified by the Mannich reaction with two different amino acids, glycine and iminodiacetic acid. The capacity of the lignins to bind Cu^{2+}, Cd^{2+} and Zn^{2+} ions was determined using unbuffered 5 mM aqueous solutions of $CuSO_4$, $CdSO_4$ and $ZnSO_4$ separately. A mixture of lignin and metal solution was shaken for 8 hours at 20-25°C. The lignin was filtered off. The binding capacity was calculated from the decrease in the metal ion concentration after the lignin treatment. It was found that individual classes of the lignin derivatives exhibit different affinities toward each of the ions. The starting lignins were almost inactive, showing very low values of the binding capacity (0.03 mmol/g toward copper ions at best). The highest values were found for iminodiacetic acid treated lignins and carboxymethylated glycine lignins toward copper ions, reaching the level of 0.6 mmol/g (38.1 mg/g) (**Brezny, 1988**).

3 Experimental

3.1 Chemicals

Acetic acid, copper sulfate, 40% dimethylamine, 1,4-dioxane, ethyl acetate, 37% formaldehyde, methanol, sulfuric acid and sodium diethyldithiocarbamate were purchased from MERCK. All of the chemicals were of *pro analysis* grade.

3.2 Lignin

The kraft lignin used in this project was an untreated softwood lignin from the demonstration plant mill in Bäckhammar. The kraft lignin is isolated from black liquor through the LignoBoost method. In this method the kraft lignin is precipitated with carbon dioxide to pH 9-10 before it is acid washed at pH 2-4.
The softwood kraft lignin has 3.5 mmol phenolic hydroxyls/g sample, the weight-average molecular mass (M_w) was 3900 and the number-average molecular mass (M_n) 700.

3.3 Mannich reaction on LignoBoost softwood kraft lignin

LignoBoost softwood kraft lignin (approx. 100 mg) was dissolved in 80 % 1,4-dioxane (10 mL) and acetic acid (4 mL). The mixture was then treated with 40 % dimethylamine (1.6 mmol) and 37% formaldehyde (1.1 mmol) and reacted at 60°C for 24 hours with continuous stirring (**Matsushita, 2004**).
The reaction mixture was poured into ethyl acetate (25 mL) and the solvents were removed through evaporation. Additional ethyl acetate (35 mL) was then poured into the remaining residue, and a precipitate was obtained. Two reactions were made in this way and gave products 1 and 2.

A second experiment was carried out on LignoBoost softwood kraft lignin (approx. 100 mg). This experiment was conducted similarly as the previous experiment except for that the reaction time was 6 hours. The reaction mixture was poured into ethyl acetate (60 mL) and the solvents were removed by filtration. Two reactions were made in this way giving products 3 and 4.

All of the samples, products 1 – 4, from the Mannich reaction were evaluated by elemental analysis of the carbon, hydrogen and nitrogen contents.

3.3.1 Experimental design of the Mannich reaction on LignoBoost softwood kraft lignin

A screening of the Mannich reaction on LignoBoost softwood kraft lignin was performed. The following parameters were varied: reaction time, temperature, amount of formaldehyde and amount of dimethylamine, see Table 2. The same amount softwood kraft lignin (100 mg), 1,4-dioxane and acetic acid as in the Mannich experiments described in section 3.3 were used for the reactions. Every screening reaction was stopped by pouring the reaction mixture into ethyl acetate (60 mL). Instead of filtering, the precipitates were centrifuged, and the supernatant liquid poured off. The results were evaluated by multivariate data analysis using Modde 8.0, Umetrics.

Table 2 – Screening plan, reaction 1-11.

Reaction	Reaction time (h)	Temperature (°C)	Formaldehyde (mmol)	Dimethylamine (mmol)
1	2	40	0.75	0.50
2	24	40	0.75	5.00
3	2	80	0.75	5.00
4	24	80	0.75	0.50
5	2	40	5.00	5.00
6	24	40	5.00	0.50
7	2	80	5.00	0.50
8	24	80	5.00	5.00
9	13	60	2.875	2.75
10	13	60	2.875	2.75
11	13	60	2.875	2.75

The products were dissolved in methanol (5 mL) and poured over into smaller flasks and the methanol was evaporated under a stream of nitrogen. The elemental compositions with respect to carbon, hydrogen and nitrogen were determined by Mikro Kemi AB, Sweden.

3.4 Condensation of LignoBoost softwood kraft lignin

7.5 g of LignoBoost softwood kraft lignin was mixed with 72 % sulfuric acid (75 mL). Deionised water was added to a final acid concentration of 2.5 % and a final lignin concentration of 0.003 %. The mixture was placed in a water bath at 80°C for one hour. The mixture was then filtered through double GF/A filters (47 mm) from Whatman. The condensed lignin was washed with hot deionised water (2150 mL) until the pH was over 5. The lignin was left on the filter overnight to air-dry, and was then dried under vacuum.

3.5 Mannich reaction on condensed LignoBoost softwood kraft lignin

Condensed LignoBoost softwood kraft lignin (7.00 g) was dissolved in 80 % 1,4-dioxane (250 mL) and acetic acid (100 mL). The mixture was then treated with 40 % dimethylamine (0.35 mol) and 37% formaldehyde (0.35 mol) and reacted at 80°C with stirring for 24 hours. The reaction mixture was then poured into 700 mL ethyl acetate.

3.6 Metal binding

3.6.1 Metal binding using LignoBoost softwood and hardwood lignins

Experiments to test the binding properties of LignoBoost softwood and hardwood kraft lignins were performed using copper ions.

0.25 g LignoBoost softwood kraft lignin was mixed with 100 mL of a solution of 126 mg copper ions/liter deionised water and was placed on a orbital shaker for 24 hours at room temperature. Samples of the copper-containing water were withdrawn at different points in time and analysed by a spectroscopic method. The first two samples were withdrawn

before the lignin was mixed into the copper solution. The first sample was taken without a micro filter while the rest of the samples were taken with a micro filter, so that there would not be any solid lignin in the sample.

Two experiments with 7.5 g LignoBoost softwood kraft lignin respective 7.5 g hardwood kraft lignin mixed with 300 mL of the solution of 126 mg copper ions/liter deionised water were made. This was a ten times greater amount of kraft lignin as used in the previous experiments.

3.6.2 Metal binding using condensed LignoBoost softwood kraft lignin

2.5 g of condensed LignoBoost softwood kraft lignin was mixed with 200 mL of a solution of 63 mg copper ions/liter deionised water and placed on a orbital shaker for 24 hours at room temperature. This is the same concentration of copper ions versus amount of lignin as used previously (2.5 g lignin/12.6 mg copper ions). Samples of the copper-containing water were withdrawn at different points in time and analysed with a spectroscopic method. The first two samples were withdrawn before the lignin was mixed into the copper solution. The first sample was taken without a micro filter while the rest of the samples were taken with a micro filter, so that there would not be any lignin in the sample. The metal-binding experiment of condensed softwood kraft lignin modified by the Mannich reaction was carried out in the same way as described above.

3.7 Analytical methods

The LignoBoost softwood and hardwood kraft lignin, Mannich reaction products and the metal binding experiments were analysed with ICP, elemental analysis and a spectroscopic method.

3.7.1 Inductive coupled plasma (ICP)

The LignoBoost softwood and hardwood kraft lignins were analysed with respect to Ca, Cd, Cr, Cu, Fe, Na, Ni, Pb, S and Zn with ICP.
Before analysis with the ICP, the lignin was wet-digested for 5 minutes under a pressure of 600 psi at 175°C. A microwave oven from MARS CEM was used in the wet digestion. In the wet-digestion of the lignin, hydrogen peroxide and nitric acid were used.
The ICP instrument used for the analysis was a Perkin Elmer instrument, Optima 2000 DV with an Optimal Emission Spectrometer.

3.7.2 Elemental analysis

The Mannich products (1-4) of LignoBoost softwood kraft lignin as well as the LignoBoost softwood kraft lignin sample were analysed by elemental analysis in regards to contents of carbon, hydrogen and nitrogen. The elemental analysis showed that when the nitrogen content increased the Mannich reaction was successful, as long as the sample was not contaminated by the amine, as a result of incomplete washing.
Analysis of C, H and N is built on the principle of combustion. Standard and sample are weighed in a tin capsule and placed in an auto sampler from which the sample is dropped into a packed combustion tube. When the sample is inside the tube, oxygen is injected into the carrier gas helium. When the tin capsule oxidises, the temperature rises

momentarily to about 1800 °C which ensures a complete combustion. The combustion gases CO_2, H_2O, NO_X, SO_2 and SO_3 are swept into a reduction chamber where NO_X are reduced to N_2 and SO_3 to SO_2. The gases are separated chromatographically and are measured with a hot wire detector. Each sample is analysed in duplicate. (**Mikrokemi, 2008**)

To establish the elemental compositions of the lignins and their respective Mannich products a normalized N_n/C_n- ratio was calculated for each analysed sample. C_n represents the moles of carbon and the N_n the moles of nitrogen per Mannich-modified guaiacyl unit. There are 13 carbon atoms and 1 nitrogen atom in the Mannich-modified guaiacyl unit. The weight-% of carbon and nitrogen from the elemental analysis of the products were normalized through division by the amounts in the expected product ($13*12=156$ g/mol for C and $1*14=14$ g/mol for N) thus giving the ratio N_n/C_n.

3.7.3 Spectroscopic analysis

A spectroscopic method was used to analyse the amount of copper remaining in the solution before and after the metal binding experiments.

The copper was determined by a spectrophotometric method utilising complexation with sodium diethyldithiocarbamate (NaDDTC), see Figure 7. (**Jankiewicz, 1998**)
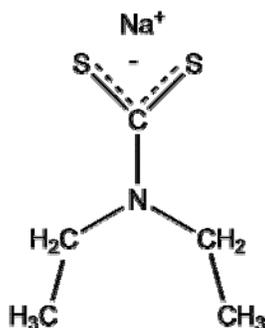
Figure 7 – *The structure of sodium diethyldithiocarbamate.*

Calibration
A 132.5 mg/L copper(II)-stock solution was prepared. The copper sulfate was dissolved in deionised water and 1 mL 65% nitric acid in a 100 mL volumetric flask.

A NaDDTC-solution was prepared. NaDDTC was dissolved in deionised water and 25 µL 4.5 M NaOH in a 100 mL volumetric flask.
Six standards were prepared in 10 mL volumetric flask. The compositions of the standards can be seen in Table 3.

Table 3 - *Standards for the calibration.*

Standard	CuSO$_4$-solution (mL)	H$_2$O (mL)	NaDDTC-solution (mL)	1,4-dioxane (mL)	Cu(II) concentration (mg/l)
1	0.8	1.2	2.0	6.0	10.6
2	0.6	1.4	2.0	6.0	7.9
3	0.4	1.6	2.0	6.0	5.3
4	0.2	1.8	2.0	6.0	2.6
5	1.0 (diluted 10 times)	1.0	2.0	6.0	1.3
6	0.5 (diluted 10 times)	1.5	2.0	6.0	0.7

CuSO$_4$-solution and water were added first, and then about 5 mL of 1,4-dioxane before adding the NaDDTC-solution. The time the NaDDTC-solution was added to the mixture was noted and the spectrophotometric analysis was performed 120 ± 1 minutes after the addition. Finally the solution was diluted to 10 mL with 1,4-dioxane. The absorbance was measured at 436 nm for the standards and a calibration curve was generated, see Figure 8.
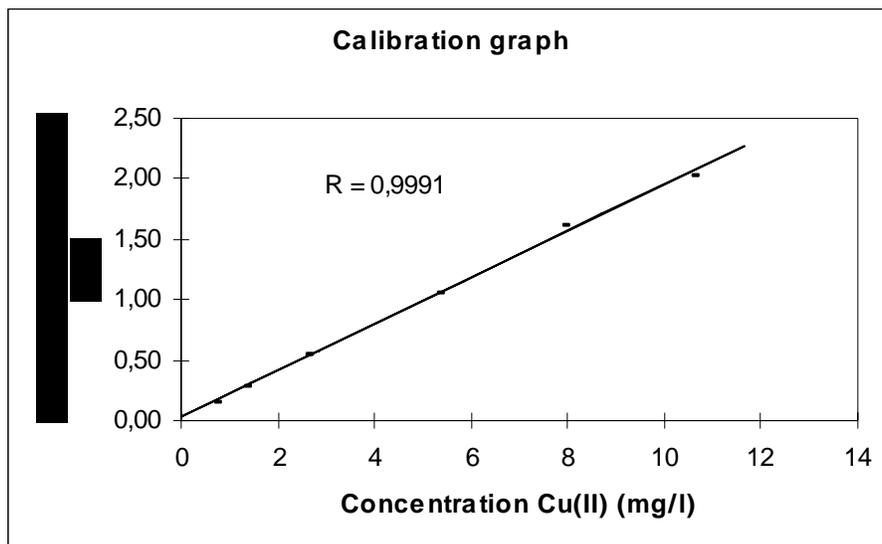
Figure 8 – *The calibration curve for the spectrophotometric method.*

Sample analysis

1500 µL water, 500 µL sample, and about 5 mL 1,4-dioxane and 2.0 mL NaDDTC-stock solution were mixed in a 10 mL volumetric flask. The mixture was diluted to 10 mL with 1,4-dioxane. The time the NaDDTC-solution was added to the mixture was noted and the spectrophotometric analysis was performed 120 ± 1 minutes after the addition. The absorbance was measured at 436 nm and the results were calculated from the calibration curve, see Figure 8.

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4 Results and discussion

4.1 Characterization of the LignoBoost kraft lignins

The softwood kraft lignin has 3.5 mmol phenolic hydroxyls/g sample, the weight-average molecular weight (M_w) is 3900 and the number-average molecular weight (M_n) 700. The samples of the softwood and hardwood kraft lignin were analyzed with regard to a range of metals by Inductive coupled plasma (ICP-AES). The results from this analysis are listed in Table 4.

Table 4 – *The contents of some elements in the samples of LignoBoost softwood and hardwood kraft lignin (mg element/kg lignin).*

Sample	Ca	Cd	Cr	Cu	Fe	Na	Ni	Pb	S	Zn
Softwood kraft lignin	42	0.19	1.5	1.3	36.9	10745	1.0	2.0	24620	7
Hardwood kraft lignin	140	0.41	0.59	3.7	38.7	2060	0.59	2.5	26660	70

4.2 Elemental analysis of the Mannich product

Modification of LignoBoost softwood kraft lignin through the Mannich reaction was performed. The Mannich reaction was carried out to increase the nitrogen content in the lignin, thereby increasing its electron donating capacity. An increase in electron-donors should promote the metal binding capacity of softwood lignin.

The Mannich reaction was performed on LignoBoost softwood kraft lignin because this lignin contains predominantly guaiacyl propane units. In guaiacyl propane units the ortho position of the phenol is available for reaction, and it is this ortho position that is the reaction site for the Mannich reaction. Hardwood lignin contains both guaiacyl propane units and syringyl propane units. In the syringyl propane units the ortho position of the phenol is occupied by a methoxy group, and is thus not available for reaction. Thus, softwood lignin is more suitable than hardwood lignin for modification through Mannich reactions.

The carbon, hydrogen and nitrogen contents for the softwood kraft lignin as well as the normalized molar ratio of the nitrogen and carbon content (N_n/C_n) of the samples are presented in Table 5. Since the kraft lignin contains elements other than carbon, hydrogen and nitrogen, such as oxygen, sulphur and different kinds of metals, the weight-% of carbon, hydrogen and nitrogen together do not reach 100 %. The decision to analyse only the carbon, hydrogen and nitrogen content of the products depended on the availability of the analysis and a decision to gain a first insight into the incorporation of nitrogen. In future experiments, the oxygen as well as the methoxy group content should be included in the analysis.

The analysis showed an increase in nitrogen content when the lignin had been subjected to a Mannich reaction. Since the content is given in weight-% the N_n/C_n- ratio for the products were calculated from elemental analysis results. C_n represents the amount of carbon atoms in the guaiacyl units and the aminomethyl unit together. Products 1 and 2

were treated with dimethylamine and formaldehyde for 24 hours while products 3 and 4 were treated with the same amounts of reagents for only 6 hours.

Table 5 –*Elemental analysis and the N_n/C_n ratio of the softwood lignin sample as well as the Mannich products 1-4. The conditions differed in reaction time; Products 1 and 2 were reacted for 24 hours and products 3 and 4 for six hours.*

Sample id	C (weight-%)	H (weight-%)	N (weight-%)	N_n/C_n
Softwood kraft lignin	41.3	7.1	< 0.3	
Product 1	58.6	6.4	3.2	0.61
Product 2	56.8	7.0	4.1	0.80
Product 3	58.6	6.2	1.9	0.26
Product 4	59.6	5.9	1.6	0.30

The products were precipitated in ethyl acetate, but the products were difficult to isolate from the starting material and reagents. Products 1 and 2 were obtained after evaporation and a lot of work up as brown crystals. Since lignin is such a complex and heterogeneous material it was hard to separate the product from the reagents. In future experiments dialysis should be used to purify the products. The dialysis tube should have a cut-off small enough so the reagents will separate from the product.
This was not an optimal isolation as the N_n/C_n ratio of these products indicated that they were contaminated with reagents and unreacted starting material. The theoretical amount of nitrogen possible to incorporate into the kraft lignin was calculated to 2.6 weight-%. Since the nitrogen content of both products 1 and 2 was higher than that, these products must be contaminated with reagents.
Products 3 and 4, brown crystals, were isolated through filtration, which resulted in a loss of product as the product stuck to the filter paper.

The carbon content in the lignin reference in the elemental analysis is low compared to the theoretical value about 65 weight-% according to Brodin and co-workers (**2009**).

LignoBoost softwood kraft lignin had a nitrogen content of less than 0.3 weight-% and for product 2 the nitrogen content had increased to 4.1 weight-%. Product 2 has increased the most with respect of nitrogen content. Since it is difficult to perform a comparison of the element content in the different products by comparing the weight-% of the products, the N_n/C_n ratio was calculated. Since products 1 and 2 were made in the same way and there was such a difference in the N_n/C_n ratio between these two products, the products do not seem to be free from starting material and reagents. To purify future products dialysis should be tested.

A screening of Mannich reaction conditions on LignoBoost softwood kraft lignin was performed by experimental design comprising of eleven experiments. The influence of four different parameters with respect to nitrogen content was studied; reaction time, temperature, amount of formaldehyde and dimethylamine. The products from these eleven reactions were analysed by elemental analysis to determine the carbon, hydrogen and nitrogen contents and the normalized N_n/C_n ratios are shown in Table 6 below.

Table 6 – *Elemental analysis and the N_n/C_n- ratio of the products of the eleven reactions from the Mannich screening experiment (1-11).*

Exp. No	C (weight-%)	H (weight-%)	N (weight-%)	Nn/Cn
1	60.4	6.7	2.8	0.51
2	58.6	6.5	3.2	0.60
3	57.7	7.0	4.7	0.91
4	60.6	6.1	2.0	0.37
5	58.4	6.8	4.3	0.81
6	60.1	6.1	1.2	0.22
7	60.7	6.2	1.5	0.28
8	55.9	7.6	5.1	1.01
9	60.1	6.5	3.1	0.57
10	59.4	7.0	4.0	0.75
11	59.3	6.7	3.7	0.69

The products were precipitated in ethyl acetate, but the products were difficult to isolate from the starting material and reagents. It was difficult to compare these products with each other and make a judgment about the purification of the products. A product that is contaminated will not give a correct elemental analysis result, as the reagents and starting material will give a false elemental content for the product e.g. if unreacted dimethylamine is present an over-estimation of the nitrogen content will result. Dialysis should have been carried out on these products before the elemental analysis was carried out. In the future, all lignin Mannich reaction products should be dialyzed before any analysis or further experiments are carried out.

The reaction that seems to have incorporated the most nitrogen into the product was reaction 8, i.e. when 5.00 mmol dimethylamine and 5.00 mmol formaldehyde were used on 100 mg lignin at 80°C for 24 hours.

The evaluation shows that the nitrogen content of the product from the Mannich reaction depends mainly on the amount of amine in the reaction. The evaluation can be seen in Figure 9.
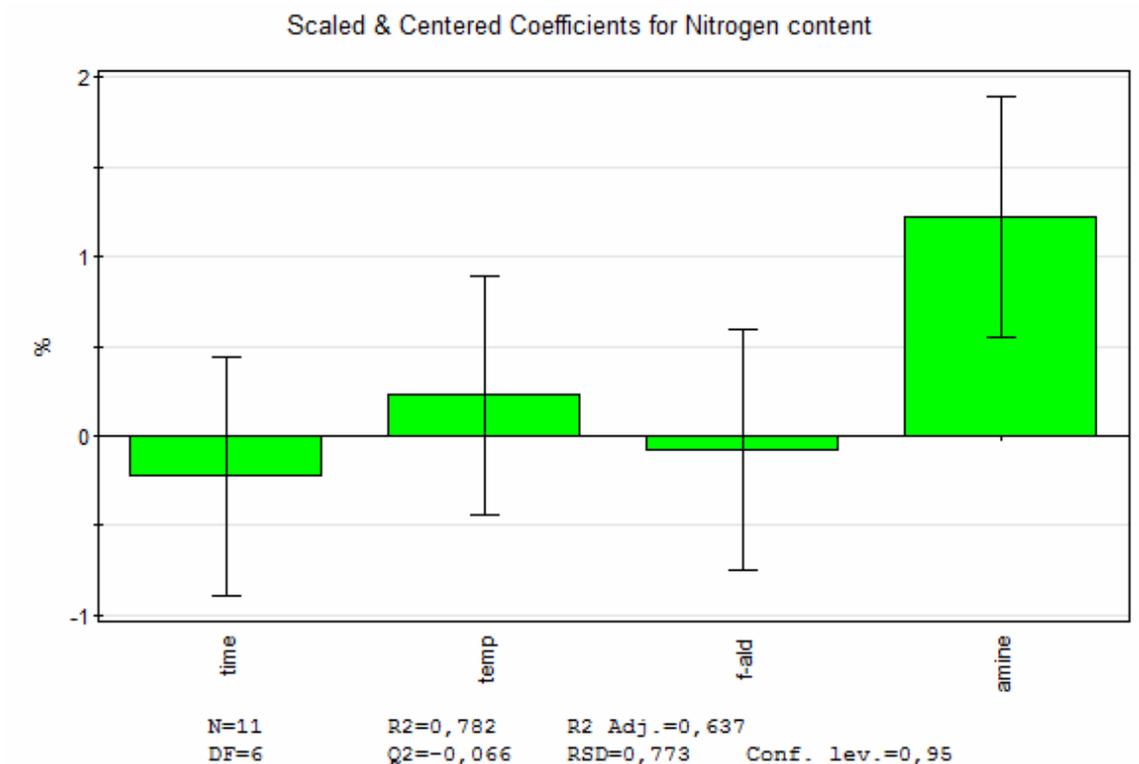
Figure 9 - *The evaluation of the screening of the Mannich reaction on LignoBoost softwood kraft lignin.*

4.3 The metal binding capacity of LignoBoost lignin

4.3.1 Metal binding capacity of softwood and hardwood kraft lignin

Analyses were made by a spectroscopic method of copper ion-containing water of the different metal binding experiments. Two experiments were made with a small amount of kraft lignin; 0.25 g lignin/12.6 mg copper. No copper binding could be seen. To test if the lignin amount had been too low to show metal binding, the amount of lignin was increased while the copper concentration was kept constant.

In the experiments where the amount of LignoBoost softwood and hardwood kraft lignin was increased ten times to 2.5 g lignin/12.6 mg copper, some copper binding was observed. The spectroscopic analysis of the two experiments is shown in Figure 10 (for details see Table A:1 in Appendix A).
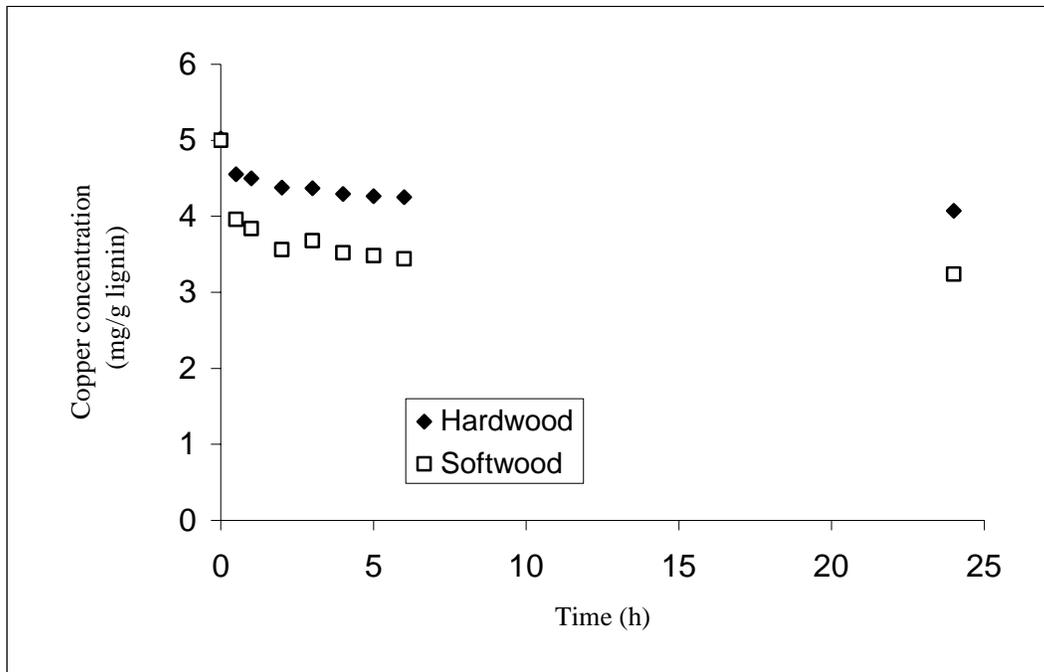
Figure 10 – *The copper concentration of the water samples when using 2.5 g LignoBoost softwood and hardwood kraft lignin, respectively, 12.6 mg Cu(II).*

The metal binding capacities are 1.76 mg Cu^{2+}/g LignoBoost softwood kraft lignin and 0.96 mg Cu^{2+}/g LignoBoost hardwood kraft lignin. The LignoBoost softwood kraft lignin had a better metal binding capacity than the LignoBoost hardwood kraft lignin, but was much lower than expected if compared to the values found in the literature, 87.05 mg Cu^{2+}/g kraft lignin (**Mohan, 2006**). Since the lignin that Mohan and co-workers used came from eucalyptus wood it will differ in particle size, purity, content and structure from the LignoBoost softwood and hardwood kraft lignin.

4.3.2 Metal binding capacity of condensed softwood kraft lignin

The LignoBoost softwood kraft lignin was condensed to make it less water soluble. When lignin is condensed, carbon-to-carbon linkages are formed between lignin entities. It has been suggested that the major part of the condensation processes occurs at the unoccupied ortho position of the phenolic units, the same site of reaction of the Mannich reaction (**Sjöström, 1993**). The spectroscopic analysis from the metal binding experiment of the condensed LignoBoost softwood kraft lignin is shown in Figure 11 (for details see Table A:2 in Appendix A).
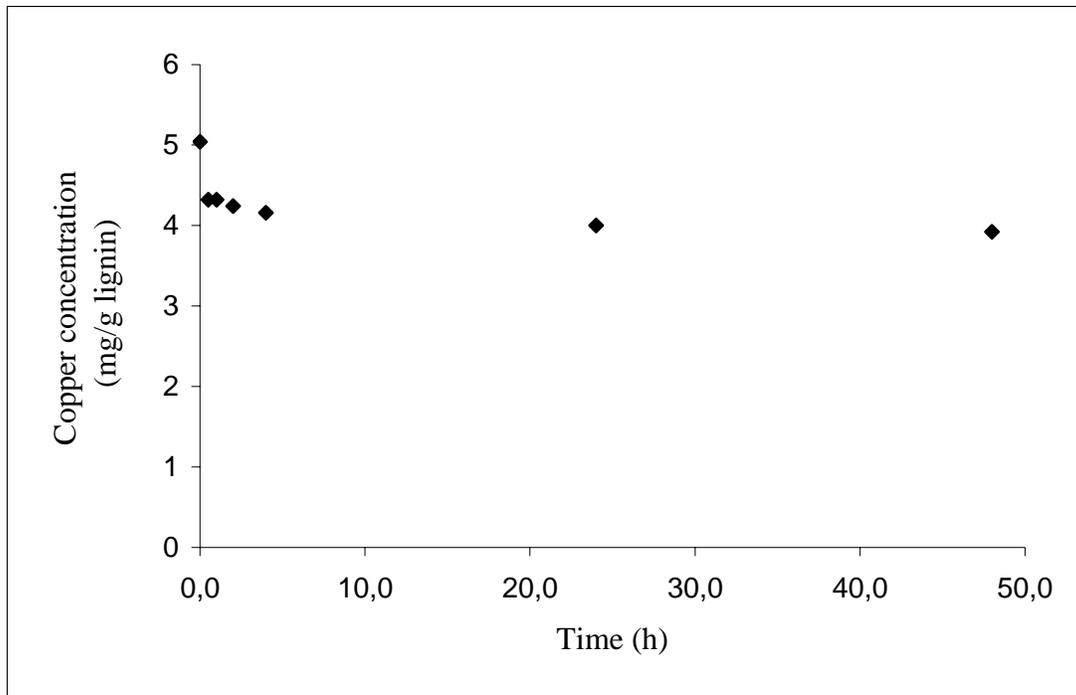
Figure 11 – *The copper concentration of the water samples when using 2.5 g condensed LignoBoost softwood kraft lignin/12.6 mg Cu(II).*

The metal binding capacity was found to be 1.12 mg Cu^{2+}/g condensed LignoBoost softwood kraft lignin, which also is lower than expected.

The lignin that had the best copper binding capacity out of the LignoBoost softwood, hardwood and condensed LignoBoost softwood kraft lignin was the softwood kraft lignin.

The metal binding capacity of the tested lignins was lower then expected. Further experiments should be performed to clarify the reason for the low metal binding capacity. One reason for the binding capacity to be lower than expected is that every type of lignin is different and the lignins that have been tested before were not exactly the same as the LignoBoost lignin. The LignoBoost lignin can, for example, differ in particle size, purity, content and structure compared to previously tested lignins.

4.3.3 pH of the copper solution in the metal binding experiments
The pH of the copper solutions used in the metal binding experiments were between 5.1 - 5.3 before the lignin was added to the solution.

When the softwood and hardwood kraft lignin (0.25 g) was added to the copper solution the pH went down to about 4.4 – 4.5 and after 24 hours it was even lower, about 4.1.

When the amount of softwood and hardwood kraft lignin was increased to 2.5 g and added to the copper solution, the pH of the copper solutions was between 4.0 - 4.1. After 24 hours the pH was around 3.5 – 3.7

When the condensed lignin was added to the copper solution the pH was 3.4 and after 24 hours it had decreased to 3.0.

The pH of the solution is not easy to control. The metal binding depends on the ionic strength, so the pH of the solution will have an impact on the metal binding capacity.

4.3.4 Metal binding capacity of Mannich-modified condensed LignoBoost softwood kraft lignin

The Mannich reaction was carried out to increase the nitrogen content in the condensed LignoBoost softwood kraft lignin, thereby increasing its electron density. An increase in electron density should promote the metal binding capacity of LignoBoost condensed softwood kraft lignin, but high nitrogen content makes the products more polar and thereby more water soluble.

When the lignin is condensed it has been suggested that the major part of the condensation processes occurs at the unoccupied ortho position of the phenolic units, the same site of reaction as the Mannich reaction (**Sjöström, 1993**). The same amount of nitrogen should thus not be possible to incorporate into condensed lignin as in regular lignin since it does not contain as many free ortho-positions at the guaiacyl units.
When the Mannich reaction was carried out on the condensed LignoBoost softwood kraft lignin the reaction conditions were the same as in reaction 8 in the screening experiments, because this reaction seemed to have incorporated the most nitrogen into the product. The condensed softwood kraft lignin (7 g) was treated with dimethylamine (0.35 mol) and formaldehyde (0.35 mol) at 80°C for 24 hours.
The reaction mixture was poured into ethyl acetate, which did not yield a precipitate, but several different phases. An attempt to separate and dry theses phases was made.

When the Mannich-modified condensed LignoBoost softwood kraft lignin was put into the copper solution for the metal binding experiment, the lignin dissolved. The lignin was water soluble and the metal binding experiment could not be carried out since the solution could not be analysed. The problem seems to be that the lignin became too polar that it dissolved immediately in the aqueous solution. The product was probably not free from reagents.

To decrease the water solubility, it seems to be necessary to lower the amount of nitrogen in the final product since the polarity contributes to increase the water solubility of lignin. Thus the amount of amine during the Mannich reaction should be lowered, even when using condensed kraft lignin. An optimisation of the conditions should include the water solubility, since this property has a great influence on the applicability of kraft lignin as a metal sequestrant. A further improvement would be to optimize the purification of the product by e.g. dialysation, or use a more lipophilic amine.

5 Conclusions

- Introduction of nitrogen to the LignoBoost softwood kraft lignin through a Mannich reaction was successful.

- The nitrogen content of products from the Mannich reaction depends mainly on the amount of amine in the reaction.

- The Mannich products were difficult to isolate, which gave an impure product. All Mannich products should be dialysed before further tests and evaluations.

- New methods to synthesise the products should be investigated.

- A new screening of the Mannich reaction conditions should also include dialysis and water solubility tests.

- The LignoBoost softwood kraft lignin had the best copper binding capacity followed by condensed LignoBoost softwood kraft lignin. The lowest binding capacity was attained for hardwood LignoBoost lignin.

- The Mannich-modified condensed LignoBoost softwood kraft lignin dissolved in the copper solution when the metal binding capacity was tested. The metal binding capacity could therefore not be evaluated.

- Less than 5.00 mmol dimethylamine / 100 mg lignin should be used in Mannich reactions on lignin, so the product will become less polar and thus less water soluble.

- A more lipophilic amine could be used instead of dimethylamine.

- The aim was not completely fulfilled since the metal binding capacity of Mannich-modified LignoBoost softwood kraft lignin was never tested due to lack of time. This should be done along with new experiments on a new Mannich-modified condensed LignoBoost softwood kraft lignin.

6 Acknowledgements

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