# USE OF GADOLINIUM CHLORIDE AS A CONTRAST AGENT FOR IMAGING SPRUCE KNOTS BY MAGNETIC RESONANCE

*Thomas L. Eberhardt*†*
Research Scientist

*Chi-Leung So*†
Postdoctoral Researcher
USDA Forest Service
Southern Research Station
Pineville, LA 71360, USA

*Amy H. Herlihy*
System Manager/MR Physicist

and

*Po-Wah So*
Biological MR Research Coordinator
Biological Imaging Centre
Imaging Sciences Department
MRC Clinical Sciences Centre
Hammersmith Hospital Campus
Imperial College London
London, W12 0NN, UK

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ABSTRACT

Treatments of knot-containing spruce wood blocks with a paramagnetic salt, gadolinium (III) chloride, in combination with solvent pretreatments, were evaluated as strategies to enhance the visualization of wood features by magnetic resonance imaging (MRI). Initial experiments with clear wood and excised knot samples showed differences in moisture uptake after pretreatments with selected solvents. For knot-containing spruce wood blocks, increased detail in the images with an ethanol pretreatment was attributed to the removal of extractives thereby resulting in higher moisture contents for the knot wood. Incorporation of the gadolinium-based contrast agent resulted in an abrupt loss in signal for a zone around each knot. Accordingly, the retention of gadolinium ions appears to be selective, thereby allowing the demarcation of what is likely to be compression wood known to surround softwood knots. Applications include studies on wood anatomy by MRI and the modeling of wood defects. The treatment of wood with contrast agents as such also shows promise as a technique to improve our understanding of the localization of different cell-wall chemistries, especially as they relate to ion exchange capacity.

*Keywords:* Compression wood, contrast agent, extractives, ion exchange, knots, magnetic resonance imaging, softwood, spruce, wood.

## INTRODUCTION

Magnetic resonance imaging (MRI) is widely used as a diagnostic tool in modern medicine. As a non-destructive technique, allowing the visualization of internal features, applications now abound in other fields of study. For conventional MRI systems, samples under analysis need to contain high levels of moisture. MRI visualizations of wood generally require moisture contents above the fiber saturation point (Muller et al. 2001, 2002). This is because the images gen-

† Member of SWST.
* Corresponding Author

erated are derived from the proton nuclear magnetic resonance (PNMR) signals due to the water rather than those from the wood (Wang and Chang 1986). The ability to detect only the PNMR signals for water within the wood allows the determination of wood moisture contents (Araujo et al. 1992; Flibotte et al. 1990). Indeed, values for magnetic resonance T2 (spin-spin) relaxation can be used to distinguish between free and bound water present in wood (Bucur 2003; Labbe et al. 2002).

Several studies have applied MRI to investigate internal wood features. For example, MRI has been used to visualize zones of wood decay based on the increase in free water in the decayed zones after water saturation (Muller et al. 2001, 2002). Zones within wood that have low moisture contents have less signal and therefore appear as darkened areas on images. Accordingly, during MRI of logs, heartwood and knots appear as dark areas surrounded by lighter areas corresponding to clear sapwood (Chang et al. 1989; Coates et al. 1998; Wang and Chang 1986). MRI has also been used to demonstrate the depth of penetration for water-based wood preservatives by monitoring the flow of water into wood (Dawson-Andoh et al. 2001). Conversely, the movement of water out of wood during drying has also been reported (Meder et al. 2003). Since the images depend upon the PNMR signals for water, substitution of deuterium oxide for water results in signal losses. Transient signal losses through the administration of deuterium oxide have been used to show the flow of water through the conducting regions of a tree trunk section (Ilvonen et al. 2001).

In many magnetic resonance experiments, especially spectroscopy and T1 (spin-lattice) relaxation experiments, the presence of paramagnetic materials can be a concern. For example, the chelating agent diethylenetriaminepentaacetic acid has been used to remove paramagnetic metal ion impurities from spruce sawdust before relaxation time determinations (Ahvazi and Argyropoulos 2000). In the case of MRI, especially for medical applications, the administration of paramagnetic contrast agents can be advantageous by enhancing differences so as to improve the delineation of healthy and diseased tissues, as well as for the assessment of organ function (Toth et al. 2002). Most contrast agents are based on chelates of gadolinium (III) which has the highest possible number of unpaired electrons, making it the most paramagnetic stable metal ion (Toth et al. 2002). While commonly used for medical applications, contrast agents have yet to be applied to MRI studies of wood.

It is well established that woody materials have an inherent capacity to bind metal ions. Applications include the use of wood to remove heavy metal contaminants from water (Marin and Ayele 2002; Shukla et al. 2002). Differences in the heavy metal ion binding capacities for various wood features (e.g., compression wood, knots, etc.) have not received much attention. Given any significant differences, it seemed plausible that the treatment of wood with an aqueous solution of a gadolinium salt might allow the enhancement of images generated by MRI. Higher levels of non-polar extractives are assumed to impart lower moisture contents for knot wood relative to the corresponding clear wood (Sahlberg 1995). Solvent pretreatments were therefore also of interest as a means to improve both moisture uptake and contrast agent penetration. Here we report our efforts to apply gadolinium-based contrast agents coupled with solvent pretreatments to increase the utility of MRI for the characterization of knots present in spruce wood.

## MATERIALS AND METHODS

### *Wood samples*

Spruce-pine-fir (SPF) dimensional lumber (nominal 2 × 4 inches) was purchased at a local lumberyard. Cross and tangential sections were prepared with a microtome and observed by light microscopy to confirm that the wood was spruce (*Picea* spp). Knots appearing on the surface of the lumber, with diameters of 8 mm or less, were selected. Wood blocks (1.5 × 1.5 × 3 cm) were cut with the cross-section of the knot on the block face having the smallest dimensions. An effort was made to have the block

encompass as much of the knot as possible in the longitudinal direction towards the pith. Samples of knot and clear wood were excised with a band saw. Moisture contents were determined by drying in an oven (103 ± 2°C) overnight.

### *Solvent uptake by knot and clear woods*

Knot and clear wood samples were stored under ambient conditions until needed. Samples of each were weighed and then transferred to glass jars containing either hexane, acetone, 95% ethanol, or water. Containers were capped after the placement of glass weights to keep the specimens submerged. Weights of the samples were periodically monitored during the 1-week exposure period. Samples were briefly removed from the jars, blotted with a tissue, weighed on an analytical balance, and promptly returned to the appropriate solvent. At the conclusion of the exposure period, samples were air-dried, weighed, and then soaked in water for 1 day with the weight change monitored as before. Solvents remaining from treatments were dried *in vacuo.* FTIR spectra were collected directly using a Thermo Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Smart Golden Gate MKII single reflection ATR accessory.

### *Treatment of wood blocks with contrast agent for MRI*

Wood blocks containing knots were weighed and transferred into each of the following solvents or solutions (2 blocks per 50 ml): hexane, 95% ethanol, water, or 50 mM gadolinium (III) chloride hexahydrate (Aldrich Chemical Co., Milwaukee, WI) in water. As before, a glass weight was placed to keep the blocks submerged. On day 6 of the 7-day exposure period, a vacuum was applied with a water aspirator to ensure impregnation. Blocks were subsequently blotted with a tissue, weighed, and allowed to dry under ambient conditions. Additional sets of blocks, previously submerged in either ethanol or hexane, were treated with solutions of gadolinium chloride as described above. The average gadolinium cation content was calculated to be 0.54% (w/w) with the weight increase corresponding to the uptake of the gadolinium chloride solution.

### *Magnetic resonance imaging*

After saturating with water, all blocks were wrapped in plastic wrap and individually placed into a $^{1}$H tuned volume birdcage coil, 25-mm ID, 25-mm length (Magnetic Resonance Laboratories, Oxford, England) for imaging at 9.4T in a horizontal bore MRI scanner (Inova, Varian Inc., Palo Alto, CA). Transverse MRI scans were performed using a conventional spin echo sequence (TR 1000ms, TE 10ms, FOV 45mm × 45mm, matrix size 256 × 128, 4 averages, 2-mm slices). Images shown were selected as being representative of the 15 total slices of data collected for each block.

## RESULTS AND DISCUSSION

It is well documented that spruce and pine knots contain high levels of hydrophobic extractives (Willfor et al. 2003a,b); thus, a means to increase the PNMR signal within a knot would be to remove said hydrophobic extractives using organic solvents. Alternatively, the incorporation of a contrast agent into a knot may aid the delineation of substructures within the knot through changes in the relaxation rates (T1 and T2) of the water protons present. Intuitively, coupling these strategies could lead to even greater image enhancement. Given the greater hydrophobic nature of the knots relative to that for the surrounding clear wood, it was also plausible that non-polar solvents could provide a selective vehicle to deposit non-polar forms of gadolinium (e.g., gadolinium octanoate) in the knot wood. Differences in the uptake of organic solvents between knot and clear woods were therefore explored.

### *Differences in solvent uptake by knot and clear woods*

Submersion and weighing of excised knot and clear wood samples in solvents with a range of

polarities provided a rough assessment of solvent uptake. Results show that the uptake of water in the clear wood was more than twice that for the knots (Table 1). Interestingly, as the polarity of the applied solvent decreased, the amount of solvent retained by the clear wood was significantly higher relative to that retained by the knots. The most striking difference was with hexane where the knots retained little of this solvent. It seemed unlikely that the greater presence of non-polar extractives in the knots would also impart significantly lower uptake levels with the organic solvents. Thus, differences in solvent uptake between knot and clear woods appeared to be influenced to a greater extent by the differences in density. Since clear wood swells less in organic solvents than in water (Stamm 1964), we attribute the slightly higher levels of solvent uptake for the clear wood in ethanol and acetone to moisture present in both the wood and solvent (especially ethanol); the moisture contents of the excised knot and clear wood samples kept under ambient conditions were approximately 8 and 10%, respectively. When small amounts of water are present, higher levels of swelling occur compared to that in the absence of water (Stamm 1964). The very low level of hexane uptake by the knots may be due to much lower levels of swelling as a function of the lower availability of moisture in this solvent. Given the above observations, it appeared that the application of non-polar forms of gadolinium in non-polar organic solvents would favor deposition in the clear wood rather than the knot wood.

After solvent pretreatment, excised knot and clear wood samples were allowed to dry under ambient conditions. The remaining treatment water was freeze-dried while the remaining organic solvents were dried *in vacuo*. As expected, extractives were removed by the organic solvent treatments. The most efficient solvent was ethanol, which gave a 13.7% yield of extractives based on the starting dry weight of knot wood (Table 1); the yield of extractives for the knot wood in acetone (6.5%) was significantly higher than that obtained with hexane (0.2%). All yields of extractives for the clear wood were low (<0.4%).

Analysis of the hexane extracts for the knot and clear woods by FTIR spectroscopy (Figs. 1a,b) gave signals consistent with the aliphatic C-H (2920, 2850, 1460 $cm^{-1}$) and carbonyl (1730 $cm^{-1}$) functionalities of softwood oleoresins. Analogous spectra were obtained for the small amounts of extractives removed from the clear wood samples with acetone (Fig. 1d) and ethanol (Fig. 1f). However, these samples showed a greater amount of aromatic functionality with signals near 1600 (C=C stretch), 1500 and 1450 $cm^{-1}$ (both C-C vibration). The aromatic signal intensities were even greater for the acetone and ethanol extracts from the knot wood samples (Figs. 1c,e). These were attributed to stilbenes and lignans which are abundant among the extractives in both pine and spruce

TABLE 1. *Moisture contents of spruce (*Picea *spp.) wood blocks soaked in water after pretreatment in water or organic solvent.*

| Sample | Pretreatment solvent | Weight increase after pretreatment (%)[a] | Extractives released during pretreatment (%)[a] | Average moisture content (%)[b] |
|---|---|---|---|---|
| Clear wood | Water | 110 | <0.1 | 73 |
| | Ethanol | 119 | 0.3 | 75 |
| | Acetone | 121 | 0.4 | 56 |
| | Hexane | 59 | 0.2 | 44 |
| Knot wood | Water | 47 | <0.1 | 28 |
| | Ethanol | 31 | 13.7 | 35 |
| | Acetone | 30 | 6.5 | 24 |
| | Hexane | 6 | 0.2 | 16 |

[a] Based on starting weight of dry wood.
[b] Based on weight of dry pretreated wood.

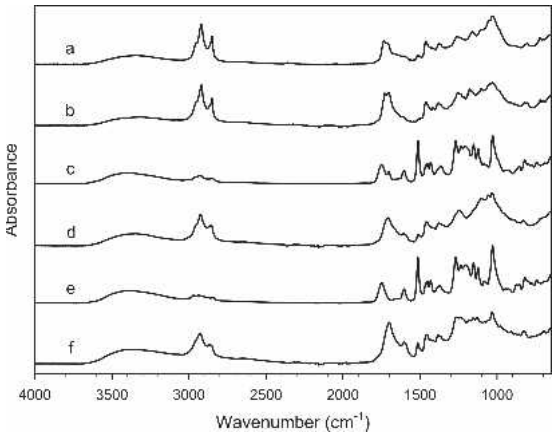


FIG. 1. FTIR spectra of excised knot extractives removed by (a) hexane, (c) acetone, and (e) ethanol treatments, and clear wood extractives removed by (b) hexane, (d) acetone, and (f) ethanol treatments.

knots (Willfor et al. 2003a,b). Such differences in the extractives compositions were anticipated given the differences in pretreatment solvent polarity as well as the differences in the extractives contents of the excised knot and clear wood samples.

Since the primary intent was to assess solvent uptake, the conditions employed here were less than ideal for extractives content determinations. Undoubtedly, the true extractives contents for both the knot and clear woods are higher than shown here. However, given the sometimes significant extractive yields, and differences in the chemical compositions of the extracts, there was particular interest in assessing the effect that such treatments would have on the moisture contents of knot and clear woods during specimen saturation with water prior to MRI.

### *Moisture contents of solvent-pretreated knot and clear woods*

The average moisture contents of the solvent-pretreated knot and clear woods are also shown in Table 1, as monitored by weight gain following submersion in water for one day. For the clear wood, pretreatment in ethanol resulted in essentially the same average moisture content as obtained with the pretreatment in water; lower average moisture contents were obtained through the pretreatments with acetone and hexane. However, with the excised knots, for which a significant amount of extractable material was passively removed by the ethanol pretreatment, the average moisture content was significantly higher relative to that for the excised knots pretreated in water. Accordingly, it was plausible that knot-containing blocks pretreated in ethanol could afford greater MRI signal intensity for the knot wood. Interestingly, for both the knot and clear woods, the pretreatment in hexane afforded significantly lower moisture contents when the samples were subsequently submerged in water. The lack of extractive removal with this solvent suggests a redistribution of the extractives that resulted in the lower uptake of water.

### *MRI of solvent-treated wood blocks*

Upon examination of the MRI image for a wood block soaked in water alone, the knot wood appears as a dark region (Fig. 2a) that coincides with the knot dimensions determined by examining the block after imaging. The pith, an area of high signal intensity, appears as a white dot in the center of the knot in the MRI image because of its low density and thus higher moisture level following saturation with water. Small cracks radiating from the pith are voids that appear essentially white because of the lack of woody material. In addition to the above features, the high sensitivity of the instrumentation allows the visualization of concentric variations in transitional tissues surrounding the knot.

Images for the organic solvent-pretreated

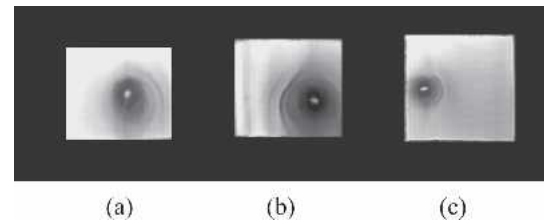


FIG. 2. MRI images of the wood blocks pretreated with (a) water, (b) ethanol, and (c) hexane, having observed average knot dimensions of 6.8, 8.0, and 4.0 mm, respectively.

blocks (Figs. 2b,c) are generally similar to that obtained with the water pretreatment, especially for a wood block pretreated with hexane (Fig. 2c). For the sample pretreated in ethanol, a more gradual transition is observed from the pith towards the outer edge of the knot. Upon closer inspection of the image, the ethanol treatment appears to allow the observation of alternating layers of high and low signal intensities coinciding with the annual growth of the branch from which the knot was derived (Fig. 2b). Such features were not readily apparent for the water- and hexane-pretreated blocks possibly due to the much lower levels of extractives removed relative to that for the ethanol pretreatment (Table 1). Increased water uptake, through the decrease in the level of knot extractives, appears to afford a greater level of detail to be observed.

### *Effect of contrast agent on MRI of wood blocks*

To evaluate the utility of contrast agents for the imaging of wood features, spruce blocks with knots were soaked in an aqueous gadolinium chloride solution following pretreatments with water, ethanol, or hexane. Gadolinium (III) octanoate was targeted as a non-polar contrast agent, but poor solubility in most common organic solvents led us to abandon its application. For all gadolinium chloride-treated samples, dark regions were observed in the images (Figs. 3a–c) that had dimensions larger than the knot dimensions which were measured upon sample removal from the instrument. Within these regions, bright areas were visible in that portion of the sample which was clearly knot wood. A lower signal to noise ratio for the contrast agent-treated samples was attributed to increased relaxation (T1 and possibly, T2) of the water as a result of the presence of the paramagnetic ions. The lack of signal in the apparent transitional region surrounding the knot suggested a greater concentration of gadolinium ions than in the clear wood. Thus, it appears that the contrast agent allowed the delineation of a zone that is neither knot nor clear wood. The stark differences in physical and chemical properties between knot and clear wood are well established. What these images appear to provide is a visualization of the transitional wood surrounding the knots that, although different from clear wood, is not readily visualized in specimens simply saturated with water.

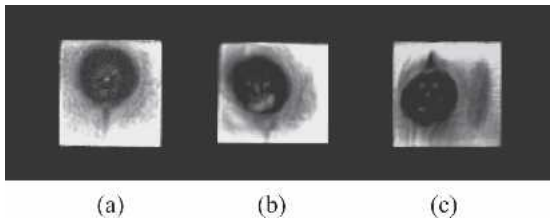


FIG. 3. MRI images of the wood blocks treated with 50 mM gadolinium chloride (a) and after pretreatment with (b) ethanol, and (c) hexane, having observed average knot dimensions of 6.8, 6.5, and 5.0 mm, respectively.

Compression wood is formed in the boles of softwoods to correct deviations from vertical growth. For the lumber industry, compression wood is not desirable because of the significant changes to wood physical properties. Likewise, for the pulp and paper industry, the compression wood tracheids are highly lignified and possess thick cell walls affording brittle pulps poorly suited to papermaking (Timell 1986). It should be recognized that compression wood is also formed around knots and along branches; although whole tree harvesting provides greater biomass utilization, pulps are generally of a lower quality (Timell 1986). Measurements of knot volumes in *Pinus taeda* suggest that the amount of compression wood surrounding a knot is 7-fold greater than the volume of the knot itself (von Wedel et al. 1968). Thus, it is imperative to visualize both the knot and surrounding compression wood tissues in order to estimate the amount of desirable clear wood present, as well as, undesirable wood in the form of knots and compression wood. The incorporation of a contrast agent provided greater contrast and demarcation of the knots and what appears to be compression wood surrounding the knots. The utilization of contrast agents therefore expands the information that can be obtained by MRI. Applications include detailed studies on wood anatomy and the modeling of wood defects. Al-

though the cell-wall chemistry of compression wood is well studied, ion exchange properties have not received much attention. Our images with the contrast agent appear to suggest a difference in the ion exchange capacities between clear wood and apparent compression wood surrounding the knots. Thus, contrast agents appear to show promise as a technique to improve our understanding of the different ion exchange capacities present in wood.

## CONCLUSIONS

The significantly lower uptake of water in knot wood relative to clear wood appears be a function of both high extractives content and high density of the knot wood. Pretreatment with ethanol can remove sufficient amounts of extractives to allow increased water uptake, thus improving somewhat the level of detail when imaging spruce knots by magnetic resonance. Treatments with a contrast agent (e.g., gadolinium chloride) can provide an increase in the level of contrast between the transitional wood between the knots and clear wood. Accordingly, the retention of this paramagnetic salt appears to show some selectivity thereby now allowing the demarcation of that which is likely the compression wood known to surround knot wood. This is well suited to studies on wood anatomy and the modeling of wood defects. Additionally, the utilization of contrast agents shows promise as a technique to improve our understanding of the localization of different cell wall chemistries and their inherent ion exchange capacities.

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