Applications of Solid State Nuclear Magnetic Resonance Spectroscopy for Non-destructive Analysis of Plant Cell Walls and Engineered Soil Surrogates

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Mark A. Werner

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The thesis of Mark Werner was reviewed and approved* by the following:

Ben Lear  
Associate Professor of Chemistry  
Thesis Advisor

Tom Mallouk  
Evan Pugh University Professor of Chemistry, Biochemistry and Molecular Biology, Physics, and Engineering Science and Mechanics

David Boehr  
Associate Professor of Chemistry

Tom Mallouk  
Evan Pugh University Professor of Chemistry, Biochemistry and Molecular Biology, Physics, and Engineering Science and Mechanics  
Head of the Department of Chemistry

*Signatures are on file in the Graduate School
ABSTRACT

Solid State NMR (SS-NMR) spectroscopy presents a widely applicable analytical method for probing interfaces and internal structure of highly complex systems. As samples are studied in powdered solid form, SS-NMR is a uniquely non-destructive technique, making it especially useful for studying naturally occurring systems without altering their chemical environment. Presented here are two preliminary studies that highlight this utility. First, a study is presented detailing the use of SS-NMR to study the internal polymer matrix of plant cell walls which are a valuable source of biofuels, but which are difficult to breakdown due to these complex polymer interactions. Second, SS-NMR was used to examine the surface chemistry of engineered soil surrogates treated with pesticide probe molecules. The complex chemistry of soils is perfectly suited to analysis by SS-NMR due to its ability to keep samples intact.
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Chapter 1

Solid-State Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) Spectroscopy is a highly versatile and widely used analytical technique that can be used to study the chemical environment of a sample as well as its dynamics. It is an atomically specific technique that utilizes radio frequency pulses to manipulate the “spin” of nuclei to make these observations.¹ This specificity allows for the isolation of certain atoms within complex molecules, making it easy to focus on components of interest. This makes it particularly useful in studying highly complex molecules.

When explaining NMR, we will use the vector model of NMR, which employs a semi-classical picture, taking into account the overall magnetization of the sample and how this net moment behaves in the presence of magnetic fields. This model allows one to easily picture the effects of radiofrequency pulses on the sample, and for this reason, much of the discussion in this section will be taken from this vector model.²

Spin

Electrons, protons, and neutrons possess an intrinsic quantum mechanical property known as spin, a form of angular momentum. In their natural state, spins are randomly oriented, however, in the presence of an external magnetic field (B₀), their position changes and results in an overall net magnetization (M) aligned with B₀ as shown in Figure 1 below.¹ ²

\[ \mu = \gamma I \]

\[ \sum \mu \]

\( \gamma \) is a characteristic called gyromagnetic ratio which is specific to each nuclei and denotes the relationship between angular momentum and magnetic moment.
In the presence of an external magnetic field ($B_0$), an overall spin alignment occurs creating a net magnetization ($M$) aligned with $B_0$.

In addition to aligning with $B_0$, in the presence of the magnetic field, the spins will precess at a constant angle with respect to $B_0$ at a rate known as the Larmor Frequency.\(^2\)

$$\omega = -\gamma B_0$$

In the absence of $B_0$, the energies of spin states are degenerate, however, in the presence of $B_0$ the energy levels are split according to the Zeeman Splitting.\(^2\)

$$E = -\gamma h B_0 m$$

In this equation, $m$ is the unique spin quantum number of a given spin state, and the difference in the energy of states is directly proportional to the strength of the external field. This energy splitting is exploited by NMR. At equilibrium these states are populated according to the Boltzmann distribution. However, when radiofrequency pulses with energy equal to the $\Delta E$ between states are applied, the absorption of energy causes a transition between spin states. Once a non-equilibrium energy state is achieved, the spins return to the more favorable equilibrium Boltzmann distribution. This relaxation takes place via two pathways that both involve a loss of energy from the spins. Spin-lattice relaxation involves the loss of energy from the spins to the lattice, and spin-spin relaxation involves the loss of energy from one spin to another spin.

These relaxation processes can be clearly depicted using the vector model of NMR.\(^2\) In the presence of $B_0$ along the $z$-axis, the net magnetization of the nucleus aligns along the $z$-axis as well. The application of a $\frac{\pi}{2}$ r.f. pulse transfers this magnetization fully into the $xy$-plane; this process is depicted in Figure 2. Initially, the spins precess coherently about the $z$-axis. Over time this precession leads to a loss of coherence as the spins diphase, ultimately resulting in a
complete loss of magnetization within the xy-plane. This is known as spin-spin relaxation, and the rate of relaxation is dictated by the spin-spin relaxation time, $T_2$.

$$M_{xy}(t) = M_{xy}(0)e^{-t/T_2}$$

While the spins are interacting with each other via spin-spin relaxation, they are also interacting with the lattice which leads to the reestablishment of magnetization along the z-axis. This process is known as spin-lattice relaxation. $T_1$ is the spin-lattice relaxation time which determines the rate of this relaxation.$^3$

$$M_z(t) = M_z(0)[1-2e^{-t/T_1}]$$

**Figure 2**: In the vector model depiction of NMR spectroscopy, magnetization is transferred from its alignment with the B<sub>0</sub> field along the z-axis into the xy-plane via radiofrequency pulses

**Solid State NMR**

Two types of NMR spectroscopy are practiced; the more common solution state NMR, and solid state NMR which is a more complicated technique due to the immobility of molecules in solid samples. Despite this added complexity associated with solid state NMR, it can offer significant advantages in certain scientific areas such as the study of naturally occurring molecules and surface chemistry studies. One of the most important advantages is the non-destructive quality of solid state NMR. Many analytical techniques involve the breakdown of samples to study them, and while solution state NMR doesn’t involve significant breakdown of samples, solid samples must be dissolved into solution which can significantly alter the chemical properties of these samples. Solid state NMR, however, involves no chemical alteration of
samples, making it ideal for observing natural products in their native configurations and for studying chemical surfaces since surface interactions will be unaltered.\textsuperscript{2, 4}

**Magic Angle Spinning**

A number of interactions affect the spins to create the resulting NMR signal. One of the largest effects results from dipole dipole interactions. This is a through space interaction between the molecular moments of individual nuclear spins.\textsuperscript{5} In solution state NMR the free motion of the molecules in a sample allows for rapid reorientation which negates much of the effect of these interactions. Solid-state NMR, however, is performed on rigid solid structures which are not able to reorient, and so, to counteract these effects, a process known as magic angle spinning (MAS) is employed.

Nuclear spin interactions are related to the molecular orientation according to the following equation.\textsuperscript{2}

\[
3 \cos^2 \theta - 1
\]

Theta is the angle of the spin interaction tensor for a specific molecule, which in a powder, will take on every value since every molecular orientation is present and fixed. This angle can be varied by spinning the sample about an axis at an angle ($\theta_R$). The molecular orientation dependence then becomes averaged as follows.\textsuperscript{2}

\[
3 \cos^2 \theta - 1 = \frac{1}{2} (3 \cos^2 \theta_R - 1)(3 \cos^2 \beta - 1)
\]

Like $\theta$, $\beta$ takes on all possible values in a powder, but ($\theta_R$) can be fixed at one value by the experiment. If it is set at 57.74° (the “magic” angle), $3 \cos^2 \theta_R - 1$ is equal to zero, and so the average is also equal to zero, removing the majority of interactions.\textsuperscript{2}
Figure 3: Magic angle spinning (MAS) places the rotating frame at an angle to the $B_0$ field to minimize nuclear spin interactions

**Pulse Sequences**

**Bloch Decay**

The simplest solid state NMR experiment is the Bloch decay experiment (direct polarization). This experiment involves directly polarizing the magnetization of a nucleus and then detecting this magnetization. After aligning with the external field $B_0$, the magnetic moment is transferred into the $xy$-plane via a $\frac{\pi}{2}$ r.f. pulse. Immediately, spin-spin and spin-lattice relaxation processes begin to take place, and this relaxation results in the free induction decay (FID) which is manipulated via Fourier Transform to produce the resulting NMR spectrum. An example of an FID and its associated NMR spectrum is shown below. Once the signal has fully relaxed back to its alignment along the $z$-axis, the experiment can be repeated and the signals added together to increase signal intensity.$^{1,2,6}$
Cross Polarization

Cross polarization (CP) is a particularly versatile solid-state NMR technique. It is widely used to increase signal intensity, and thereby reduce experiment time, and it can also be used to elucidate the chemical environment of target molecules within a sample. In CP, magnetization is transferred from abundant and highly sensitive nuclei (such as $^1$H) to less abundant or less sensitive nuclei (such as $^{13}$C) via heteronuclear dipolar coupling. As such, it can also be used to highlight nuclei that are in proton rich environments (or those nuclei in the vicinity of other sensitive nuclei).\(^7\)

CP operates by transporting the entire system to a doubly rotating frame. In this case, both nuclei in question are in a state in which all fields resulting from pulses on the respective nuclei appear static. After transferring the abundant magnetization to the xy-plane, a spin-lock
pulse is applied that acts on the rotating magnetization as \( B_0 \) does on equilibrium magnetization. Simultaneously, a spin-lock pulse is applied to the rare nuclei that acts to lock the spins in the rotating state along with the abundant spins. Figure 5 below shows the vector model behavior of nuclear spins in this spin-lock state undergoing CP.\(^2\)\(^7\)

**Figure 5:** The vector model of cross polarization illustrates how \( ^1\text{H} \) polarization is transferred to \( ^{13}\text{C} \) to conserve angular momentum
INADEQUATE

The Incredible Natural Abundance Double Quantum Technique is given the humorous acronym INADEQUATE. This pulse sequence suppresses isolated $^{13}$C signals selectively detecting natural pairs of $^{13}$C atoms within molecules, so called double quantum coherences. Such pairs are rare due to the abundance of $^{12}$C isomers and the relative scarcity of $^{13}$C; therefore naturally occurring $^{13}$C pairs are extremely rare. This selectivity makes the INADEQUATE pulse sequence a highly useful technique for correlating $^{13}$C atoms within complex organic structures. This J-coupling depicts nuclei whose electrons are interacting within the molecule. This means that the nuclei must be close together within the bonding network. By employing a 2D INADEQUATE experiment, a correlation map is created that depicts these J-coupling interactions. A “correlation walk” can be done in which correlation along the x-axis indicates a J-coupling interaction and correlation along the y-axis indicates the same nuclei. The spectrum below is a representative INADEQUATE spectrum of a plant cell wall with the correlation walks shown in the colored lines. An additional benefit of the INADEQUATE experiment is that these spectra lack the typical diagonal peaks seen in 2D correlation spectra. The absence of this diagonal makes for a cleaner spectrum and makes small, crowded peaks easier to differentiate.
Figure 6: Representative 2D INADEQUATE spectrum and correlation walks of a plant cell wall
References

Chapter 2

Solid-State NMR Analysis of Plant Cell Walls

Plant cell walls (PCWs) are essential in providing strength and protection as well as contributing to numerous physiological processes of plants such as the transport of nutrients.\textsuperscript{1,2} Recently, interest in PCWs has risen due to their potential use as a source of lignocellulose-based biofuel. Lignocellulose is a raw material composed of polysaccharides and the aromatic polymer lignin. The abundance of polysaccharides contained within this biomass makes it an excellent potential energy source; however, the complexity and cohesive nature of these polysaccharides presents a complication due to the cell walls recalcitrance to being broken down into liquid biofuels. This complexity benefits the plant by allowing the wall to perform the tasks necessary for a plant’s life and providing the mechanical strength and protection it needs to survive, but it is a major obstacle to efficient conversion of cellulosic biomass to biofuels which involves the depolymerization of the PCW followed by the fermentation of the starches contained in the wall.\textsuperscript{3} Unfortunately, the complex PCWs are recalcitrant to depolymerization. In order to overcome this difficulty, increased knowledge of the growth, structure, and internal interactions and dynamics of PCWs must be gained.\textsuperscript{3,4} One important aspect that must be studied is the interaction between cellulose, the chief component of the cell wall, and the polysaccharide matrix in which it is embedded.\textsuperscript{3,5} This interaction plays a large role in the recalcitrance of PCWs to depolymerization. If a better understanding of what components of the cell wall are bound together and interacting with each other, the efforts to breakdown the cell wall can become more focused on these interactions and will lead to more efficient conversion of biomass to liquid fuel.\textsuperscript{4}

Although much work has attempted to study the interactions between cell wall polymers, there is no current consensus within the plant cell wall community about the internal structure of cell walls. One reason for discord lies in the complex sample treatment and breakdown of the cell wall which is required for many characterization techniques. Many studies of the PCW were carried out using biochemical methods such as alkaline extraction that break down the wall in order to study it, and microscopic techniques that require dissection of the PCW to gain access to the internal components.\textsuperscript{5,6} Among these past studies are NMR studies of individual cell wall components and creep studies. As a result, the current description of the inner workings of the
PCW is likely incomplete or incorrect. One promising new approach toward studying these interactions is the use of multi-dimensional solid state magic angle spinning (ss-MAS) NMR experiments. Solid state NMR samples require less treatment and preparation than other commonly used techniques. This allows for the study of intact cell wall samples in states that are similar to the natural state of the cell wall in nature.²

**Figure 7: Formation of a secondary plant cell wall, containing aromatic lignin polymers, after cell wall growth has stopped³**

PCWs are categorized in two classes based on the developmental stage of the cells that form them: primary and secondary cell walls. Primary cell walls are formed by growing cells, and primary cell walls of different cells display common properties such as polysaccharide content. Secondary cell walls are formed after cell growth has stopped, and different types of cells can form secondary cell walls with different properties. For example, xylem, the water conducting tissue of plants, forms secondary cell walls that are thickened and waterproofed by a compound known as lignin, which does not appear in all secondary cell walls.⁴ A cell with both primary and secondary cell walls is shown in Figure 7. Most of the current progress in the study of PCWs has been made on primary cell walls, which are largely made up of three components: cellulose, hemicellulose, and pectins. The latter two components comprise a polysaccharide matrix in which cellulose fibrils are embedded.² Widely accepted models of primary PCWs have proposed a construction wherein the cellulose microfibrils are coated with hemicelluloses that bind the microfibrils together. Pectins are dispersed throughout this matrix controlling microfibril spacing while having little direct interaction with the cellulose. This primary cell wall model is depicted in Figure 8.¹² Secondary PCW models, as seen in Figure 7, depict a similar cellulose-hemicellulose matrix with lignin taking the place of pectin filling in the space within
the matrix. Some recent findings, however, have provided evidence of a significantly different primary cell wall model.

As noted above, a number of NMR studies have been carried out on individual PCW components. Ralph, et al. have studied whole PCWs via solution-state NMR with a focus on structural characterization of lignin. Newman, et al. have used solid state MAS NMR to study model composites of plant hemicellulose and bacterial cellulose. Results from these studies show evidence for the cellulose-hemicellulose matrix, but show less coating than expected. These studies, however, are insufficient, because they focus on only one component of a PCW, or because they utilize solution state NMR which puts the cell wall in an unnatural state. Initial ss-MAS NMR analysis of intact cell wall samples was carried out by Dick-Perez, et al. A series of $^{13}$C-$^{13}$C correlation experiments was carried out on intact primary cell walls of *Arabidopsis thaliana*. Based on their findings, Dick-Perez, et al. proposed a modified cell wall model, shown in Figure 9, that exhibits a significantly higher level of cellulose-pectin interactions and less prevalent cellulose-hemicellulose (xyloglucan) interactions. This model suggests a more complicated internal structure of the PCW with interactions occurring between cellulose, hemicellulose, and pectins in a three-component matrix. A more complex model could lead to
greater reluctance of the cell wall to breakdown into liquid biofuel; however, a significant issue with this study has been raised. The *A. thaliana* cell wall samples were dehydrated by washing with acetone and subsequently rehydrated in the sample preparation. This drying is believed to contract the cell wall leading to the observation of cellulose-pectin interactions that do not occur naturally.

![Diagram showing proposed revision to primary plant cell wall model](image)

*Figure 9: Proposed revision to primary plant cell wall model; cellulose-hemicellulose interactions are not as prevalent, and much higher levels of cellulose-pectin interaction occur than in previous models.*

The work presented here aims to provide a base understanding of primary and secondary cell walls. Importantly, the samples that were analyzed for this study were “never-dried”. This means that no acetone wash was used in the preparation of the samples. It is hoped that this lack of drying will leave the cell walls closer to their naturally occurring state by not removing the water that exists between components within the wall. Additionally, experiments carried out on secondary cell walls will provide initial findings about the differences between primary and secondary cell wall components, in particular, the presence of lignin in these secondary cell walls. Past studies of intact cell walls have made identification of lignin difficult due to the complexity of the spectra. It is predicted that the lignin signals overlap with the cellulose signals which are the principle component of both the primary and secondary cell walls.

**Plant Cell Wall Samples**

**Plant Cell Wall Components**

The samples studied for this project were taken from *Arabidopsis thaliana* plants, a commonly studied dicotyledonous plant. The main hemicellulose in primary cell walls of dicotyledonous plants is xyloglucan (XG). In *Arabidopsis thaliana* the main pectin is
homogalacturonan (HGA), which consists of linear chains of 1,4-α-D-galacturonic acid (GalA). Small amounts of rhamnogalacturonin (RG) are present as well. The main hemicellulose of secondary cell walls is xylan with lignin taking the place of much of the pectin found in primary cell walls. Structures of these compounds can be seen in Figure 10.\textsuperscript{12} The leaves and stems of \textit{A. thaliana} plants will be collected in order to study primary and secondary cell walls. Leaves contain mostly primary cell walls, while the stems contain mostly secondary cell walls.\textsuperscript{1,2} All samples will be “never-dried” to avoid any contraction of the PCW, and all PCW samples will be uniformly \textsuperscript{13}C labeled.

\textbf{Figure 10: Major polysaccharides of \textit{Arabidopsis thaliana}; (right) lignin occurs only in secondary cell walls}\textsuperscript{7}

\textbf{\textsuperscript{13}C Labeling}

Due to the complexity of PCWs, a strong NMR signal is imperative for the experiments. Unfortunately, the relative natural scarcity of \textsuperscript{13}C makes obtaining strong signal difficult and time consuming for naturally occurring samples. To combat this, samples can be \textsuperscript{13}C labeled to increase the samples NMR sensitivity. In order to leave PCW samples as intact as possible for these studies, this labeling was carried out during the growth of the \textit{A. thaliana} plants.

Two pathways were employed to achieve this labelling. In the first method, plants were grown in a liquid glucose media which was enriched with \textsuperscript{13}C. While effective, this method is too dissimilar to natural conditions to consider the samples comparable to naturally occurring PCWs. The second labelling method utilizes a \textsuperscript{13}C enriched atmosphere in which the plants are grown while using a natural soil rather than a liquid media. This produces more natural growing
conditions while still incorporating $^{13}$C via intake from the enriched atmosphere. This method presents its own difficulties as an enriched atmosphere is difficult to maintain without contamination from the natural atmosphere. Ultimately, the enriched atmosphere did not provide sufficient $^{13}$C abundance, so the following experiments were carried out with the liquid media samples. Future experiments, however, should focus on improving the efficiency of the more natural enriched atmosphere method.

NMR Sample Prep

Samples were taken from various regions of plants by separating the stems and two types of leaves, cauline leaves which grow out from the stem and rosette leaves which form earlier and grow at the base of the stem. This allowed for the observation of various developmental stages in the growth of the plant by separating older and younger leaves as well as isolation of primary and secondary cell walls. Primary cell walls occur in both types of leaves as well as in the stem material, while secondary cell walls occur exclusively in the stems.

These samples were treated to remove carbohydrates and proteins with little impact on the overall PCW matrix. This reduced the complexity making isolation of $^{13}$C signals of interest easier while leaving the walls largely unaltered from their natural state.

NMR Experiments

Mobility Selectivity

Several types of NMR experiments were employed to study the cell wall material, each with a particular goal. One-dimensional experiments provided baseline characterization of the PCW systems. Direct polarization (DP) and cross polarization (CP) were used for selective excitation of mobile polysaccharides and rigid polysaccharides respectively. Since cross polarization relies on dipolar coupling interactions for the transfer of polarization, these experiments excite rigid polysaccharides, or those which experience more direct interaction with additional polysaccharides and therefore experience greater dipolar coupling. Direct polarization excites all polysaccharides including the highly mobile species by exciting the magnetization of carbon nuclei and then detecting that signal.$^{13, 14}$

NMR Studies
Samples taken from the stems and rosette and cauline leaves of *A. thaliana* were analyzed via CP and DP $^{13}$C NMR. The primary cell walls contained in the rosette leaves produced the spectra shown below. The complexity of these cell wall samples is clearly evident from an initial viewing of these spectra. By comparing the rigid selective CP spectra and the unselective DP spectra the most mobile components can be identified. The reduced intensity of the glycoprotein sidechain signals between 0 and -25 ppm and those around 87 ppm indicates that these are mobile components and therefore they will likely not be involved in the rigid polymer matrix that composes the major structure of the cell wall. The strongest peaks in both spectra occur at approximately 30 ppm. These peaks are attributed to internal cellulose microfibrils. As expected there is little difference between these peaks in the CP versus the DP spectra due to the rigidity of these microfibrils.

![Figure 11: DP (top) and CP (bottom) $^{13}$C spectra of rosette leaves](image)
Figure 12 compares DP spectra from rosette and cauline leaves. This figure shows that there are few significant differences between the older rosette leaves and the younger cauline leaves indicating that primary cell wall development occurs relatively quickly in the life of the plant.

![Figure 12: DP 13C spectra of rosette (top) and cauline (bottom) leaves](image)

A final comparison is shown in Figure 13. Here inflorescence stems (top) which contain lignin rich secondary cell walls are compared to cauline leaves (bottom) which contain only primary cell walls. There are a number of significant observations to be made from these spectra. First, the mobile regions of the inflorescence spectrum between -10 and -50 ppm and above 50 ppm display much lower intensity than the cauline leaf spectrum. This indicates a lack of these mobile components in the secondary cell wall which is expected given its stronger, more rigid structure. Additionally, signals are observed in the stem spectrum between 15 and 30 ppm that are not present in the cauline leaf spectrum. These signals can be initially attributed to the lignin
present only in secondary cell walls. Future experiments utilizing both 1D and multidimensional NMR should be carried out to confirm this finding.

![Inflorescence stems and Cauline leaves spectra](image)

**Figure 13:** DP 13C spectra of inflorescence stems (top) and cauline leaves (bottom)

**Further Studies**

At this time, efforts to develop INADEQUATE pulse sequences on the instruments within Penn States NMR facilities. These efforts should be continued in order to allow for efficient correlation studies to be carried out on plant cell wall samples. Additionally, samples should be collected from different developmental stages of plant stems in order to observe the development of secondary cell walls. These secondary cell walls should be only beginning to form in young samples taken from the top of the stem, while the samples from the base of the stem should contain fully formed secondary cell walls.
References

Chapter 3

Surface Chemistry of Engineered Soil Surrogates

A growing population places increasing demands on food production; but with this population increase, more and more land is needed to house the surplus, so this food must come from an increasingly limited area of agricultural land.\(^1\),\(^2\) To improve the efficiency of food production a better understanding of soil, its chemical composition, and its interactions with a variety of other chemical species such as pesticides, fertilizers, and other additives must be gained.\(^1\),\(^3\)

In the following ongoing study, the interaction of soil samples with pesticides is explored utilizing inorganic/organic systems of increasing complexity which mimic real soil systems and a variety of pesticide probe molecules. After the probe molecules are sorbed onto the surfaces, a variety of solid state NMR experiments are used to probe the chemical environments of the interface to determine what interactions are taking place and how the pesticides are bonding to the soils. Since these pesticides can be harmful to humans, it is imperative that they can be removed from the soil after plant treatment. Ideally, one would desire a highly effective pesticide, to maximize food production, that can be easily removed from the soil, to avoid any harmful effects on humans. By studying the soil/pesticide interaction, more efficient methods of removal can be developed.\(^4\),\(^5\),\(^6\)

The initial work outlined below is focused on determining ideal conditions for loading phosphates onto artificial soil samples, engineered soil surrogates which are detailed in section 3.1.1 and silica surfaces. Due to the complexity of these artificial soils, an efficient method of preparation and phosphate loading must be determined before additional in depth interaction studies are undertaken.

Engineered Soil Surrogates

Soil consists of complex combinations of both inorganic and organic components. The organic matter of soils is largely made up of decayed plant materials and so mainly consists of lipids, lignin, and cellulose. This highly complex and heterogeneous nature makes soils extremely difficult to study and correlate functional group properties. Therefore, engineered soil
surrogates (ESS) have been developed to mimic real soils. These ESS’s are complex yet by separating functional groups into distinct layers, they are simplified enough to allow for correlation of bulk properties. Silica particles, which mimic the inorganic component of soil, are functionalized with organic layers to mimic the organic components of soil. Aliphatic chains of various lengths correlate to the lipids in soil, phenolic oligomers mimic the lignin, and cyclic glucose oligomers stand in for the soils cellulosic components.7, 8, 9

These ESS’s are categorized by the number of layers of organic functionalization placed on them. Single layer ESS’s are achieved by modifying a silica surface with hydrocarbon chains of various lengths (C\textsubscript{6}H\textsubscript{14}, C\textsubscript{12}H\textsubscript{26}, and C\textsubscript{18}H\textsubscript{30}) via reaction with a siloxane terminus. As noted above, this layer mimics lipid components that make up part of the organic matter of soil. 2 layer ESS’s contain 2 distinct layers of organic functionalization above the silica surface. An 11 carbon aliphatic hydrocarbon chain is attached to the surface as the first layer. The second layer is an aromatic oligomer attached to the first layer to mimic lignin. Three aromatic oligomers have been used; styrene, 4-vinylphenyl acetate, and 4-vinylphenol. These acetates were subsequently hydrolyzed to the phenol. While unsuccessful as of the time of this writing, efforts have been undertaken to synthesize the desired third layer comprised of a hydrophilic oligomer to mirror the cellulose components of soil. Examples of these 2 and 3 tiered systems are shown in Figure 14 below.
A series of ESS samples synthesized with styrene and loaded with phosphates as outlined in Section 3.2.1 below. These ESSs were designed, synthesized, and loaded with phosphates by Robert Cook and group at Louisiana State University (LSU).

**Sorption of Pesticide Probe Molecules**

A series of ESS materials and silica samples were prepared with either AlPO₄, PO₄³⁻, dAMP, or GMP loaded onto the surface. The silica samples were made by first combining fumed silica (cab-o-sil) with a buffer solution (3xSSC). Phosphate compounds, deoxyadenosine monophosphate (dAMP) or guanosine monophosphate (GMP), were then added to this solution to mimic pesticides.¹⁰,¹¹ Phosphates were chosen for this mimic because organophosphates make up about 50% of killing agents in chemical pesticides. These organophosphates inhibit neuromuscular enzymes making them highly effective against insects and therefore widely used. However, these enzymes also function in humans and other animals making these organophosphate pesticides act similar to some nerve agents.¹²

**Sample Preparation**

The base silica was prepared by combining 0.5g cab-o-sil with 20 mL 3xSSC. For sample 2, 150 mg dAMP was added to the silica-SSC mixture. For sample 3, 121 mg GMP was added to
the silica-SSC mixture. These 3 samples were left to sit for 24 hours. At this point, the solvent was separated via centrifuge, then a 0.1% aqueous solution of sodium dodecyl sulfate (NaC\textsubscript{12}H\textsubscript{25}SO\textsubscript{4}) was added and the mixture was left to sit for 30 minutes. This process was repeated with DI water, and then with a 70:30 ethanol in water solution. The samples were then dried in an oven over night and stored in a freezer while awaiting analysis.

A series of silica and ESS samples were also obtained from collaborators at LSU. These samples similarly employed a series of phosphate compounds to mimic pesticides and used a variety of acid/base buffer solutions. A series of silica samples were prepared. The first was not loaded with phosphate, but was only combined with water in its preparation. Three silica samples were loaded with PO\textsubscript{4}\textsuperscript{3-}; one with no buffer, one in HCl, and one in NaOH. An additional silica sample was loaded with AlPO\textsubscript{4} in SSC buffer, and a final silica sample was loaded with dAMP in SSC. Two different ESS surfaces were also included in the LSU samples. One of these was loaded with AlPO\textsubscript{4} in SSC and the second was used in two samples, one loaded with AlPO\textsubscript{4} and the other with dAMP, both in SSC.

**NMR Studies**

These phosphate loaded samples discussed above were analyzed via \textsuperscript{31}P direct polarization and cross polarization solid-state NMR for the presence of phosphates on the silica and ESS surfaces. These experiments were chosen because they easily highlight the presence of \textsuperscript{31}P in samples. Since the ESS and silica bases contain no phosphorus, this signal can only come from phosphate loaded onto the surface. These initial tests, therefore, indicated the ideal conditions for loading phosphates onto surfaces by identifying what samples contained the most phosphorus on the surface. Once these conditions are determined, further studies can focus on the phosphate surface interactions.
As seen in Figure 15, the ESS loaded with dAMP displays virtually no $^{31}\text{P}$ signal, indicating no presence of phosphates loaded onto the surface. In comparison, both ESS1 and ESS2 display evidence of phosphates when treated with AlPO$_4$. Figure 16 below shows the spectra from these AlPO$_4$ treated samples overlaid. These spectra are very similar, while the central ESS2 peak is roughly 27% stronger (intensity of 7362 compared to an intensity of 5779 from ESS1). These spectra were collected using the same number of scans, which means that the relative peak intensities reflect the strength of the signal from each sample. Since only one phosphorous peak is present in each spectrum, and since no phosphorous was present before loading it onto the surface, these relative intensities then indicate the relative amounts of phosphorous loaded in each trial. With no additional ESS1 or ESS2 samples to compare, this difference in intensity cannot be attributed solely to differences in the ESS materials. Slight variations in experimental conditions may have caused this. However, the conclusion can be made that both ESS materials interact with AlPO$_4$ in a similar manner with one phosphorous species ($\text{PO}_4^{3-}$) uniformly bonding to the surface. If more than one phosphorous species had
bonded, or if it was loaded nonuniformly, multiple peaks would have been observed in the spectrum.

Figure 16: Spectral overlay of AlPO$_4$ on ESS 1 (red) and AlPO$_4$ on ESS 2 (blue)
The silica samples from LSU that were loaded with dAMP and AlPO$_4$ agree with the results from the ESS samples. That is, the dAMP showed little evidence of significant bonding with the surface, while the AlPO$_4$ loaded sample displayed strong $^{31}$P signal. These results are shown in Figure 17. These results are unsurprising; due to its bulkier structure, dAMP would be more reluctant to interact with the silica or ESS surface to deposit the phosphate.

The 3xSSC samples prepared at Penn State also agreed with these findings. They showed no DP signal, indicating very little phosphate loaded onto the surface from either dAMP or GMP under the conditions employed in these trials. No samples were prepared with AlPO$_4$ to confirm that these experimental conditions were conducive to phosphate loading from a smaller, more susceptible molecule.
Figure 18 depicts spectra resulting from the LSU silica samples that were treated with PO$_4^{3-}$ under different conditions; acidic, basic, and neutral. These spectra clearly show that acidic conditions (HCl) yielded the greatest results with a strong central $^{31}$P peak. On the other hand, neutral conditions yielded virtually no phosphorous on the silica surface.

Finally, Figure 19 below compares ESS and silica surfaces loaded with AlPO$_4$. As discussed above, ESS2 displayed slightly better efficiency at phosphate interaction. By adding the LSU silica sample to this comparison, we can see that both ESS samples are much more efficient than the silica at bonding to phosphates. The peak intensity of the silica sample is 2214 compared to 5779 or 7362 for ESS1 and ESS2 respectively.
From the results discussed above, it can be concluded that acidic conditions are best for treating surfaces with phosphates. Additionally, it can be said that bulkier phosphates such as dAMP and GMP are less efficiently loaded onto surfaces than AlPO$_4$ or PO$_4^{3-}$. Finally, we can see that ESS surfaces bond to phosphates much more efficiently than standard silica surfaces.

**Further Studies**

Going forward, studies can be undertaken to probe the interactions between the artificial soil surfaces and the phosphates loaded onto the surface under the conditions outlined above. These studies should take the form of NMR experiments that correlate $^{31}$P with $^{13}$C and $^{29}$Si. These experiments will indicate what layer of the ESS samples are bonding to the phosphate and how these interactions are occurring.
References


Chapter 4

Conclusions and Future Directions

The work covered above highlights the breadth of areas in which solid state NMR can serve as a useful analytical tool. Thanks to the nondestructive quality of the studies, which are carried out on solid powder samples, ss-NMR is ideal for studying natural products and surface interactions. Here we have discussed such studies of the internal polymers of plant cell walls and the surface interactions between soil surrogates and organophosphate pesticide probe molecules.

Chapter 2 focused on the ongoing debate over plant cell wall structure and efforts to determine an agreed upon model for the polymer interactions that yield the strength and stability of plant cell walls. Primary cell walls were observed by analyzing rosette and cauline leaves from *Arabidopsis thaliana*, and secondary cell walls were observed by studying inflorescence stems from the same plants. These initial experiments focused on differentiating rigid and mobile polymer substances within primary cell walls and observing lignin signals which are only present in secondary cell walls. A comparison of CP and DP spectra of rosette leaves shows that the most rigid components are internal cellulose microfibrils and that the most mobile components are glycoprotein side chains. A comparison of rosette leaves and cauline leaves shows few significant differences between the two indicating that primary cell walls develop very quickly and don’t evolved over the course of a plants development. Finally, a comparison of cauline leaves and inflorescence stems indicates the absence of highly mobile side chains and the presence of unique signals in secondary cell walls that can be attributed to lignin which is not present in primary cell walls.

Chapter 3 discussed initial experiments carried out to investigate the interactions of pesticides and soils. Silica and ESS materials served as soil surrogates and the phosphates *AlPO*$_4$, *PO*$_4$$_3^-$, dAMP, and GMP served as pesticide probe molecules. Soil surrogates loaded with these phosphates under acidic, basic, and neutral conditions were analyzed and compared to determine ideal conditions for synthesizing soil-pesticide samples in future experiments. These comparisons indicated that acidic conditions were the ideal conditions for phosphate loading. Additionally, it was found that the size of phosphate is indirectly correlated to the efficiency of
loading onto the soil surface. Finally, it was determined that ESS samples were more susceptible to phosphate loading than silica surfaces.

**Future Directions**

The work discussed here displays the great variety of solid state NMR applications. However, these experiments only represent the initial steps that should be taken to answer the questions of cell wall polymer interactions or soil-organophosphate pesticide interactions.

Both primary and secondary cell wall samples should be studied more in depth using multi-dimensional solid state NMR. Efforts to develop a 2D INADEQUATE pulse sequence on the instruments in Penn State NMR facility have been unsuccessful at this time, but these efforts should continue, and in the meantime these experiments should carried out using facilities with these capabilities. These experiments will be very valuable in determining interactions occurring between cell wall components. Higher field experiments should also be carried out on secondary cell wall samples to further isolate the lignin signals from the overlapping cellulose signals. Once these lignin components are fully identified, the development of the secondary cell wall and the lignification process can be studied by carrying out experiments on plant material from various stages of the plants life.

Like the plant cell wall experiments, the soil surrogate experiments have only laid the foundation for further studies. The experiments discussed in chapter 3 have been used to determine the ideal phosphate sources, soil surrogates, and experimental conditions under which to load the samples. Further studies should use these conditions to develop soil/phosphate samples to be analyzed for a greater understanding of the interactions taking place. Specifically, correlation experiments should be carried out to determine what layer of the ESS materials are interacting with the phosphates. Additionally, further efforts should be made to synthesize 3-tiered ESS materials that will more closely approximate natural soils.