Deciphering the Interaction between Wood Components and Preservative Chemicals: A Comparison of Analytical Techniques

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Applied Science

Department of Chemical Engineering and Applied Chemistry University of Toronto

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Masters of Applied Science
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Abstract
The durability of preservative-treated wood is highly dependent on the interaction of wood components (cellulose, hemicellulose, lignin, and extractives) with preservative chemicals. Wood preservatives are generally comprised of metal ions, commonly copper, chromium, and arsenic, with iron as a pigment. This work establishes methods to quantify interactions between preservative chemicals and main wood components, to aid efforts in formulation development that extend the weathering performance of wood. Analytical methods for isothermal titration calorimetry (ITC) and quartz crystal microbalance with dissipation monitoring (QCMD) were developed. Iron was found to interact with organosolv softwood lignin and beechwood glucuronoxylan, whereas copper, arsenic, and chromium were found to interact with the glucuronoxylan only. Additionally, the impact of alkaline copper quaternary (ACQ)-type preservative treatment on the degradation of wood structures by UV-A light was studied using Fourier transform infrared spectroscopy (FTIR) and the distribution of copper ions visualized using time-of-flight secondary ion mass spectrometry (ToF-SIMS).
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To Timber Specialties, thank you for supporting this fundamental research and providing samples for testing. I hope this work can help guide your future formulation development efforts.

To my family and friends, thank you for having faith in me and never doubting that I could succeed.
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Chapter 1 – Background

1.1 Chemical composition of wood

Wood is comprised mainly of cellulose (40-50%), hemicellulose (25-35%), and lignin (18-35%), with small amounts of organic extractives (4-10%) and inorganic trace elements (<1%) (Humphrey, 2002). Cellulose is a linear polymer of (β-1→4)-D-glucopyranose which can be amorphous or crystalline (Figure 1). Approximately 60-70% of wood cellulose forms crystalline structure permitted by alternating β-glycosidic bonds with hydroxyl groups in the equatorial plane (Rowell, 2012).

![Figure 1. Chemical structure of cellulose.](image1)

Hemicelluloses do not form crystalline structures and are polymers of 150-200 (β-1→4)-linked sugar units with short side substitutions that can include additional sugars or acetyl groups (Figure 2) (Rowell, 2012). The particular composition of a given hemicellulose depends on its botanical source. For example, the main hemicelluloses in hardwoods, such as maple or oak, are acetylated glucuronoxylan and glucomannan, whereas the main hemicelluloses in softwoods, such as spruce or pine, are galactoglucomannan and arabino-glucuronoxylan (Rowell, 2012).

![Figure 2. Chemical structure of glucuronoxylarabin, an example of hemicellulose.](image2)
Finally, lignin is a three-dimensional cross-linked polymer consisting of phenyl propane units with no regular structure (Figure 3) (Rowell, 2012). Similarly to hemicelluloses, lignin composition differs in hardwoods and softwoods. Often, the phenyl propane units in softwoods have a hydroxyl and methoxy group giving rise to a methoxy-phenyl propane (guaiacyl propane) (Rowell, 2012). Hardwoods have an additional methoxy group so they consist of both syringyl and guaiacyl units (Obst, 1982).

Figure 3. Chemical structure of lignin (example).
1.2 Wood morphology

Wood cells contain a system of cell walls with different compositions. The outermost cell wall is called the primary cell wall and is characterized by comparatively amorphous cellulose and xyloglucan as the dominant hemicellulose (Lawoko, 2005; Sjöström, 1993). The secondary cell wall is comprised of three layers: S1 (the first layer), S2 (the second layer), and S3 (the third layer). The S2 layer is the thickest, approximately 5 µm, and the other two layers are quite thin, approximately 0.1-0.2 µm (Booker & Sell, 1998; Sjöström, 1993). The cells are held together by the middle lamella, which is rich in lignin and pectin (Lawoko, 2005; Sjöström, 1993).

![Cell wall structure of a wood cell](image)

Figure 4. The cell wall structure of a wood cell. S1 = secondary cell wall 1st layer, S2 = secondary cell wall 2nd layer, S3 = secondary wall 3rd layer.

1.3 Weathering of wood

Weathering of wood is surface degradation in response to environmental factors such as solar radiation, precipitation, temperature changes, abrasion, and atmospheric pollution (Rowell, 2012). Degradation is primarily initiated by the ultraviolet (UV) portion of solar radiation causing photooxidation of the surface (Feist & Hon, 1984; Rowell, 2012). The UV light interacts with lignin initiating a complex free-radical sequence of reactions. The free radicals interact with oxygen creating hydroperoxides that cause deterioration and discolouration (Feist & Hon, 1984). The extent of weathering is subject to many factors of wood, such as species density, growth rate, and texture differing in early and late woods (Rowell, 2012).
1.4 Chemicals in wood preservative formulations

The effects of weathering can be mitigated by applying a wood treatment process and/or wood finishing (Ozdemir, Temiz, & Aydin, 2015). Currently, wood preservatives are categorized into three types: (1) water-borne preservatives, (2) oil-borne preservatives, and (3) light organic solvent preservatives. Water-borne preservatives tend to be the least expensive and most widely used (Rowell, 2012). This section briefly discusses the use of chromic acid, copper, chromated copper arsenate (CCA), and iron oxide in water-borne wood preservatives.

1.4.1 Chromic acid

Treatment of wood with inorganic salts, such as chromic acid, is often used to protect wood against weathering. Wood treated with 5-10% aqueous solution of chromium trioxide (CrO₃) is known to result in a wood surface that is highly resistant to photochemical degradation (Pandey & Khali, 1998; Rowell, 2012). Some studies suggest that chromium ions interact with lignin and form complexes that may be able to emit effective energy from wood surfaces (Chang, Hon, & Feist, 1982). This complex decreases light energy absorption by the wood consequently decreasing the number of initiated reactions. Another theory is that the complex causes a shift of the absorbance zone to shorter wavelengths, decreasing the amount of photooxidation (Chang et al., 1982). Chromium (VI) functions as a fixing agent in a complex series of reactions undergoing reduction to chromium (III) that are discussed in more detail in section 1.5 (Wilkinson, 1979).

1.4.2 Copper-based

Copper (II) acts as a fungicide causing the non-specific inhibition of certain enzymes or denaturation of proteins by coordination with groups such as thiols, hydroxyls, amines, or carboxylic acids (Humphrey, 2002; Lupsea, Mathies, Schoknecht, Tiruta-Barna, & Schiopu, 2013). A variety of copper-based preservatives exist, such as alkaline copper quartenary (ACQ) and copper azole preservatives. These types of preservatives have become popular as many countries have placed restrictions on the use of chromated copper arsenate (CCA) preservatives, discussed in Section 1.4.3 below (Rowell, 2012). ACQ preservatives consist of copper, which acts as a fungicide, and a quaternary ammonium compound (QAC) which acts as an insecticide. QACs
penetrate cell walls reacting with lipid and protein portions of the membrane which cause disorder and lysis of cell walls (Gerba, 2015). The ammonium compound also enhances the fungicidal effect of the copper (Rowell, 2012).

More recently, copper carbonate nanoparticles and microparticles have been used in wood preservatives. The ability to tailor the size of particles can be advantageous for obtaining controlled release of bioactive agents, potentially extending the wood preservation time (Matsunaga, Kiguchi, & Evans, 2009). It should be noted that the effectiveness of particle-based wood preservatives depends on proper dispersion of particles in the wood and the conversion of particles to copper (II) ions in order to protect against weathering (Matsunaga et al., 2009).

### 1.4.3 Chromated copper arsenate (CCA)

The presence of chromium (VI) improves the fixation of other metal salts in wood, such as copper and arsenic ions, although the mechanism is not well understood (Radivojevic & Cooper, 2007). CCA preservatives, in which chromium is used in conjunction with copper and arsenic, have been used since the mid-1930s and are still used today in external applications such as telephone poles (Ferrarini et al., 2016; Thompson, 1991).

Arsenic acts as an insecticide in the preservative. As (III) is considered most toxic to insects but the less soluble As (V) is generally used in preservatives to prevent arsenic leaching. Arsenic has a secondary role as a fungicide limiting a cell’s ability to form ATP, the energy molecule, by interfering with oxidative phosphorylation (Humphrey, 2002).

Possible formulation components for CCA preservatives are summarized in Table 1 (Humphrey, 2002).

Table 1. CCA formulation components registered with the American Wood Preservers’ Association.

<table>
<thead>
<tr>
<th>Active component</th>
<th>Formulation compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr(VI)</td>
<td>CrO₃, K₂Cr₂O₇, Na₂Cr₂O₇</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>CuO, CuSO₄·H₂O, Cu(OH)₂, CuCO₃·Cu(OH)₂</td>
</tr>
<tr>
<td>As(V)</td>
<td>As₂O₅, H₃AsO₄, Na₃AsO₄, Na₄As₃O₇</td>
</tr>
</tbody>
</table>
Current formulations can be subdivided into three types (A, B, C) although most CCA preservatives are type C (>90%). Type A, B and C differ by the ratio of CrO$_3$, CuO, and As$_2$O$_5$ as shown in Table 2 below (Humphrey, 2002).

Table 2. CCA formulation types and component ratios from the American Wood Preservers’ Association.

<table>
<thead>
<tr>
<th>Type</th>
<th>% CrO$_3$</th>
<th>% CuO</th>
<th>% As$_2$O$_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>65.5</td>
<td>18.1</td>
<td>16.4</td>
</tr>
<tr>
<td>B</td>
<td>35.3</td>
<td>19.6</td>
<td>45.1</td>
</tr>
<tr>
<td>C</td>
<td>47.5</td>
<td>18.5</td>
<td>34.0</td>
</tr>
</tbody>
</table>

Various studies have been performed to determine the optimum ratio for maximum fixation but studies are difficult to compare since different methods, species of wood, sample size, and other factors are employed (Choi, Ruddick, & Morris, 2004; Humphrey, 2002; D. N. R. Smith & Williams, 1973).

1.4.4 Iron oxide

Iron oxides are typically used in stains and paints as pigments to augment the colour of wood. Copper-based and chromated copper arsenate preservatives give wood a green tinge upon treatment, which is undesired, therefore, iron oxides are added to change the colour to brown. It can also be used in conjunction with carbon black pigment (Matsunaga et al., 2009). The iron oxides may also have an additional beneficial effect of UV protection, slowing the weathering process of wood by reducing the loss of lignin (Schauwecker, Mcdonald, Preston, & Morrell, 2014). The shape of iron oxide particles affects its protective qualities (Schauwecker et al., 2014).

1.5 Interaction of wood and preservative components

1.5.1 Chromated copper arsenate (CCA) fixation

Fixation refers to a series of complex reactions that result in the immobilization of components onto the wood surface. Chromium, copper, and arsenic react with wood components at different rates, with copper and arsenic fixing to the wood matrix faster than chromium. Since the fixation of chromium is the slowest, it is often used as a marker of the extent of fixation of...
CCA-preservative on wood (Kazi & Cooper, 2000). Qualitatively, the fixation of CCA-type preservatives on wood is often estimated by determining the presence of chromium (VI) with chromotropic acid since the level of unreduced chromium (VI) decreases as the preservative is fixed onto the wood (Foster, 1988). Quantitatively, the degree of fixation can be determined by comparing the chromium, arsenic, and copper content of the preservative solution before and after treating the wood by techniques such as atomic absorption spectroscopy (Guo, Ung, & Cooper, 2000).

Currently two models exist which attempt to describe the interaction of CCA and wood components: (1) Dahlgren and Hartford fixation model and (2) Pizzi fixation model.

1.5.1.1 Dahlgren and Hartford fixation model

Dahlgren and Hartford reacted finely ground wood flour (Pinus sylvestris) with a CCA preservative (Dahlgren & Hartford, 1972). They identified that the process undergoes three identifiable steps:

(1) initial reactions in which
   a. a sudden increase in pH occurs due to the adsorption of HCrO$_4^-$ onto wood (specific component unknown) as H$_2$CrO$_4$
   b. and Cr(VI) is reduced to Cr(III) likely by solubilised wood extractives

(2) primary precipitation fixation in which
   a. Cr(VI) is reduced to Cr(III) with second order rates with respect to Cr (VI)
   b. the pH gradually increases (over hours or days) causing precipitation of various compounds such as [Cr(Cr$_2$O$_7$)$_3$]$^{3-}$ and [Cr(CrO$_4$)$_3$]$^{3-}$ at pH <3.0, Cr(OH)$_2$$_2$CrO$_4$ at pH >3.0, and [Cr$_4$(OH)$_{10}$]CrO$_4$ at pH >3.5
   c. copper and chromium arsenates are predicted to be formed such as CuHAsO$_4$ and Cu$_3$(AsO$_4$)$_2$ between pH 2 and 2.8, and CrAsO$_4$ at pH >2.8

(3) final conversion reactions which yield final fixation product and basic copper arsenates

The final fixation products (Cu(II)-wood, CrAsO$_4$, Cu(OH)CuAsO$_4$ and Cr(OH)$_3$ were proposed based on known chemistries but were not confirmed (Dahlgren & Hartford, 1972; Humphrey, 2002).
1.5.1.2 Pizzi fixation model

Pizzi revised the Dahlgren and Hartford fixation model using selected combinations of model structures with lower molecular weights (Pizzi, 1983). D(+) glucose and guaiacol were used as a structural models for cellulose and lignin, respectively (Pizzi, 1983). The work has been challenged by other researchers since simple mixtures of wood and chromium (VI) behave significantly differently to complex mixtures with all CCA components (Plackett, 1983). The chemical composition of wood varies between and within species, therefore, model compounds may not accurately depict the mechanism of wood preservative interactions (Humphrey, 2002).

Pizzi proposed that chromium (VI) is initially adsorbed to cellulose and is then reduced to chromium (III). Contrary to the Dahlgren and Hartford model, Pizzi proposes that cellulose is oxidized and not extractives. The reduced chromium then forms CrAsO$_4$ when $\text{H}_3\text{AsO}_4$ is present, which could be bound to lignin or precipitated. Any unreduced Cr(VI) complexes with copper forming CuCrO$_4$ which is thought to be bound to lignin (Pizzi, 1983).

1.5.2 Factors affecting fixation

Fixation rate is highly dependent on the temperature. Kazi and Cooper studied the rate of “Initial reactions” between wood and CCA solution (marked by a spike in pH) at different temperatures for red pine samples (2000). The decrease in chromium (VI) concentration was monitored over time at 4ºC, 13ºC, and 22ºC. Although the final concentration of chromium (VI) was constant in all experiments, more time was required to reach equilibrium at lower temperatures than higher temperatures (Kazi & Cooper, 2000). Treatment times for coating wood with preservative should be optimized based on the temperature.

Guo et al. compared the fixation rates of CCA on earlywood and latewood from sapwood, whole sapwood, and heartwood from Douglas-fir, southern pine, and eastern larch at different temperatures (21ºC and 60ºC). The chromium (VI) content at sampling points over several days was determined by the diphenylcarbazide method to determine the percent chromium fixation. They found faster fixation rates in latewoods than earlywoods of each species indicating that wood density affects fixation. In addition, heartwood rates were faster than sapwood for all species. Heartwoods are higher in extractive content, which likely accelerates the chromium reduction
process. Variation was also seen between species (Guo, Cooper, Ung, & Ruddick, 2002). This study shows that wood type must be clearly defined when comparing preservative interactions.

1.5.3 Chromium interactions

As mentioned above, chromium is suspected to reduce to Cr (III) by oxidizing cellulose. This process results in a coordination change from tetrahedral to octahedral providing many sites for coordination with wood components (lignin or carbohydrates). The coordination makes insoluble complexes that greatly retard photochemical degradation (Rowell, 2012).

Electron spectroscopic (ESCA) studies were performed to evaluate the oxidation of earlywood surfaces after chromic acid treatment (Williams & Feist, 1984). The studies were compared to fixation on cellulose alone. The results showed fixation to both wood and cellulose with no significant differences in interaction observed between the two (Williams & Feist, 1984). This supports Pizzi’s fixation model in which chromium is adsorbed onto cellulose and cellulose is oxidized.

The chromium trioxide complex is then thought to stabilize lignin, to mitigate its degradation due to weathering. It was found that chromium-guaiacol complexes were highly insoluble, thermally stable, and UV protecting (Schmalzl, Forsyth, & Evans, 2003). In many studies, guaiacol is used as a lignin model compound to study interactions with chromium compounds; however, since this simplified model of lignin, guaiacol may have behavioural differences when compared to lignin found in wood samples. A more recent study analyzed the adsorption of Cr (III) on lignin isolated from black liquor (Wu, Zhang, Guo, & Huang, 2008). The researchers found that “Cr (III) adsorption was strongly dependent on pH and adsorbent dosage, but independent of ionic strength and other metal ions” meaning chromic acid many work best in combination with other CCA preservative components (Wu et al., 2008). It should be noted that lignin isolated from black liquor often contains additional chemical compounds from the isolation process, which may interfere with studies (Kim et al., 2017; Zhu & Theliander, 2015).
1.5.4 Copper interactions

Copper (II) fixation is thought to occur through complexation with weak acidic groups in wood fractions. Interactions through a weaker ion-pair or associations between Cu(H₂O)₆²⁺ and polar functional groups in wood are also suggested. It is currently unclear as to which wood fraction is responsible for binding of Cu (II) since some studies suggest carboxylic acids or uronic acids of hemicellulose, whereas others suggest cellulose, lignin, or various extractives (Bland, 1963; Michie, 1961).

Lignin hydroxy or methoxy groups are potentially suitable for chelation with Cu (II) since complexes with the model compound, guaiacol, have been observed (Bullock & Jones, 1968). On the other hand, Plackett et al studied the interaction of CuSO₄·5H₂O with cellulose, wood lignin, and radiata pine sapwood finding that copper (II) was bound by oxygen donor ligands at the centre of an octahedral environment both cellulose and pine sapwood indicating that cellulose might interact with Cu (II) (Plackett, 1983).

Ung and Cooper found that the stabilization of copper in ACQ preservatives, monitored by X-ray fluorescence spectroscopy, occurs much faster at higher temperatures (50°C vs 22°C). Although several wood species were analyzed (spruce, fir, pine, etc.), no significant species effects were detected, other than Douglas-fir in which copper reacted more quickly than in other species (Ung & Cooper, 2005).

1.5.5 Iron interactions

The interaction of iron (III) and lignin was studied using the model compound, guaiacol, by Schmalzl, Forsyth, and Evans (1995). A few complexes with guaiacol were isolated by HPLC then analyzed by ¹H NMR and mass spectrometry. Since complexes were formed between the guaiacol and iron, it was assumed that iron (III) and lignin also form complexes. As previously discussed, the use of guaiacol as a model for lignin may not accurately describe the interaction of lignin with metal ions in a wood fiber matrix.
1.5.6 Summary

A summary of studies and respective limitations discussed is provided in Table 3 below.

Table 3. Summary of wood fraction and preservative component interaction studies.

<table>
<thead>
<tr>
<th>Preservative type or components</th>
<th>Wood fraction</th>
<th>Analysis method</th>
<th>Main findings</th>
<th>Limitations</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA</td>
<td>wood flour (Pinus sylvestris)</td>
<td>pH variation</td>
<td>Dahlgren and Hartford fixation model (see section 1.5.1.1)</td>
<td>speculation on reactions and precipitation products</td>
<td>(Dahlgren &amp; Hartford, 1972)</td>
</tr>
<tr>
<td>CCA</td>
<td>D(+)‐glucose (cellulose model)</td>
<td>pH variation, Cr(VI) and Cr(III) concentration variation</td>
<td>Pizzi fixation model (see section 1.5.1.2)</td>
<td>simple mixtures (ex. wood and Cr(VI)) behave different than complete mixtures of CCA</td>
<td>(Pizzi, 1983)</td>
</tr>
<tr>
<td>CCA</td>
<td>wood (Pinus patula)</td>
<td>Cr(VI) concentration variation at different temperatures</td>
<td>fixation rates are slower at lower temperatures</td>
<td>only describes “initial reactions” (ie. step 1 of Dahlgren and Hartford fixation model)</td>
<td>(Kazi &amp; Cooper, 2000)</td>
</tr>
<tr>
<td>CCA</td>
<td>earlywood and latewood from sapwood, whole sapwood, and heartwood from Douglas-fir, southern pine, and eastern larch</td>
<td>Cr(VI) concentration variation by diphenylcarbazide method</td>
<td>faster fixation rates in latewoods than earlywoods</td>
<td>no information on interaction between components</td>
<td>(Guo et al., 2002)</td>
</tr>
<tr>
<td>Chromic acid</td>
<td>cellulose</td>
<td>electron spectroscopy for chemical analysis (ESCA)</td>
<td>at least 75% Cr(VI) to Cr(III) reduction on all substrates</td>
<td>no interaction studies on lignin-chromium (limitations on extraction of lignin without degradation)</td>
<td>(Williams &amp; Feist, 1984)</td>
</tr>
<tr>
<td></td>
<td>wood (Sequoia sempervirens and Pinus sp.)</td>
<td></td>
<td>modified cellulose and wood exhibited greatly improved water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromic acid</td>
<td>guaiacol</td>
<td>reaction of chromium trioxide with guaiacol</td>
<td>amorphous chromium (III) complexes with guaiacol were highly insoluble, thermally stable, and UV protecting</td>
<td>use of simple guaiacol model compound</td>
<td></td>
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<td>------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Chromic acid</td>
<td>guaiacol</td>
<td>characterization of products by chromium analysis, FTIR, magnetic susceptibility</td>
<td>postulated that chromic acid oxidizes lignin phenols in wood affording related chromium (III) quinone complexes that confer weather stability to the treated wood surface</td>
<td>opposite of other studies which show cellulose is oxidized by chromic acid</td>
<td></td>
</tr>
<tr>
<td>Cr(III) solution</td>
<td>lignin isolated from black liquor (acidified to pH 2–3 with sulfur dioxide and precipitate collected)</td>
<td>influences of pH, lignin dosage, contact time, ionic strength, Cr(III) concentration and other metals were investigated</td>
<td>Cr(III) adsorption was strongly dependent on pH and adsorbent dosage, but independent of ionic strength and other metal ions</td>
<td>lignin extract used in this study is only 80 ± 2% lignin – other components could be participating in reaction</td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>wood (Eucalyptus regnans)</td>
<td>IR spectra comparison of different treated samples with copper solution</td>
<td>only a fraction of carboxyl groups react with copper</td>
<td>unclear as to which wood fraction is responsible for binding of Cu(II) ex. carboxylic acids or uronic acids of hemicellulose, or cellulose, lignin, or various extractives</td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>wood α-cellulose</td>
<td></td>
<td>other lignin component are responsible for sorption of copper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>cotton – purified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>E. regnans milled wood lignin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>E. regnans methanol lignin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>E. goniocalyx milled wood lignin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>P. radiata compression wood milled lignin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper acetate solution</td>
<td>P. radiata compression wood milled lignin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Cu(II)</td>
<td>• guaiacol</td>
<td>• analysis of complexes formed by diffuse reflectance spectroscopy</td>
<td>• hydroxy or methoxy groups of lignin are potentially suitable for chelation with Cu(II) since complexes with guaiacol</td>
<td>• use of model compounds which likely does not represent lignin in wood</td>
<td>• no interaction studies between wood fractions</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>• ACQ-D</td>
<td>• spruce (hardwood)</td>
<td>• effect of ACQ retention and wood species on percentage of copper stabilized and time to stabilization at conditioning temperatures of 22°C and 50°C monitored by X-ray fluorescence spectroscopy</td>
<td>• stabilization faster at higher temperatures</td>
<td>• no significant species effects were detected other than Douglas-fir in which copper reacted more quickly than in other species</td>
<td>• no information on individual wood fractions or interactions between ACQ components</td>
</tr>
<tr>
<td>• Fe (III)</td>
<td>• guaiacol</td>
<td>• complexes with guaiacol were isolated by HPLC then analyzed by 1H NMR and mass spectrometry</td>
<td>• reaction results in a complex mixture of coupled guaiacol oligomers (ex. symmetrical carbon-carbon coupled dimer 3,3′-dimethoxy-[1,1′-biphenyl]-4,4′diol and the trimer 3,3″,5″-trimethoxy-[1,1′:3′,1″-terphenyl]-4,4′,4″-triol)</td>
<td>• use of model compounds which likely does not represent lignin in wood</td>
<td>• no interaction studies between wood fractions</td>
</tr>
</tbody>
</table>
1.6 Analytical techniques

1.6.1 Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) is a quantitative chemical analysis technique in which thermodynamic parameters such as the equilibrium association constant ($K_a$) and enthalpy ($\Delta H$) can be determined experimentally. ITC is a titrimetric method in which a ligand is injected into a closed adiabatic sample cell containing a macromolecule and the heat of interaction between the two is monitored by comparison to a reference cell. The injection of ligand is repeated in known aliquots. After each injection, there is an exothermic or endothermic heat flow change in the sample cell. The feedback system then adjusts the heat flow to return sample and reference cell to the sample temperature, providing a quasi-isothermal process. Each subsequent injection causes a smaller change in heat flow in the system until an equilibrium state is reached. The entire process is performed under isobaric conditions (Grolier & del Río, 2012). A schematic of the system is shown below (Figure 5).

![Figure 5. Isothermal titration calorimetry system schematic.](image-url)
It should be noted that a background experiment, in which the ligand is injected into a buffer solution without the macromolecule, must be performed. The results of the background experiment will determine the heat of dilution, which must be subtracted from the ligand-macromolecule experiment to determine the heat of interaction, or enthalpy.

The enthalpy and association constant determined can be used to calculate the Gibbs free energy and entropy of the interaction using the following relationship:

\[ \Delta G = -RT \ln K_a = \Delta H - T\Delta S \]

where \( R \) is the gas constant and \( T \) is the absolute temperature. A strong binding affinity is represented by a large \( K_a \) and very negative \( \Delta G \).

ITC is advantageous since many thermodynamic parameters can be determined in a single binding experiment and, no tags or markers are required for monitoring interactions. Although this method is primarily used for solution-based systems, it is possible to use ITC for dispersions, two-phase systems, and others. Conversely, certain systems are difficult to evaluate using ITC. For example, kinetically slow systems or systems with very low enthalpies such as non-covalent complexes may be overlooked using this technique. It should be noted that ITC provides a global heat measurement and elucidating interactions in complex systems, with many binding sites or several macromolecules, may be difficult (Callies & Hernández Daranas, 2016; Dutta, Rösgen, & Rajarathnam, 2015).

1.6.2 Quartz crystal microbalance with dissipation monitoring

Sauerbrey first established the use of quartz crystal resonators for quantitative mass measurement in 1959. The piezoelectric properties of the thin quartz disc cause oscillation of the sensor when voltage is applied across the electrodes. The resonance frequency is dependent on the mass oscillating on the sensor. If a thin rigid film is adsorbed to the sensor, the change in resonance frequency (\( \Delta f_n \)) will be directly proportional to the change in mass, according to the Sauerbrey equation (Reviakine, Johannsmann, & Richter, 2011; Sauerbrey, 1959):
\[ \Delta f_n = -\frac{n}{C} m_f = -\frac{n}{C} \rho_f h_f \]

where \( m_f \) is the areal mass density of adsorbed film (mass per unit area), \( C \) is 17.7 ng/Hz for a 5 MHz quartz crystal, \( n \) is the overtone number (1,3,5,7, etc.), \( \rho_f \) is the density of the absorbed film, and \( h_f \) is the thickness of the adsorbed film.

It should be noted that in cases of solvated adsorbants, the areal mass density obtained is the sum of the adsorbate \( (m_{ads}) \) and the solvent \( (m_{solvent}) \):

\[ m_f = m_{ads} + m_{solvent} \]

The sensor can be coated with a variety of materials for interaction and binding studies. The Sauerbrey equation provides a good estimation of mass and film thickness for thin and rigid adsorbed film on the sensor. However, during many QCM measurements, water may add to the mass through entrapment, direct hydration, or viscous drag, which will create a thick soft layer that dampens the sensor’s oscillation, causing a dissipation effect. In this case, the Sauerbrey model will not apply (Höök et al., 2001).

Monitoring dissipation in addition to frequency changes can help in assessing the viscoelastic properties of the film. An increase in dissipation during the binding experiment is indicative of a viscoelastic film. The Kelvin-Voigt viscoelastic model can be used to describe the adsorbed film by the complex shear modulus \( (G) \) with \( G' \) being the storage modulus (material elasticity) and \( G'' \) being the loss modulus (viscous energy dissipation) (Reviakine et al., 2011; Voinova, Rodahl, Jonson, & Kasemo, 1999):

\[ G = G' + iG'' = \mu_f + i2\pi f n_f = \mu_f (1 + i2\pi f \tau_f) \]

where \( \mu_f \) is the elastic shear (storage) modulus, \( n_f \) is the shear viscosity (loss modulus), \( f \) is the oscillation frequency, and \( \tau_f \) is the characteristic relaxation time of the film.
Under no-slip conditions and a Newtonian bulk fluid, the changes in frequency $\Delta f$ and dissipation can be approximated by the following equations (Reviakine et al., 2011; Voinova et al., 1999):

$$\Delta f_n \approx -\frac{n}{C} m_f \left(1 - n\omega f \rho_1 \eta_1 \left(\frac{G''_f}{\rho_f (G'_{f}^2 + G''_{f}^2)}\right)\right)$$

where the subscripts 1 and $f$ refer to the liquid and film, respectively, $m_f = \rho_f h_f$ is the areal mass density of the film, $\omega$ is the angular frequency of deformation, and $\eta$ is the viscosity.

1.6.3 Time-of-flight secondary ion mass spectrometry

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) is a surface analysis technique that is commonly used to determine the chemical composition of solid materials. It is often applied in polymer, pharmaceutical, and semi-conductor industries (Mahoney, 2009; Scarazzini, 2017; Sodhi, 2004). In a vacuum chamber, a pulsed ion beam removes secondary ions from the sample surface. The secondary ions are accelerated towards a detector where the mass-to-charge ratios are recorded at the time of arrival (time-of-flight). The mass-to-charge ratios and time-of-flight are used to determine the chemical composition of the surface (Bertrand & Lu-Tao, 1996; Griffiths, 2008; Weickhardt, Moritz, & Grotemeyer, 1996). The spectral sensitivity of ToF-SIMS ranges from ppm to ppb, making it applicable to ions of low concentration (Goacher, Edwards, Yakunin, Mims, & Master, 2012).

In addition to chemical composition information, ToF-SIMS can be used to gather ion images, which display secondary ion intensities as a function of location on the sample surface. The ion images provide both elemental and chemical mapping on a sub-micron scale. Other advantages of ToF-SIMS include high mass resolution, meaning molecules with similar mass-to-charge ratios can be distinguished, and high sensitivity to trace elements (ppm to ppb levels). It should be noted that each pixel of an ion image contains a full mass spectrum, and a large amount of data analysis is often required for each sample (Belu, Graham, & Castner, 2003; Kiss, Jungmann, Smith, & Heeren, 2013; Sodhi, 2004).
1.6.4 Fourier transform infrared spectroscopy

Infrared spectroscopy techniques, sometimes known as vibrational spectroscopy, are commonly used to identify chemical compounds in solids, liquids, or gases. Infrared red light in the mid-IR region (4000-400 cm⁻¹) can be used to study fundamental vibrations and associated rotational-vibrational structure. A number of vibrational modes exist in the radial, latitudinal, and longitudinal planes of chemical bonds. Structures can be identified because functional groups give rise to bands of characteristic wavelength position and intensity (B. C. Smith, 2011; Stuart, 2015).

Fourier-transform infrared spectroscopy (FTIR) determines the absorption or emission of infrared light at a range of wavelengths in the mid-IR region. Different from other spectroscopy techniques, which shine a monochromatic light beam at the sample and determine the light absorbed at each wavelength, an FTIR beam contains many wavelengths simultaneously. The beam is then modified by a configuration of mirrors called an interferometer, to contain a different combination of wavelengths and the absorbance measured again. The process is repeated creating a collection of combinations from which an algorithm, called the Fourier transform, determines the absorbance at each wavelength (typical resolution of 4 cm⁻¹). The wavelengths at which absorbances occur match the vibrational frequency of structures in the compound (B. C. Smith, 2011; Stuart, 2015).

1.7 Statistical techniques – principal component analysis

Principal component analysis (PCA) is a method of dimensionality reduction which aims to reduces the number of variables while still containing the most relevant information of the original data set. Mathematically, PCA is an orthogonal linear transformation that convert the data set to a new coordinate system with the first coordinate (the first principal component) being a projection of the data with the largest variance. Similarly, the second principal component has the second greatest variance on its coordinate, and so on. The new variables, or principal components, are linear combinations of the original variables (Abdi & Williams, 2010; Bro & Smilde, 2014; Jolliffe & Cadima, 2016).

For a given data matrix, \( X \), that is mean-centered with \( n \) rows representing different experiment runs and \( p \) columns describing a particular feature, the linear transformation can be written as:
\[ t_{k(i)} = x_{(i)} \cdot w_{(k)} \] for \( i = 1, ..., n \) and \( k = 1, ..., l \)

where \( t_{k(i)} \) is a new vector of principal component scores, \( x_{(i)} \) is each row vector of \( X \) and \( w_{(k)} \) is the set of \( p \)-dimensional unit vectors of weights. The maximum possible variance of \( X \) is contained in \( t_1 \) followed by \( t_2 \), and so on. Generally, \( l \) is chosen to be less than \( p \) to reduce the dimensionality of \( X \) (Bro & Smilde, 2014).

To determine the first principal component, the variance must be maximized, and the following must be satisfied:

\[
\begin{align*}
w_{(1)} &= \arg \max_{||w||=1} \left\{ \sum_i (t_1)_{(i)}^2 \right\} = \arg \max_{||w||=1} \left\{ \sum_i (x_{(i)} \cdot w)^2 \right\} \\
&= \arg \max_{||w||=1} \left\{ \frac{w^T X^T X w}{w^T w} \right\}
\end{align*}
\]

Equivalently in matrix form and expressed as the Rayleigh quotient:

\[
\begin{align*}
\begin{align*}
&= \arg \max_{||w||=1} \left\{ \frac{w^T X^T X w}{w^T w} \right\}
\end{align*}
\end{align*}
\]

The maximum value of the quotient \( \frac{w^T X^T X w}{w^T w} \) is the largest eigenvalue, representing the largest variance, of the matrix occurs when \( w \) is the corresponding eigenvector, representing the first principal component (Bro & Smilde, 2014).

The remaining \( k \) principal components can be found by subtracting the first principal component \( (k - 1) \) from the data matrix \( X \):

\[
\begin{align*}
\bar{X}_k &= X - \sum_{s=1}^{k-1} X w_{(s)} w_{(s)}^T \\
with the weight vector represented by:
\end{align*}
\]

\[
\begin{align*}
w_{(k)} &= \arg \max_{||w||=1} \left\{ \|\bar{X}_k w\|^2 \right\} = \arg \max_{||w||=1} \left\{ \frac{w^T \bar{X}_k^T \bar{X}_k w}{w^T w} \right\}
\end{align*}
\]

The remaining eigenvectors of \( X^T X \) are found with the above expression which represents the principal components with the Rayleigh quotient representing the corresponding eigenvalues (or variance) (Bro & Smilde, 2014). The final truncated transformation of the matrix with \( L \) principal components is represented by:

\[
T_L = X W_L
\]
It should be noted that there is another method used to determine principal components called the covariance method. The mean-centered covariance matrix of $X$ is used to determine the eigenvalues (variance) between the data variables and Gaussian Elimination used to determine the respective eigenvectors. The percent variance due to each principal component can be determined by the dividing the respective eigenvalue by the sum of the eigenvalues. The final converted matrix is achieved by the same equation as above (Jolliffe & Cadima, 2016). This method is not typically used for large data sets because of the higher computational power requirements.
Chapter 2 – Motivation and objectives

2.1 Motivation

Fundamental understanding of interactions between wood fractions (lignin, hemicellulose, and lignin) and preservative components (metal ions) can help drive targeted formulation development. In recent years, the wood preservation industry has shifted away from traditional CCA-type preservatives due to environmental and health concerns with arsenic leaching (Coles, Arisi, Organ, & Veinott, 2014; Hasan et al., 2010; Hingston, Collins, Murphy, & Lester, 2001). Our industrial research collaborator is focused on the development of micronized copper preservatives with the addition of iron oxide as a pigment. Understanding the interaction of iron and copper with wood can help direct industrial research and development efforts.

Several strategies for preservation exist. For example, preservative compounds can interact with lignin minimizing its photooxidation, which initiates a series of reactions leading to UV-degradation of the wood (Pandey & Khali, 1998; Rowell, 2012). Alternatively, the preservative component can act as a fungicide, inhibiting enzyme activity and preventing rot of wood (Humphrey, 2002; Lupsea et al., 2013). In both cases, the interaction and distribution within the wood is important (Matsunaga et al., 2009).

As mentioned in the previous section, there are many limitations to current studies attempting to elucidate the interaction between wood fractions and preservative components. Primarily, many studies use model compounds such as guaiacol in place of lignin and D(+)-glucose in place of cellulose (Pizzi, 1983; Schmalzl et al., 1995, 2003). The results of studies have contradicting conclusions. For example, the two leading models for CCA-type preservatives, the Dallgren-Hartford model and the Pizzi model, present conflicting theories as to the role of chromium (III) in binding (Dahlgren & Hartford, 1972; Pizzi, 1983). In some studies on copper-based preservatives, copper is thought to interact with only lignin; whereas others indicate that hemicellulose or cellulose binds copper (II) (Bland, 1963; Michie, 1961).

The localization and dispersion of preservative components in the wood can greatly influence degradation prevention. The depth of penetration of preservatives is commonly determined in the industry but, this does not provide accurate information on dispersion on the cellular level (Ibach, 1999).
2.2 Research objectives and proposed approach

The objective of this study is to systematically investigate the interaction of wood preservative components with specific wood fiber fractions using surface analysis and calorimetric techniques. The analytical methods developed, can be used as a resource for future studies of wood coatings. Since studies on the mechanism of preservation have mostly been performed using CCA-type preservatives, CCA preservatives will be included in this study as a validation tool when developing novel analytical techniques.

Isothermal titration calorimetry (ITC) is commonly used to study protein-ligand interactions as well as other chemical reactions, however, it has not been previously used to study preservative interactions with wood or wood fractions. Through the measurement of heat flow, we can make suppositions as to the fraction of wood (cellulose, hemicellulose, or lignin) that interacts with metal ions of preservatives (copper, chromium, iron, arsenic). For example, if the injection of a copper ion solution into a sample cell containing lignin causes a change in heat flow in the system, we can assume that copper interacts with lignin.

To complement the isothermal titration calorimetry studies, a quartz crystal microbalance with dissipation monitoring (QCMD) method will be developed. QCMD has previously been used in the ERM lab group to study enzyme activity on lignocellulosic surfaces (Vilbik, 2016). This study will use techniques known to the group to coat sensors with wood fractions, such as lignin, then, determine if metal ions found in preservative formulations binding to the coated surface. It will be assumed that if the metal ion binds to the wood fraction, it must also bind to the wood fraction in the wood matrix during preservative application processes.

The ITC and QCMD studies above will provide insight into the interactions of wood fractions with metal ions found in preservative formulations; however, lignin, hemicellulose, and cellulose are contained in a complex structure in the wood matrix. To confirm the conclusions made from the ITC and QCMD studies using isolated wood fractions, it is beneficial to study preservative compounds in complete wood samples. Time-of-Flight Secondary Ion Mass Spectroscopy (ToF-SIMS) has previously been used to monitor wood decay and enzyme activity on wood (Goacher et al., 2012; Mahajan, Jeremic, Goacher, & Master, 2012), however, it has not been be used to study metal ions from preservatives in a cross-section of treated wood. Since ToF-SIMS can provide ion images with high mass resolution spectra and is sensitive enough to detect
metal ion at concentrations at the ppb/ppm level, the distribution of metal ions in the wood can be visualized using this analytical technique. Cell walls of wood have higher cellulose content whereas as the region between the cells, known as the middle lamella, has higher relative lignin content. By studying the localization of metal ions, the validity of the QCMD and ITC work can be determined.

Finally, the purpose of treating wood with preservatives is to reduce the photodegradation effect. Upon exposure to UV, chemical changes to the structure of wood occur which should be minimized by treating with a preservative. Fourier transform infrared spectroscopy (FTIR) can be used to study changes to chemical structure in materials. In this study, FTIR will be used to determine any structural changes in wood due to treatment with preservatives as well as changes upon exposure to UV light. Since FTIR spectrums can be difficult to interpret, principal component analysis will be used to help elucidate differences in wood samples.
Chapter 3 – Materials and methods

3.1 Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) is used to determine thermodynamic parameters of a reaction. This study focused to developing a method to understanding the heat flow due to the interaction of preservative components with wood fractions.

3.1.1 Experimental set-up

The ITC instrument contains two identical cells, the sample cell and reference cell. The sample cell is equipped with a gold agitator and a syringe for injecting the ligand solution. The syringe is attached to a pump, which is calibrated to inject an accurate volume of ligand into the sample cell. A feedback system between the reference cell and sample cell controls the heater which ensures that the two cells are kept at a similar temperature. The power and related heat flow required to maintain a quasi-isothermal system, are recorded by the software.

![Isothermal titration calorimetry system schematic.](image)

Figure 6. Isothermal titration calorimetry system schematic.
3.1.2 Wood samples and fractions

Ball-milled spruce sapwood wood samples and organosolv softwood lignin were used for method development in ITC studies.

3.1.3 General method

The two sample cells were 1.344 mL in volume. The system was set to maintain a temperature of 25 °C for the cells. The agitation speed in the sample cell was set to 50 rev/min. The sample cell was filled with 2 mg of wood powder and 800 µl of MilliQ water. After the system has reached a steady-state baseline, a diluted iron standard solution (10 µg/mL Fe) was added by injecting 10 µL of solution through the syringe every 5 minutes. The injection process was repeated 20 times to achieve a final volume of 200 µl of iron solution added to the sample cell. After each injection, a change in heat flow is observed and the feedback system returns the temperature of the cell to the baseline state.

The above procedure was repeated without the addition of the wood as a background experiment. The heat evolved during the background experiment represents the heat of dilution of the iron oxide solution in water.

3.1.4 Method development

To determine the appropriate experimental conditions, the type of wood sample, preservative type or component, agitation speed, injection interval and temperature of the experiment was altered. A summary of experimental conditions tested is provided below:

Table 4. Summary of experimental conditions used in isothermal titration calorimetry method development.

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Wood fraction in solvent</th>
<th>Preservative/Metal ion solution injected</th>
<th>Agitation speed (rev/min)</th>
<th>Injection interval</th>
<th>Injection amount</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Inorganic Ventures iron standard solution in HNO₃ matrix (10 µg/mL Fe)</td>
<td>50</td>
<td>5 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td></td>
<td>2 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Micro Shades Natural Brown® commercial preservative (Yellow iron oxide, Red iron oxide) estimated iron concentration by ICP = 350 µg/mL Fe</td>
<td>50</td>
<td>5 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>2 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Inorganic Ventures copper standard solution in HNO₃ matrix (10 µg/mL Cu)</td>
<td>50</td>
<td>5 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td>4</td>
<td>2 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Micro Shades Natural Brown® commercial preservative (Yellow iron oxide, Red iron oxide) estimated iron concentration by ICP = 350 µg/mL Fe</td>
<td>80</td>
<td>10 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td>5</td>
<td>2 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Micro Shades Natural Brown® commercial preservative (Yellow iron oxide, Red iron oxide) estimated iron concentration by ICP = 350 µg/mL Fe</td>
<td>100</td>
<td>10 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td>6</td>
<td>800 µL Milli-Q water</td>
<td>200 µL Milli-Q water</td>
<td>80</td>
<td>10 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td>7</td>
<td>800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Micro Shades Natural Brown® commercial preservative (Yellow iron oxide, Red iron oxide) estimated iron concentration by ICP = 350 µg/mL Fe</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td>8</td>
<td>800 µL 0.5% organosolv softwood (OSW) lignin in 1,4-dioxane</td>
<td>200 µL 100x water-diluted Inorganic Ventures copper standard solution in HNO₃ matrix (10 µg/mL Cu)</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
<td>25.000</td>
</tr>
<tr>
<td>No</td>
<td>Comment</td>
<td>Micro-Pro</td>
<td>Water</td>
<td>Min</td>
<td>µL</td>
<td>µg/mL</td>
</tr>
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<td>----</td>
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</tr>
<tr>
<td>9</td>
<td>800 µL 0.5% organosolv softwood (OSW) lignin in 1,4-dioxane</td>
<td>200 µL 100X water-diluted MicroPro 200C commercial preservative (copper carbonate) estimated copper concentration by ICP = 3800 µg/mL</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td>10</td>
<td>800 µL 0.5% organosolv softwood (OSW) lignin in 1,4-dioxane</td>
<td>200 µL 100X water-diluted Wolman E (CA-C) commercial preservative (copper ethanalamine complex, tebuconazole) estimated copper concentration by ICP = 1250 µg/mL</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td>11</td>
<td>800 µL 0.5% organosolv softwood (OSW) lignin in 1,4-dioxane</td>
<td>200 µL 100X water-diluted NW100® commercial preservative (mixed copper ethanalamine complexes) estimated copper concentration by ICP = 850 µg/mL</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td>12</td>
<td>800 µL 1,4-dioxane</td>
<td>200 µL 100x water-diluted Inorganic Ventures copper standard solution in HNO₃ matrix (10 µg/mL Cu)</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td>13</td>
<td>2 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Inorganic Ventures copper standard solution in HNO₃ matrix (10 µg/mL Cu)</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td>14</td>
<td>2 mg OSW lignin in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Inorganic Ventures copper standard solution in HNO₃ matrix (10 µg/mL Cu)</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td>15</td>
<td>2 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Inorganic Ventures copper standard solution in HNO₃ matrix (10 µg/mL Cu)</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td>16</td>
<td>800 µL Milli-Q water</td>
<td>200 µL 100x water-diluted Inorganic Ventures copper standard solution in</td>
<td>80</td>
<td>30</td>
<td>20x10</td>
<td>25.000</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Condition</td>
<td>Time</td>
<td>Volume</td>
<td>Concentration</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------------------------</td>
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<tr>
<td>17</td>
<td>5 mg OSW lignin in 800 µL Milli-Q water</td>
<td>200 µL undiluted Inorganic Ventures copper standard solution in HNO₃ matrix (1000 µg/mL Cu)</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
<td></td>
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<tr>
<td>18</td>
<td>800 µL Milli-Q water</td>
<td>200 µL undiluted Inorganic Ventures copper standard solution in HNO₃ matrix (1000 µg/mL Cu)</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
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<tr>
<td>19</td>
<td>10 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL undiluted Inorganic Ventures copper standard solution in HNO₃ matrix (1000 µg/mL Cu)</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>10 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL undiluted Inorganic Ventures copper standard solution in HNO₃ matrix (1000 µg/mL Cu)</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>10 mg ball-milled white spruce sapwood in 800 µL Milli-Q water</td>
<td>200 µL 100X water-200 µL 100X water-diluted NW100® commercial preservative (mixed copper ethanolamine complexes) estimated copper concentration by ICP = 850 µg/mL</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>800 µL Milli-Q water</td>
<td>200 µL 100X water-diluted NW100® commercial preservative (mixed copper ethanolamine complexes) estimated copper concentration by ICP = 850 µg/mL</td>
<td>80</td>
<td>30 min</td>
<td>20x10 µL</td>
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</table>
3.2 Quartz crystal microbalance with dissipation monitoring

3.2.1 Sensor preparation

Biolin Scientific SiO$_2$ sensors (AT cut, 14 mm diameter, 0.3 mm thickness, frequency 4.95 MHz ± 50 kHz) were cleaned in an ozone UV cleaner for 10 minutes, then spin-coated with 30 µL of organosolv softwood lignin (0.5 % in 1,4-dioxane). The sensors were cured in an oven at 90 ºC for 24 hours to ensure tight binding of lignin to SiO$_2$. Similarly, clean SiO$_2$ sensors were spin-coated with 2x30 µL of beechwood xylan (2 % in water; purchased from Sigma, USA) and cured for 24 hours at 90 ºC. Sensors were allowed to cool to room temperature before placing in the Biolin Scientific QSense Analyzer.

3.2.2 Binding experiment

Two coated (either xylan or lignin) and two uncoated SiO$_2$ sensors were placed in the running chambers of the Biolin Scientific QSense Analyzer (Experiments were performed in duplicates for both uncoated and coated sensors). The changes in frequency and dissipation were monitored at $n = 3, 5, 7, 9, \text{ and } 11$ overtones. A baseline measurement was established by flowing Milli-Q water over the sensors until the change in frequency over 30 minutes was less than 1 Hz. Once a steady baseline was reached, the Milli-Q water was replaced with a metal ion solution to observe the interaction with lignin or xylan. Each of the standard metal ion solutions tested (Cu (II), Fe (III), As, and Cr (VI)) were obtained from Inorganic Ventures and diluted 100X with Milli-Q water before running QCM-D experiment. In addition to the single metal ions tested, commercial CCA preservative concentrate was diluted 100X and the binding assessed for xylan and lignin. Once the frequency change had stabilized, the metal ion (or commercial) solution was replaced with Milli-Q water to wash the sensors and assess the reversibility of the binding.

3.3 UV exposure

Spruce wood planks (1 in x 4 in x 12 in) treated with ACQ-type preservative from Timber Specialties were split lengthwise with a band saw to create planks of dimensions 0.5 in x 4 in x 12 in. The visual depth of penetration of the ACQ-type preservative is only a few millimeters deep; therefore, the inner portion of the split wood was used as a control (untreated) sample.
The wood was placed against a UV-exposure treatment chamber (UVA-340 nm) at a distance of 5 cm from the bulbs. Two split wood planks were placed treatment side towards the UV lamps and two planks were places un-treated side towards the lamps. The samples remained in the chamber for 2000 hours to determined effect of UV exposure (simulated weathering) on sample composition and wood structure. Samples not exposed to UV were kept as controls.

![Figure 7. Treated and untreated wood samples exposed to UVA-340 nm light.](image)

### 3.4 Time-of-flight secondary ion mass spectrometry

#### 3.5.1 Sample preparation

The treated surface of ACQ-treated wood planks provided by Timber Specialties was cut into slivers of approximately 2 mm x 2mm x 0.75 cm using a razor blade. The slivers were then embedded epoxy prior to cutting with microtome. Buehler EpoThin epoxy (100 parts resin to 36 parts hardener) was poured over wood slivers in silicone molds of approximately 0.5 cm x 0.5 cm x 1 cm dimensions. The samples were left to cure for 24 hours at room temperature, as per manufacturer’s instructions.

The embedded samples were cut using a Leica EM UC6 Ultra-microtome (Leica Microsystems GmbH, Vienna, Austria) with a glass knife to expose a smooth cross-sectional surface of the treated wood, suitable for ToF-SIMS analysis. A final cut was made using a diamond knife prior to analysis by ToF-SIMS.
3.5.2 Acquisition

ToF-SIMS acquisition was performed using ToF-SIMS IV instrument (ION-TOF GmbH, Münster, Germany) with a bismuth liquid metal ion source and reflectron-type analyzer. Positive ion spectra were acquired using 50 keV Bi₃²⁺ primary ions with an incidence of 45°. A delayed extraction approach was used to improve mass resolution. The extraction voltage was set to 2 kV, the rise time to 40 ns and the delay to +10 μs. The pressure during analysis was maintained between 1×10⁻⁸ and 1×10⁻⁷ mbar. The spectra were gathered over a 100 μm x 100 μm area in a raster pattern with 200 scans per ion image pixel. The spectra were calibrated to CH₃⁺, C₂H₅⁺ and C₃H₅⁺ ions using SurfaceLab 6.7 software (IONTOF GmbH, Münster, Germany).

3.5 Fourier-transform infrared spectroscopy

3.4.1 Sample preparation

The wood samples from section 3.3 above can be summarized as the following:

Table 5. Summary of wood samples generated in from section 3.3 used in FTIR studies.

<table>
<thead>
<tr>
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<th>ACQ-treatment</th>
<th>UV-exposure</th>
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</thead>
<tbody>
<tr>
<td>Treated+UV (TU)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Untreated+UV (NU)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Untreated (N)</td>
<td>No</td>
<td>No</td>
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The top millimeter of the samples above was shaved off then passed through a Wiley mill with a 40 mesh screen to achieve powder samples appropriate for FTIR analysis.

3.4.2 Spectrum acquisition

A Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory was used to obtain a spectrum for powdered wood samples described in Table 5. The spectrum was obtained by performing 32 scans for wavenumbers 4000-600 cm⁻¹ at a resolution of 4 cm⁻¹. A total of 10 replicates per sample type were performed.
Chapter 4 – Isothermal titration calorimetry

The objective of this study was to gain understanding into the interaction between wood or wood fractions and preservative formulations along with relevant metal ion solutions (iron, copper, chromium, and arsenic) by measuring heat flow during a titration calorimetry experiment. From a comprehensive literature review, it does not appear that ITC has been previously used to study the interaction of wood fractions with metals ions or preservative formulations. A typical plot from an ITC experiment is shown below (Figure 8). The panel on the left shows typical raw data and the right shows the binding isotherms created by plotting the integrated heat peaks against the molar ratio of the ligand to macromolecule. The graph on the right can then be used to calculate kinetic parameters such as enthalpy and the association constant.

Figure 8. Sample expected data for an isothermal titration calorimetry experiment. The panel on the left shows the raw data and the right shows the binding isotherms created by plotting the integrated heat peaks against the molar ratio of the ligand to macromolecule. The graph on the right can then be used to calculate kinetic parameters such as enthalpy and the association constant.

4.1 Instrument calibration

4.1.1 Control studies

To ensure the instrumentation was operating as expected, water was first injected into a sample cell containing only water, as per manufacturer’s recommendations. Each injection caused a heat flow peak of similar magnitude and the baseline was quickly regained, as expected (Table 4 - Experiment 6). The heat flow is due to differences in temperature between the syringe
temperature and cell temperature. The heat flow is very minimal since there is no dilution or reaction occurring.

Then, a diluted commercial iron-based preservative was injected into the sample cell containing only water (Table 4 - Experiment 7). Similarly, the peaks exhibited were sharp and the baseline regained after each injection. The heat flow in this case can be attributed to slight differences in temperature as well as heat of dilution of the preservative in water. Since both experiments yielded typical expected results, it was concluded that the instrument is operating correctly.

4.1.2 Agitation rate

The agitation rate was adjusted so that the wood particles would remain dispersed throughout the experiment and not stick to the bottom (Table 4 – Experiments 3, 4, 5). The agitation rate cannot be too high, otherwise bubbles may be introduced into the system, causing uncharacteristic fluctuations in the heat flow measurements due to differences in heat transfer properties. An agitation rate of 80 rev/min was determined to be suitable. A comparison between 100 rev/min, which was too vigorous, and 80 rev/min is shown below (Figure 9).

Figure 9. Raw data from isothermal titration calorimetry (ITC) agitation rate study monitoring the heat flow over time. Two milligrams of ball-milled white spruce sapwood were added to the sample cell containing 800 µL of Milli-Q water. The temperature of the cell was set to 25.000 °C and 10 µL of 100x diluted Micro Shades Natural Brown® solution was injected into the sample cell every 10 min. A total of 20 injections were made. The agitation rate of the impeller was set to 100 rev/min in (a) and 80 rev/min in (b), Table 4 – Experiment 4 and 5, respectively.
From the data in Figure 9b, a few immediate conclusions can be made. Firstly, the baseline is not regained after each injection, meaning the injection interval of 10 minutes is likely too short. In other words, the reaction rate is slow and equilibrium cannot be reached in the given amount of time before the next injection. The injection interval was increased to 30 min (Table 4 – Experiment 7 to 22) which alleviated this concern. Secondly, there appears to be multiple or split peaks which is also characteristic of slow reaction kinetics. Alternatively, it can also be an indication of multiple reactions. Understanding that wood is a complicated matrix with different types of polymers and small molecules, it is very likely that there are multiple reactions occurring at the same time. Finally, the expected heat flow changes are in the high µW or low mW range, much higher than what is observed above. The next section will focus on using simpler systems to improve the ITC results.

4.2 Optimization studies

4.2.1 Lignin in 1,4-dioxane

To reduce the number of reactions in the system, a simpler system with a organosolv softwood (OSW) lignin solution (2 wt% in 1,4-dioxane) in the sample cell with a diluted copper standard solution injected by the syringe, was tested (Table 4 – Experiment 8). The injection interval time was set to 30 min to ensure there was enough time between injections for the baseline to regain and system to reach equilibrium. The raw data for the experiment is provided in Figure 10a with the respective heat of dilution experiment (Table 4 – Experiment 12) in Figure 10b.
Figure 10. Raw data from isothermal titration calorimetry (ITC) experiment on organosolv softwood (OSW) lignin in dioxane with copper standard solution monitoring heat flow over time. (a) 800 µL 0.5% organosolv softwood (OSW) lignin in 1,4-dioxane was added to the sample cell. The temperature of the cell was set to 25.000 ºC and 10 µL of 100x diluted Inorganic Ventures copper (II) standard solution was injected into the sample cell every 30 min. A total of 20 injections were made. The agitation rate of the impeller was set to 80 rev/min (Table 4 – Experiment 8). (b) Heat of dilution experiment was performed by injecting 10 µL of 100x diluted Inorganic Ventures copper (II) standard solution was injected into the sample cell every 30 min for a total of 20 injections into a sample cell containing 800 µL 1,4-dioxane. Temperature 25.000 ºC, agitation rate 80 rev/min (Table 4 – Experiment 12).

The peaks in Figure 10 are very sharp and the baseline is clearly regained after each injection. There are no multiple/split peaks after each injection and the magnitude of the heat flow is as expected, in the low mW range. The magnitude of the peaks after the third injection are similar, this means the equilibrium of the reaction has likely been reached at this point. Similar binding profiles were obtained when testing commercial copper-based preservatives solutions (Table 4 – Experiment 9, 10, 11).

The effect of the heat of dilution (Figure 10b) on the total heat flow after each injection must be removed from the results in Figure 10a to determine the heat due to interaction between lignin and the copper (II) ion solution. In best practices, the heat of dilution should be low in comparison with experimental binding heat flow measurements to ensure the correction of the binding heat flow is accurate (Lewis & Murphy, 2005). In this case, the heat of dilution is quite large. In addition, the peaks are initially negative, then flip to positive heat flow indicating there is likely a reaction occurring between the copper ions and the 1,4-dioxane. These reactions are interfering with potential reactions between the lignin and copper. Due to the interference, it is recommended that 1,4-dioxane is not used as a solvent.
4.2.2 Lignin in water

To avoid issues with 1,4-dioxane interfering with binding experiment, organosolv softwood (OSW) lignin (2mg) was dispersed in water and diluted copper standard was injected into the cell (Table 4 – Experiment 14). The heat flow was very low and a typical profile, in which the magnitude of the peaks decreases after each injection until an equilibrium is reached, was not observed. It was hypothesized that lignin may not react with copper, and therefore, the experiment was repeated with wood (2 mg) in water (Table 4 – Experiment 15). A similar spectrum was observed. Since copper should react with some fraction of wood and we did not observe a change in heat flow in this experiment, it was hypothesized that the concentration must be too low.

Lignin sample concentration of 2 up to 5 mg dispersed in water with undiluted copper standard solution yielded similar results (Table 4 – Experiment 17). Wood at concentrations up to 10 mg in 800 ul of water was tested with undiluted copper standard solution (Table 4 – Experiment 19), and yielded the raw data in Figure 11a with the respective heat of dilution experiment in Figure 11b (Table 4 – Experiment 18).

Figure 11. Raw data from isothermal titration calorimetry (ITC) experiment on organosolv softwood (OSW) lignin in water with copper standard solution monitoring heat flow over time. (a) 10 mg organosolv softwood (OSW) lignin in Milli-Q water was added to the sample cell. The temperature of the cell was set to 25.000 ºC and 10 µL of undiluted Inorganic Ventures copper (II) standard solution was injected into the sample cell every 30 min. A total of 20 injections were made. The agitation rate of the impeller was set to 80 rev/min (Table 4 – Experiment 19). (b) Heat of dilution experiment was performed by injecting 10 µL of undiluted Inorganic Ventures copper (II) standard solution into the sample cell containing 800 µL Milli-Q water every 30 min for a total of 20 injections. Temperature 25.000 ºC, agitation rate 80 rev/min (Table 4 – Experiment 19).
The heat of dilution (Figure 11b) is providing the bulk of the heat flow in the binding experiment (Figure 11a). The selected conditions are not suitable for studying the binding of wood to copper (II) ions through titration calorimetry. It should be noted that many wood treatment processes are performed at higher temperature; therefore, it would be beneficial to determine the effect of temperature of the reaction (MacLean, 1952; Williams, 2005). Unfortunately, the ITC system is not equipped with a heater for the injection syringe. Attempts to perform binding experiments at higher temperatures (40 °C and 50 °C), were unsuccessful leading to uncharacteristic heat flow profiles (Table 4 – Experiment 20, 21, 22).

4.3 Conclusions

Binding experiments between wood or OSW lignin samples and preservative or metal ion preservatives were performed to determine if ITC techniques could be used to better understand chemical interactions. Throughout the method development experimentation, the heat of dilution accounted for a large portion of the binding heat flow signal, indicating the heat flow due to binding kinetics was too low to accurately determine interactions between the tested fractions and metal ions.

There are many limitations in our study. Firstly, it is possible that the concentration of wood is too low to achieve adequate heat flow for analysis. There is a balance between the concentration of wood and the injection interval. Essentially, the more the concentration of wood and/or preservative is increased, the longer it will take for equilibrium to be reached between each injection. If the concentration is increased to improve the heat flow, the interval between injections must also be increased. It is known that the binding processes of wood can take over 24 hours with many slow reactions (Williams, 2005).

Concrete conclusions about the interaction of lignin and copper could not be made during isothermal titration calorimetry experiments. An alternative method, discussed in the next Chapter, was developed to elucidate the interaction between lignin and preservative metal ions using a Quartz Crystal Microbalance with Dissipation Monitoring (QCMD). QCMD allows for longer reactions times between wood fractions and metal ion solutions, a limitation of the ITC method.
Chapter 5 – Quartz crystal microbalance with dissipation monitoring

5.1 Binding frequency and dissipation profiles

The interaction of lignin and xylan with preservative components (Cu (II), Fe (III), As, and Cr(VI)) as well as a commercial copper chromated arsenic (CCA) preservative was studied by monitoring the change in resonance frequency and dissipation with quartz crystal microbalance. A sample of the raw data in which arsenic was binding with xylan is shown in Figure 12.

Figure 12. Xylan-coated SiO₂ QCM-D sensors were exposed to 100X diluted Inorganic Ventures Arsenic standard solution (pH 6) as indicated by the braces. After approximately 21 hrs, the sensors were washed with Milli-Q water to assess the reversibility of binding. The changes in frequency (a) and dissipation (b) were measured at n = 3, 5, 7, 9, and 11 overtones over time indicated in the legend. The black line represents the response of the uncoated sensor in which frequency and dissipation values for all overtones overlapped. Experiments were performed in duplicates for both uncoated and coated sensors.

The decrease in resonance frequency observed during exposure to arsenic solution in Figure 12a was indicative of an increase in mass on the oscillating sensor. Since the mass on the sensor increased, we can deduce that arsenic interacted and adhered to the xylan-coated surface. When the sensors were washed with water, an increase in the frequency was observed, indicating that some of the arsenic on the xylan surface was removed. Since the frequency did not return to the baseline value, the binding of arsenic to xylan was not fully reversible. Uncoated sensors (shown by the black line in Figure 12) did not exhibit these changes in frequency, indicating that
the binding was indeed due to interactions with the xylan coating and not interactions between SiO$_2$ and the metal ion.

The dissipation was monitored over the course of the binding and washing experiment (Figure 12b). An increase in the dissipation was observed when the xylan-coated sensors were exposed to the arsenic solution. During the water washing step, the dissipation decreased as was expected if some of the arsenic was removed from the surface.

A sample of the raw data in which iron was binding with lignin is shown in Figure 13 below. Similarly to the arsenic example (Figure 12), a decrease in frequency was observed upon exposing the lignin-coated sensors to an iron solution, indicating that the iron (III) solution was adsorbed onto the surface. Minimal changes in frequency were observed when the sensors were washed with water indicating that the interaction between iron (III) and the lignin was not reversible. Uncoated sensors (shown by the black line in Figure 13) did not exhibit large shifts in frequency compared to lignin-coated sensors, therefore, the adsorbance of the iron solution was likely due to interactions with the lignin coating and not interactions between SiO$_2$ and the iron. The dissipation was also monitored, however, contrary to the arsenic-xylan example, minimal changes in dissipation occurred throughout the experiment.

Figure 13. Lignin-coated SiO$_2$ QCM-D sensors were exposed to 100X diluted Inorganic Ventures Iron standard solution (pH 6) as indicated by the braces. After approximately 2 hrs, the sensors were washed with Milli-Q water to assess the reversibility of binding. The changes in frequency (a) and dissipation (b) were measured at $n = 3, 5, 7, 9,$ and $11$ overtones over time indicated in the legend. The black line represents the response of the uncoated sensor in which frequency and dissipation values for all overtones overlapped. Experiments were performed in duplicates for both uncoated and coated sensors.
5.2 Modelling of mass and layer thickness

Monitoring of the dissipation was essential in determining the applicability of the Sauerbrey model, which describes a linear relationship between the change in resonance frequency and change in mass. The Sauerbrey model is only applicable for thin, rigid, and evenly distributed films. It should only be used when there are no significant dissipation shifts and the frequency shifts do not spread between overtones. Since Figure 12 shows an increase in dissipation during exposure to arsenic; thus the Sauerbrey model does not apply. Biolin Scientific’s QSense DFind software was used to confirm that the Sauerbrey model poorly fit the data ($R^2 = 0.0$). In the case of the iron-lignin experiment (Figure 13), minimal changes in the dissipation were observed during the experiment, however, the large amount of noise could indicate uneven distribution of iron on the sensor. Using the Biolin Scientific’s QSense DFind software, the Sauerbrey model was applied to the data which yielded an $R^2$ of 0.15 indicating that the Sauerbrey model was not a good fit in this case.

The data in Figure 12 shows an increase in dissipation as the adhering layer was formed meaning a viscoelastic model would better describe the data. Biolin Scientific’s QSense DFind software was used to model the binding of arsenic to xylan using the Kelvin-Voigt model. The model was used to estimate the mass adsorbed to the surface and the thickness of the layer created during the binding of arsenic to xylan and the subsequent washing step as shown in Figure 13 ($R^2=0.73$).
Figure 14. Kelvin-Voigt viscoelastic modelling of layer thickness and mass changes over time. Xylan-coated SiO$_2$ QCM-D sensors were exposed to 100X diluted Inorganic Ventures Arsenic standard solution (pH 6) as indicated by the braces. After approximately 21 hrs, the sensors were washed with Milli-Q water to assess the reversibility of binding. Modelling was fit with Biolin Scientific’s QSense DFind software ($R^2 = 0.73$) assuming that the density of the adhering layer was equivalent to the density of arsenic (1970 g/L) and the bulk fluid density is 1000 g/L.

The Kelvin-Voigt model was also applied to the iron-lignin data from Figure 13. The mass adsorbed to the surface and the thickness of the layer created during the binding and subsequent washing step was estimated ($R^2 = 0.87$). The results are shown in Figure 15.

Figure 15. Kelvin-Voigt viscoelastic modelling of layer thickness and mass changes over time. Lignin-coated SiO$_2$ QCM-D sensors were exposed to 100X diluted Inorganic Ventures Iron standard solution (pH 6) as indicated by the braces. After approximately 2 hrs, the sensors were washed with Milli-Q water to assess the reversibility of binding. Modelling was fit with Biolin Scientific’s QSense DFind software ($R^2 = 0.87$) assuming that the density of the adhering layer was equivalent to the density of iron (7874 g/L) and the bulk fluid density is 1000 g/L.
Similarly to the two examples described above, the adsorption of xylan or lignin by other metal ion solutions and the commercial CCA solution was explored by QCM-D. In all cases, the Sauerbrey model was a poor fit to the data and the Kelvin-Voigt viscoelastic model was used to estimate the mass adsorbed and the layer thickness. The results are summarized in Figure 16 below.

![Figure 16](image_url)

**Figure 16.** Thickness (a) and mass of the adhering layer (b) of preservative (CCA) or preservative component (Cu (II), Fe (III), As, and Cr(VI)) on xylan- or lignin-coated SiO$_2$ QCM-D sensors (pH 6 for all solutions). The Kelvin-Voigt viscoelastic model was used to estimate the layer thickness after binding and after washing the sensors with water. Modelling was performed with Biolin Scientific’s QSense DFind software assuming that the density of the adhering layer was equivalent to the density of the metal ion (ie. As = 7874 g/L, Cu = 8960 g/L, Fe = 7874 g/L, Cr = 7140 g/L, CCA = 5436 g/L) and the bulk fluid density is 1000 g/L. The $R^2$ for each model fit is indicated above the bars. No binding was observed for As, Cu (II), and Cr (VI) with lignin.

As mentioned, arsenic was found to adsorb to glucuronoxylan, where adsorption was only partially reversible. By contrast, adsorption of arsenic to lignin was not detected. Like arsenic, copper (II) also adsorbed to glucuronoxylan, where adsorption was partially reversible. This observation is consistent with studies that show the carboxylic acids or uronic acids of glucuronoxylan bind to Cu(II) (Bland, 1963; Michie, 1961). Past studies suggest that hydroxyl or methoxyl groups within lignin are potentially suitable for chelation with Cu(II) since complexes with the model compound, guaiacol, have been observed (Bullock & Jones, 1968); however, adsorption of copper to the organosolv lignin used in the current study was not observed.

Iron was found to irreversibly bind both xylan and lignin. Notably, the observed interaction between iron (III) and lignin is consistent with a previous study by Schmalzl et al., in which interaction between the model compound, guaiacol, and iron (III) was observed (Schmalzl et al., 1995).

The binding of chromium (VI) to xylan was partially reversible whereas binding to lignin was not observed. Chromium (VI) is found as CrO$_4^{2-}$ in some preservative formulations, therefore,
our study may not represent true interactions between chromium and wood components in preservative applications (Rowell, 2012).

To compare interactions between wood components and individual preservative chemicals with a complete preservative formulation, the binding of a commercial CCA-type with lignin and xylan was studied. Figure 16 shows the adsorbance of a CCA-type preservative to both lignin- and xylan-coated sensors. Binding had some reversibility for binding with lignin but was irreversible for binding with xylan. All individual components of this preservative (copper, chromium, arsenic) bound xylan so it was expected that the CCA preservative would bind to xylan. However, the binding of lignin with these individual components was not observed. It should be noted that in CCA preservative solutions, chromium is found as trivalent chromium, arsenic as arsenic pentoxide, and copper as copper oxide. The oxidation of these metals may be affecting the interaction with xylan and lignin in our study. In addition, other chemicals in the preservative formulation could be affecting the interaction with wood components.

5.3 Conclusions

The interaction between preservative chemicals (Cu, As, Cr, and Fe) and wood components (lignin and xylan) was investigated using quartz crystal microbalance with dissipation (QCM-D) monitoring. Iron was found to interact with both organosolv softwood lignin and beechwood glucuronoxylan whereas copper, arsenic, and chromium were found to interact with the xylan only. This preliminary study did not consider the effect of pH, temperature, and interaction between metal ion solutions, which are known to be factors influencing binding of commercial preservatives.

Wood is a complex matrix of lignin, hemicellulose, and cellulose, along with extractives and small amounts of inorganic compounds. Although the QCMD studies provide insight into which wood fractions interaction with the metal ions of preservatives, it is important to validate the results in industrially-relevant unfractonated wood samples. Time of Flight-Secondary Ion Mass Spectroscopy (ToF-SIMS), a surface analysis technique, was used to determine the localization of copper ions in alkaline copper quaternary (ACQ) preservative-treated wood. The results of the ToF-SIMS study are summarized in Chapter 6.
Chapter 6 – Time-of-flight secondary ion mass spectrometry

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a highly sensitive surface analysis technique that can determine chemical composition of a material. Ion images can be used to visualize the localization of specific chemical compounds. For the purpose of this study, ToF-SIMS was used to determine the distribution of copper ions in ACQ preservative-treated wood before and after UV exposure.

ToF-SIMS requires a very smooth surface to acquire ion images and determine localization of compounds. Typically, samples are cut using a microtome with either a glass or/and diamond knife. Sample preparation for biological materials, such as wood, can be challenging. If the surface is not rigid, the knife can slip against the surface, damaging the sample and the knife.

Initially, wood samples were adhered to SEM mounts and cross-sectional slices of the wood removed to smooth the surface using the glass-knife. Due to the flexibility of wood fibers, precise cuts on the edges of the samples could not be achieved. Upon ToF-SIMS analysis, it was apparent that the wood cell structures had been damaged during the cutting process. Since the ACQ-preservative and copper ions are localized to the edge of the wood samples, it was imperative that the samples were supported prior to microtome cutting. Supporting the biological samples can be achieved by embedding in ice or epoxy. Since embedding in ice would likely lead to leaching of the preservative into the water, changing the concentration of copper in the wood samples, epoxy-embedding was chosen as the support method. The microtome cutting process was repeated and no issues with wood fibers were observed. The time to achieve a visually smooth surface was greatly reduced after embedding in epoxy.

A ToF-SIMS acquisition was performed on epoxy-embedded samples before and after UV exposure. The respective ion images are provided in Figure 17 below:
Figure 17. ToF-SIMS ion images of ACQ preservative-treated wood cross-sections. Samples were embedded in epoxy resin and cross-sections cut using a microtome. Intensities of mass-to-charge (m/z) ratio peaks given by black to white gradient on the side of images. The total secondary ion image is shown on the left with (a) being before UV 340 nm exposure for 2000 hrs and (c) being after. The sum of the two copper signals (63 m/z and 65 m/z) is shown on the right with (b) being before UV 340 nm exposure for 2000 hrs and (b) being after. A delayed extraction acquisition method was used on post-UV exposure samples (c) and (d) which improved image resolution.

The top row of ion images (Figure 17a and Figure 17b) represent ACQ preservative-treated wood before UV-exposure and the bottom row of images (Figure 17c and Figure 17d) represent ACQ preservative-treated wood after UV-exposure. It should be noted, that a delayed extraction acquisition method was used in post-UV exposure samples (Figure 17c and Figure 17d), in which ions are allowed to move away from the surface before the extraction is applied to the system. This improved the image resolution of the wood cell structure compared to pre-UV samples (Figure 17a and Figure 17b). The left panel (Figure 17a and Figure 17c) represents total secondary ions detected during ToF-SIMS acquisition whereas the right panel (Figure 17b and Figure 17d) represent only the sum of copper signals at 63 m/z and 65 m/z. The total concentration of copper in the samples was 1.3-1.4 %, based on the sum of the copper secondary ions over the total secondary ions in the sample analyzed. This is comparable to results from x-ray photoelectron
spectroscopy analysis of the treated wood, which indicated a copper concentration of 1.2%. The copper ions appear to be evenly distributed in the wood with higher concentration on the cell wall than the middle lamella. There does not appear to be any major differences between pre- and post-UV exposure samples in terms of copper distribution of localization. By obtaining an average spectrum for a region of the lumen, cell wall, and middle lamella, the relative intensities for copper in these regions are depicted in Figure 18.

Figure 18. ToF-SIMS ion intensities from UV-exposed ACQ preservative-treated wood cross-sections. A subset of the total secondary ion intensities acquired for a region of the middle lamella (blue), lumen (orange), and cell wall (green) is shown. The intensities of the region of interest were normalized to the number of pixels in the area. Copper secondary ions are represented by peaks at 63 m/z and 65 m/z. The most copper is found in a region representing the cell wall and the least in the lumen.

A short range of mass-to-charge ratios in the region of copper (63 m/z and 65 m/z) is shown in Figure 18 to view the relative amount of copper in the middle lamella, lumen, and cell wall. Since each pixel in an ion image represent an average spectrum of 200 scans, the intensity of each region of interest was normalized per pixel to compare regions. The highest relative concentration of copper was present in the cell wall, with decreasing concentrations in the middle lamella, and very little in the lumen (Figure 18). The middle lamella, between wood cells, is an area that is more
lignin rich, acting as a binder between the cells (Lawoko, 2005; Sjöström, 1993). Since there is a lower concentration of copper in the middle lamella than the cell wall, it is possible that copper interacts more with hemicellulose and cellulose structures than lignin.

In Chapter 5, the QCMD studies indicated that copper does not interact with lignin whereas all metal ion solutions interact with glucuronoxylan. Glucuronoxylan, a type of hemicellulose, is more prevalent in the cell wall than the middle lamella, whereas, lignin is more enriched in the middle lamella. The ToF-SIMS can provide some support to this conclusion since the cell wall appears to have a higher concentration of copper than the middle lamella. Past studies, discussed in Section 1.5.4, have shown that both lignin and cellulose have the potential for interaction with copper (Bullock & Jones, 1968; Plackett, 1983). Our studies have shown that interaction with cellulose structures is more likely than lignin. As previously discussed, Bullock & Jones used guaiacol as a model compound, which may not be an accurate representation of lignin and could be a source of difference between that study and this study.

Wood degradation is typically initiated by UV exposure causing free radical formation and degradation of the lignin (Chang et al., 1982; Rowell, 2012). The degradation can be visualized by SEM imaging, which shows the collapse of the integrity of the wood matrix and cell (Rowell, 2012). In the ToF-SIMS images acquired (Figure 17), degradation due to UV-exposure cannot be detected by visual inspection. Since UV light only penetrates the top ~100 µm of the wood surface, it is possible that the cross-section examined by ToF-SIMS was not impacted by the UV-exposure treatment (Kataoka, Kiguchi, Williams, & Evans, 2007; Živković, Arnold, Radmanović, Richter, & Turkulin, 2014). To further examine the effect of UV exposure on ACQ preservative-treated samples, the top surface of the wood was analyzed by Fourier-Transform Infrared Spectroscopy (FTIR) and compared to untreated samples.
Chapter 7 – Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) is an analytical technique that elucidates structural information of compounds by monitoring vibrational frequencies upon incidence of infrared light at a range of wavenumbers in the mid-IR region (B. C. Smith, 2011; Stuart, 2015). Wood samples treated with ACQ-type preservative were exposed to UV light to induce weathering reactions that lead to degradation of wood. The samples were analyzed by ATR-FTIR and compared to non-exposed samples as well as control samples that were not treated with ACQ-type preservative (summary of samples in Table 5 - Section 3.5).

7.1 Effect of UV-exposure on structure of Wood by FTIR

The effect of UV-exposure on preservative-treated and untreated wood samples was studied using ATR-FTIR. An average IR spectrum for each sample type of wood is shown in Figure 19.

![Figure 19. ATR-FTIR spectrum of preservative-treated and untreated wood before and after UV-exposure. ACQ-treated wood and an untreated control were exposed to UV-A 340 nm light for 2000 hr and compared to unexposed samples. The exposed surface was removed, milled, and passed through a 40 mesh screen. Powdered samples were analyzed by a Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory. The spectrum was obtained by taking 32 scans for wavenumber 4000-600 cm⁻¹ at a resolution of 4 cm⁻¹. A total of 10 replicates per sample type were performed. The average of replicates, baseline corrected and normalized according to default software settings is shown.](image-url)
Differences in the absorbance peaks are most obvious around wavenumbers of 2924-2921 cm\(^{-1}\) and 1737 cm\(^{-1}\). The region of 2924-2921 cm\(^{-1}\) represents methylene groups of either lignin or hemicellulose (Decocq et al., 2005; Müller, Schöpper, Vos, Kharazipour, & Polle, 2015). There appears to be some modification of this region upon treatment with preservative that can be identified in both UV-exposed and non-exposed samples (blue and orange line in Figure 19). From the QCMD and ToF-SIMS studies previously presented, it is possible that copper is interacting with hemicellulose in the cell wall to cause the modifications in this region; however, there could be other components of the preservative formulation that are interacting with methylene groups of lignin or hemicellulose.

The region of 1737 cm\(^{-1}\) which represents C=O carbonyls in ester groups and acetyl group in xylan (hemicellulose) (Traoré, Kaal, & Martínez Cortizas, 2018). It appears that the absorbance of this peak is relatively increased in untreated UV-exposed samples (yellow line in Figure 19). Exposure to UV light causes oxidative degradation of lignin and leads to a relatively higher concentration of xylan on the surface of the wood (Lionetto, Del Sole, Canoletta, Vasapollo, & Maffezzoli, 2012; Xie et al., 2005). Comparatively, the other three samples types (blue, orange, and grey lines in Figure 19) have lower absorbance for the peak at ~1737 cm\(^{-1}\). Since the intensity of the treated UV-exposed peak (orange) is comparable to the control samples, which have not been exposed to UV light, the treatment is protecting the wood from some structural modifications.

Additional differences between the four sample types are difficult to observe from visual inspection of the IR spectrum in Figure 19. To determine which peaks account for the major differences between the samples, a multivariate analysis tool, called principal component analysis, was implemented.

7.2 Principal component analysis

Principal component analysis (PCA) is a method of dimensionality reduction which aims to reduces the number of variables while still containing the most relevant information of the original data set. The IR spectrum obtained from ATR-FTIR analysis of wood samples contained an absorbance value for wavenumbers between 4000 and 600 cm\(^{-1}\) at an interval of 4 cm\(^{-1}\). Since there is a large amount of data, PCA was applied to the data to identify the peaks which account for the most variation in the data.
First, the untreated samples before and after UV exposure were compared to each other. Then, the treated samples before and after UV exposure, and finally all four samples types together. For all analyses, several normalization techniques were tested and multiple signal correction (MSC-Mean) was determined to be optimal, since the first two principal components accounted for the most variation in the data. The data was also mean-centered before building the model so that absorbance peaks can have either positive or negative effect on the principal components.

7.2.1 Untreated samples

The cumulative variance for the first 20 principal components is given by Figure 20 below. The first principal component (PC1) accounts for 52.64% of the variation between UV-exposed and non-exposed untreated samples, whereas, the second principal component (PC2) accounts for 29.85% of the variation in the data. Since the goal is to determine only the major contributors to the difference in peak intensities, only the first two principal components were considered for analysis, which represent a cumulative variance of 82.49%.

Figure 20. Cumulative variance captured by principal components of untreated wood samples before and after UV-exposure analyzed by ATR-FTIR spectroscopy. Wood samples exposed to UV-A 340 nm light for 2000 hr were compared to unexposed samples using a Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory. The spectrum was obtained by taking 32 scans for wavenumber 4000-600 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). A total of 10 replicates per sample type were performed. Pre-processing for principal component analysis was performed using MATLAB® with Eigenvalue Research’s PLS Toolbox. Multiple signal correction (MSC-Mean) followed by mean-centering was performed as a pre-processing step.
The first two principal components with respective loadings plots are shown in Figure 21 for all untreated samples. Samples exposed to UV light are denoted by green squares (NU) and un-exposed samples are denoted by red diamonds (N). The principal component plot (Figure 21a) shows good separation between exposed and unexposed samples. Untreated (N) samples have a negative effect on PC1, whereas untreated samples that have been exposed to UV (NU) have a positive effect (Figure 21b). Similarly, untreated samples (N) have a negative effect on PC2 and untreated UV exposed (NU) samples have a positive effect on PC2 (Figure 21c). The loadings plot for PC1 and PC2 are shown in Figure 21d and Figure 21e, respectively. The peaks in each loadings plot represent the absorbance peaks which account for the separation of data on that principal component. Peaks of greatest magnitude, either positive or negative, have the most influence on the principal component.
Figure 21. Principal component analysis of untreated wood samples before and after UV exposure. Wood samples exposed to UV-A 340 nm light for 2000 hr (NU) were compared to unexposed samples (N) using a Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory. The spectrum was obtained by taking 32 scans for wavenumber 4000-600 cm$^{-1}$ at a resolution of 4 cm$^{-1}$. A total of 10 replicates per sample type were performed. Multiple signal correction (MSC-Mean) followed by mean-centering was performed as a pre-processing step using MATLAB® with Eigenvalue Research’s PLS Toolbox. (a) Principal component plots of each sample’s score on the first principal component (PC1) and the second principal component (PC2). (b) Scores on PC1 for each sample. (c) Scores on PC2 for each sample. (d) Loadings plot for PC1 against wavenumber (cm$^{-1}$). (e) Loadings plot for PC2 against wavenumber (cm$^{-1}$).
The loadings plot for the first principal component (Figure 21d) is most strongly influenced by the peak at 1736 cm\(^{-1}\) (largest positive peak) and the peak at 1509 cm\(^{-1}\) (largest negative peak). The peak at 1736 cm\(^{-1}\), discussed in section 7.1, likely represents C=O carbonyls in ester groups and acetyl group in xylan (hemicellulose) (Traoré et al., 2018). This absorbance peak is opposed by the peak at 1509 cm\(^{-1}\), which likely represents aromatic skeletal vibrations in lignin (Popescu et al., 2007; Traoré et al., 2018; Zhou, Jiang, Cheng, & Via, 2015). As previously mentioned, there can appear to be relatively higher amount of hemicellulose on the surface as the lignin is degraded by UV, so it is expected that the UV exposed samples (NU) are opposed by the unexposed samples (N) on the first principal component plot.

The loadings plot for the second principal component (Figure 21e) is similarly influenced by the peak at 1736 cm\(^{-1}\) (largest positive peak). UV-exposed (NU) samples have absorbance values relatively larger at this wavelength than the unexposed samples (N). The opposing, largest negative influence, peak is at 1029 cm\(^{-1}\) which can represent C–O deformation in primary alcohols of cellulose and aromatic C–H in-plane deformation in guaiacol lignin (Chen et al., 2010; Mahajan et al., 2012; Traoré et al., 2018). Lignin is typically degraded upon UV exposure enriching cellulose on the surface, giving the exposed wood a “tanned” colouring and protecting the wood from further UV-degradation (Lionetto et al., 2012; Volkmer, Noël, Arnold, & Strautmann, 2016). Because of the opposition, it is difficult to make a concrete conclusion about this result.

7.2.2 Treated samples

The cumulative variance for the first 20 principal components, comparing ACQ-type preservative-treated samples before and after UV exposure, is given by Figure 22 below. The first principal component (PC1) accounts for 62.22% of the variation between UV-exposed and non-exposed treated samples, whereas, the second principal component (PC2) accounts for 18.50% of the variation in the data. The cumulative variance for the first two principal components is 80.72%.
Figure 22. Cumulative variance captured by principal components of ACQ-type preservative treated wood samples before and after UV-exposure analyzed by ATR-FTIR spectroscopy. Wood samples exposed to UV-A 340 nm light for 2000 hr were compared to unexposed samples using a Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory. The spectrum was obtained by taking 32 scans for wavenumber 4000-600 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). A total of 10 replicates per sample type were performed. Pre-processing for principal component analysis was performed using MATLAB® with Eigenvalue Research’s PLS Toolbox. Multiple signal correction (MSC-Mean) followed by mean-centering was performed as a pre-processing step.

The first two principal components with respective loadings plots are shown in Figure 23 for all preservative treated samples. Samples exposed to UV light are denoted by green squares (TU) and un-exposed samples are denoted by red diamonds (T). The principal component plot (Figure 23a) shows samples are well separated across the second principal component but poorly separated across the first principal component (PC1). The PC1 plot (Figure 23b) shows that both sample types fall above and below the mean (0). For the second principal component (PC2), UV-exposed samples appear to have a negative effect on PC2 compared to unexposed samples (Figure 23c). It should be noted that two of the treated unexposed samples (T) fall outside of the 95% confidence interval and may be outliers skewing the results. The outliers may be due to impurities in the samples accumulated during the milling process or others external factors. A simulation was performed in which the outliers (sample 5 and sample 7) were removed, however, this did not improve or affect the main loadings peaks in the influence plots. The loadings plot for PC1 and PC2 are shown in Figure 23d and Figure 23e, respectively.
Figure 23. Principal component analysis of ACQ-type preservative treated wood samples before and after UV exposure. Wood samples exposed to UV-A 340 nm light for 2000 hr (TU) were compared to unexposed samples (T) using a Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory. The spectrum was obtained by taking 32 scans for wavenumber 4000-600 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). A total of 10 replicates per sample type were performed. Multiple signal correction (MSC-Mean) followed by mean-centering was performed as a pre-processing step using MATLAB® with Eigenvalue Research’s PLS Toolbox. (a) Principal component plots of each sample’s score on the first principal component (PC1) and the second principal component (PC2). (b) Scores on PC1 for each sample. (c) Scores on PC2 for each sample. (d) Loadings plot for PC1 against wavenumber (cm\(^{-1}\)). (e) Loadings plot for PC2 against wavenumber (cm\(^{-1}\)).
The major positive peak for the PC1 loadings plot (Figure 23d) is the broad absorbance peak at approximately 3361 cm\(^{-1}\) which likely represents hydroxyl stretching in cellulose and lignin (Esteves, Marques, Domingos, & Pereira, 2013). The major negative peak represents absorbance variation at a wavenumber of 1029 cm\(^{-1}\), discussed in the previous section as C–O deformation in primary alcohols of cellulose and aromatic C–H in-plane deformation in guaiacyl lignin (Chen et al., 2010; Mahajan et al., 2012; Traoré et al., 2018). It is difficult to make conclusions about the relation of these peaks to the exposure to UV of treated samples since samples are not well separated across this principal component.

The loadings plot of the second principal component (Figure 23e) shows that PC2 is most greatly influenced by absorbance at a wavelength of 1509 cm\(^{-1}\) (largest positive) and 1720 cm\(^{-1}\) (largest negative). The peak at 1509 cm\(^{-1}\) likely represents aromatic skeletal vibrations in lignin, whereas the peak at 1720 cm\(^{-1}\) likely represents C=O carbonyls in ester groups and acetyl group in xylan (hemicellulose) (Popescu et al., 2007; Traoré et al., 2018; Zhou et al., 2015). The treated unexposed samples have relatively higher absorbance values at 1509 cm\(^{-1}\) compared to UV-exposed samples. As is the conclusion made in section 7.2.1, lignin is being degraded in UV-exposed samples and hemicellulose is relatively enriched. Although the preservative treatment is protecting the wood to some extent, it is not perfect, as the PCA can detect differences between the samples.

7.2.3 All sample types

Finally, principal component analysis was used to determine if treated and untreated samples could be differentiated from each other, before and after UV exposure. The cumulative variance for the first 20 principal components, comparing all four samples types (n=10 per sample type), is given by Figure 24 below. The first principal component (PC1) accounts for 50.24% of variation in the data, whereas, the second principal component (PC2) accounts for 22.30% of variation in the data. The cumulative variance for the first two principal components is 72.54%. Since the cumulative variance of the first two principal components is lower than analysis in 7.2.1 and 7.2.2, the data is likely not as well separated.
Figure 24. Cumulative variance captured by principal components of untreated wood samples as well as ACQ-type preservative treated samples, before and after UV-exposure analyzed by ATR-FTIR spectroscopy. Wood samples exposed to UV-A 340 nm light for 2000 hr were compared to unexposed samples using a Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory. The spectrum was obtained by taking 32 scans for wavenumber 4000-600 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). A total of 10 replicates per sample type were performed. Pre-processing for principal component analysis was performed using MATLAB® with Eigenvalue Research’s PLS Toolbox. Multiple signal correction (MSC-Mean) followed by mean-centering was performed as a pre-processing step.

The first two principal components with respective loadings plots are shown in Figure 25 for the four different sample types: treated (T, dark-blue triangles), treated+UV (TU, light-blue triangles), untreated (N, red diamonds), and untreated+UV (NU, green squares). The principal component plot (Figure 25a) shows NU samples well separated from other samples, focused on the top-left quadrant of the plot where the first principal component (PC1) is negative and the second principal component (PC2) is positive. The N samples are somewhat concentrated in the bottom left quadrant where PC1 and PC2 are both negative. Treated samples, both before and after UV exposure (T and TU, respectively), are found on the positive side of PC1 but are not well separated along PC2. Since the T, TU, and N samples are all somewhat clustered, the preservative must be maintaining a structure that is similar to innate wood samples. It should be noted that some samples fall outside of the 95% confidence interval (Figure 25b, Figure 25c). As discussed in in section 7.2.2, these may be outliers causing large amount of variability within a sample type. The loadings plot for PC1 and PC2 are shown in Figure 25d and Figure 25e, respectively.
Figure 25. Principal component analysis of untreated wood samples as well as ACQ-type preservative treated samples, before and after UV-exposure. Wood samples exposed to UV-A 340 nm light for 2000 hr (TU – treated, NU - untreated) were compared to unexposed samples (T – treated, N - untreated) using a Perkin Elmer Spectrum One with UATR Single Bounce with ZnSe/Diamond Crystal accessory. The spectrum was obtained by taking 32 scans for wavenumber 4000-600 cm$^{-1}$ at a resolution of 4 cm$^{-1}$. A total of 10 replicates per sample type were performed. Multiple signal correction (MSC-Mean) followed by mean-centering was performed as a pre-processing step using MATLAB® with Eigenvalue Research’s PLS Toolbox. (a) Principal component plots of each sample’s score on the first principal component (PC1) and the second principal component (PC2). (b) Scores on PC1 for each sample. (c) Scores on PC2 for each sample. (d) Loadings plot for PC1 against wavenumber (cm$^{-1}$). (e) Loadings plot for PC2 against wavenumber (cm$^{-1}$).
In the loadings plot for the first principal component (Figure 25d), the major positive peak is found at a wavenumber of approximately 2923 cm\(^{-1}\) and the major negative peak is found at a wavenumber of approximately 1735 cm\(^{-1}\). These regions were also visually identified as differences in the IR spectra from section 7.1 (Figure 19). The region of 2924-2921 cm\(^{-1}\) represents methylene groups of either lignin or hemicellulose and appears to be modified in preservative treated samples (T and TU) (Decocq et al., 2005; Müller et al., 2015). The region of 1735 cm\(^{-1}\) which likely represents C=O carbonyls in ester groups and acetyl group in xylan (hemicellulose) (Traoré et al., 2018). This region appears to be enriched in UV-exposed samples that have not been treated with preservative (NU).

The loadings plot of the second principal component (Figure 25e) shows that PC2 is most greatly influenced by absorbance at a wavelength of 1733 cm\(^{-1}\) (largest positive) and 1029 cm\(^{-1}\) (largest negative). As previously discussed, the peak at 1029 cm\(^{-1}\) can represent C–O deformation in primary alcohols of cellulose and aromatic C–H in-plane deformation in guaiacol lignin (Chen et al., 2010; Mahajan et al., 2012; Traoré et al., 2018). More lignin is expected in samples not exposed to UV than the untreated UV-exposed samples, conversely, more hemicellulose on the surface is expected in untreated UV-exposed samples, than other samples, which is what is observed.

7.2.4 Conclusion

Principal component analysis (PCA) was used to determine the distinguishing absorbance peaks of the four samples types: treated (T), treated+UV (TU), untreated (N), and untreated+UV (NU). A summary of the relevant peaks identifies is provided in Table 6 below.
Table 6. Summary of key ATR-FTIR absorbance peaks determined by principal component analysis. Samples treated with ACQ-type preservative before and after UV exposure are denoted by T and TU, respectively. Untreated samples before and after UV exposure are denoted by N and NU, respectively.

<table>
<thead>
<tr>
<th>Peak Wavenumber (cm(^{-1}))</th>
<th>Assignment</th>
<th>Dominant in Sample</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2924-2921</td>
<td>methylene groups of either lignin or hemicellulose</td>
<td>T, TU</td>
<td>Modification to region upon preservative treatment</td>
</tr>
<tr>
<td>1740-1720</td>
<td>C=O carbonyls in ester groups and acetyl group in xylan (hemicellulose)</td>
<td>NU</td>
<td>Relatively enriched in untreated UV-exposed samples</td>
</tr>
<tr>
<td>1509</td>
<td>aromatic skeletal vibrations in lignin</td>
<td>T, N</td>
<td>Relatively enriched non-exposed samples</td>
</tr>
<tr>
<td>1030</td>
<td>C–O deformation in primary alcohols of cellulose and aromatic C–H in-plane deformation in guaiacol lignin</td>
<td>T, TU, N</td>
<td>Relatively depleted in untreated UV-exposed samples</td>
</tr>
</tbody>
</table>

Previously, FTIR in combination with PCA has been used to differentiate various wood species and wood from different regions (Chen et al., 2010; Traoré et al., 2018). In addition, structural changes during composite formation and structural changes during fungal degradation has been studied (Mahajan et al., 2012; Müller et al., 2015). This study adds to current research by using PCA to study differences due to chemical preservative treatment and the effect of exposing wood samples to UV.
Chapter 8 – Summary, recommendations, and impact

8.1 Summary and recommendations for future work

The objective of this study was to systematically investigate the interaction of wood preservative components with specific wood fiber fractions using surface analysis and calorimetric techniques. Isothermal titration calorimetry (ITC) was used to perform binding experiments between lignin and metal ion solutions that are commonly used in preservative formulations. Throughout the method development experiments, the heat of dilution accounted for a large portion of the binding heat flow signal, indicating the heat flow due to binding kinetics was too low to accurately determine interactions between the tested fractions and metal ion. This study was limited to experiments with wood powder and organosolv softwood lignin. Although appropriate reaction conditions for the system tested could not be found, it is possible that binding kinetics could be determined for other systems such as hemicellulose or cellulose, with metal ions. In addition, if a heating element for the injection syringe could be outfitted, binding above room temperature could be explored. Many preservative treatment processes involve heat and pressure, so testing kinetics at industrially relevant temperature would be beneficial.

A quartz crystal microbalance with dissipation monitoring (QCMD) was used to study the adsorbance of preservative chemicals (Cu, As, Cr, and Fe) on wood components (lignin and xylan). Iron was found to interact with both organosolv softwood lignin and beechwood gluconoxylan whereas copper, arsenic, and chromium were found to interact with the xylan only. This preliminary study did not consider the effect of pH, temperature, and interaction between metal ion solutions, which are known to be factors influencing binding of commercial preservatives. It should be noted that most wood is treated with preservatives under conditions of high temperature and pressure, therefore, the results of this study should be validated using alternative methods to assess the applicability in industrially-relevant systems.

Weathering of wood was simulated by exposing ACQ-type preservative-treated and untreated wood samples to UV-A light. Since the distribution of preservatives in a wood sample can greatly affect the wood protection efficacy, time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to visualize the distribution of copper ions in the wood. Copper was found to be well distributed throughout the wood, however, the ions were found primarily on the cell
wall surface, and not in the lignin-rich middle lamella. This observation supports conclusions from QCMD studies in which lignin does not interact with copper (II) ions. Currently, wood treated with a new class of preservatives, called micronized copper, undergoes additional treatment with iron oxide as a pigment. The cosmetic appeal of the wood is dependent on the distribution of the iron in the wood. ToF-SIMS could be applied to determine the distribution of iron at various concentrations or treatment techniques.

Wood structural changes due to UV degradation, that were not observable by ToF-SIMS, were studied using Fourier transform infrared spectroscopy (FTIR). Principal component analysis (PCA) was applied to determine the key absorbance peaks that differentiate preservative-treated and untreated samples, before and after UV exposure. The peaks identified (2924-2921 cm\(^{-1}\), 1740-1720 cm\(^{-1}\), 1509 cm\(^{-1}\), 1030 cm\(^{-1}\)) primarily correspond to lignin and hemicellulose structures, where hemicellulose structures are relatively enriched on the surface and lignin is depleted upon UV exposure. A large amount of variability was observed in some of the samples. To improve the method and possibly decrease variability, a sliver of the wood sample could be directly analyzed by ATR-FTIR without milling into a powder. In future work, the techniques could also be used to compare structural changes after treatment with different types of wood preservatives and different wood species compared. This could then be a method of screening the efficacy of new preservative formulations.

8.2 Impact and benefits to industry

Fundamental understanding of interactions between wood fractions (lignin, hemicellulose, and lignin) and preservative components (metal ions) can help drive targeted formulation development. In recent years, the wood preservation industry has shifted away from traditional CCA-type preservatives due to environmental and health concerns with arsenic leaching and chromium toxicity (Coles et al., 2014; Hasan et al., 2010; Hingston et al., 2001). As new formulations are being developed, there is a need for standardization testing and analytical methods of comparing different types of preservatives. The techniques developed can also be used to study adjacent wood technologies, such as composite materials.
The localization and dispersion of preservative components in the wood can greatly influence degradation prevention. Understanding how preservatives components interact with wood and how they are distributed can help improve formulations. In addition, this can prevent the overuse of preservatives which leads to leaching into the environment. If less preservative is required for wood preservation, the process will become more efficient. From an engineering perspective, the production cost could be decreased requiring smaller equipment, or same size equipment that can be used to produce larger volumes of material. Improving the weathering performance of wood is beneficial to the wood industry since it increases the life span of wood products, decreasing the amount of wood required to maintain structure and possibly diversifying the applications for wood products. The global wood preservative chemical markets is predicted to grow to approximately $2.6 billion USD by 2023 (Trent, 2018). One of the major driving factors of growth is the development of new environmentally friendly preservative formulations, therefore, it is imperative that research into wood chemistry continues (Trent, 2018).
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