

FOREST PRODUCTS LABORATORY

Rooted in Research

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Improved Methods for Gel-State 2D NMR of Plant Cell Walls

Lignocellulose, found in plant cell walls, is primarily composed of cellulose, hemicelluloses, and lignin. Lignin is the largest renewable source of aromatics on Earth. Understanding the composition and structure of lignocellulose is essential for elucidating the molecular architecture of plant cell walls and chemical features governing polymer interactions, but lignocellulose is arranged in a complex matrix that resists dissolution and structural analysis.



Lignocellulosic biomass from poplar that has been dried in an oven at 50°C and ground in a ball mill in preparation for analysis with gel-state 2D NMR. Forest Service photo by Nathaniel Sapp.

Traditional analytical approaches to understanding lignocellulose structure involve dissolving cell walls and often require harsh chemical treatments or fractionation steps, such as isolation of lignin, that risk altering native structures and adding time and cost. Use of gel-state 2D nuclear magnetic resonance (NMR), which swells the whole cell wall material rather than fully dissolving it, enabled important advances. However, this approach has historically suffered from challenges,

including a limited ability to resolve and distinguish features in the NMR spectrum (poor spectral resolution), an unstable main magnetic field across the sample (unstable shimming), and inadequate sample homogenization, which can be influenced by

PUBLIC BENEFITS

Improved methods for conducting structural analysis of plant cell walls will help facilitate conversion of plant biomass into fuels, chemicals, and other materials.

high moisture content of the sample disrupting the effectiveness of ball milling.

Research chemist Hoon Kim, research forest products technologist Daniel Yelle, and Peter Kitin forest products technologist from the Forest Service Forest Products Laboratory, along with collaborators from two universities, advanced NMR analyses for whole cell walls by developing a finely pulverized low-moisture gel-state 2D NMR method. This new approach integrates controlled drying, optimized ball milling, and a mixed Dimethyl sulfoxide- d_6 /Dimethylformamide- d_7 (DMSO- d_6 /DMF- d_7) solvent system. This method produces sharp, high-resolution spectra across hardwoods, softwoods, and grasses, enabling simultaneous detection of lignin subunits, interunit linkages, and polysaccharides directly from intact biomass.

KEY SCIENTIFIC IMPLICATIONS

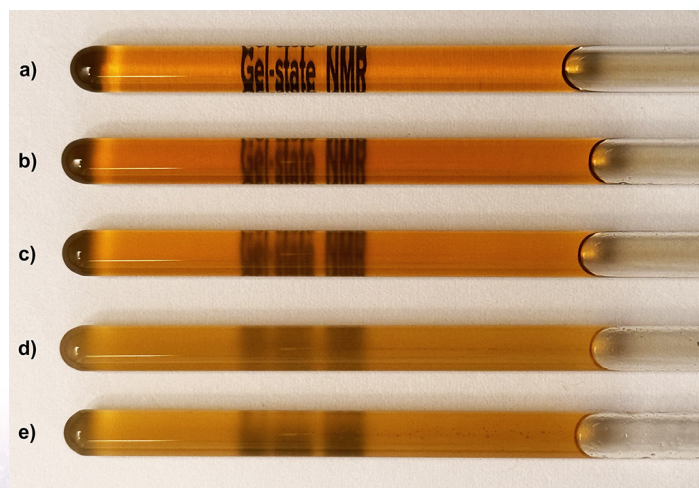
- This improved method offers a robust, reproducible framework for whole cell-wall 2D NMR analysis.
- The method improves resolution and chemical-shift consistency, reducing errors in structural assignment.
- Cell-wall structure can be compared reliably across different species and chemical treatments, making the method suitable for routine screening and profiling the chemical structure of many biomass samples simultaneously. This supports research into transgenic plant development, biomass conversion, polymer chemistry, and material design.
- The method provides a foundation for sample preparation. How dry and fine a biomass sample is controls the quality of the resulting NMR spectra, so other laboratories can reproduce the results.

Method Improvement

The method that Kim, Yelle, Kitin, and their colleagues developed improves the following main steps:

Improved drying and milling of samples: Maintaining low residual moisture improves ball-milling efficiency to obtain finer, more uniform cell wall powders accessible to perdeuterated solvents, which is essential for achieving reproducible gel formation. Oven drying at 50 °C (122 °F) or freeze-drying consistently produced biomass with low residual moisture, formed uniform, translucent gels upon swelling in DMSO- d_6 /DMF- d_7 , and these gels provided improved shimming performance and enhanced spectral resolution.

Improved cosolvent system: The improved DMSO- d_6 /DMF- d_7 (4:1, v/v) cosolvent system almost completely dissolves cell walls, stabilizes chemical shifts, and preserves native structural features without introducing spectral interference of the cell wall polymer peaks. DMF- d_7 also replaces the pyridine- d_5 used in the previous gel-state method, removing a toxic, strong-smelling component while keeping the gel clear and stable. Spectral reproducibility is markedly improved, and shimming issues associated with earlier solvent systems are resolved, resulting in better spectral quality across diverse biomass types. Dimethylacetamide- d_9 (DMAc- d_9) was also tested and produced similar results to DMF- d_7 , but the latter is more widely available and is less expensive.



Ball-milled aspen cell wall samples in NMR tubes from different preparation conditions. Forest Service photo by Hoon Kim.

Benefits of the Improved Method

The finely pulverized low-moisture gel-state 2D NMR method is an improvement over traditional methods for multiple reasons, including:

- Enabling high-resolution analysis of intact lignocellulosic biomass without harsh chemical treatment and structural modification.
- Improving shimming stability and spectral reproducibility through controlled moisture reduction and fine milling.
- Detecting fundamental lignin aromatic units (syringyl, guaiacyl, and *p*-hydroxyphenyl), interunit linkages (β -O-4, β -5, and β - β), and polysaccharide structures simultaneously.
- Allowing application across diverse biomass types including hardwoods, softwoods, and grasses.
- Providing more complete structural information for bioenergy, wood products, and plant science research.

FOREST SERVICE CONTACTS

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FURTHER READING

Kim, H.; Mao, Y.; Yelle, D. J.; Rencoret, J.; Del Río, J. C.; Kitin, P. 2026. [Finely pulverized low-moisture \(FPL\) gel-state NMR for near-completely solubilized whole plant cell walls](#). *Magnetic Resonance in Chemistry*. 64(8): 672-681. DOI: 10.1002/mrc.70106.

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For questions on Rooted in Research, please contact [Matthew Richardson](#). Technical editing by [Susan Barro](#). Design and layout by [Jeremy Siegrist](#).



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