HSQC (heteronuclear single quantum coherence) $^{13}$C-$^1$H correlation spectra of whole biomass: A powerful tool for evaluating pretreatment

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**Introduction**

The lignocellulosic matrix of native plant biomass is naturally recalcitrant which poses a significant challenge for the cost-effective conversion of plant polysaccharides to 2nd generation biofuels. Recent advances in genetic plant engineering indicate that it is possible to decrease the recalcitrance of plants with obvious benefits to the conversion of cellulosics to bioethanol. The production of next generation low-recalcitrant plants has placed a new challenge to high-resolution facile characterization of plant cell wall chemistry. The traditional method for the characterization of lignin, cellulose and hemicellulose involves the isolation of the individual components by lengthy separation procedures followed by spectroscopic techniques. We have utilized a simple ionic liquid,perdeuterated pyridinium chloride from commercially available deuterated pyridine-d5 and established that a 1:3 mixture of deuterated pyridinium chloride-DMSO-d6 solvent is an effective solvent system for whole cell biomass dissolution and NMR characterization. Employing this solvent system, heteronuclear single quantum correlation (HSQC) spectroscopy of different pretreated biomass samples was readily accomplished and established this methodology as a semi-quantitative procedure for the analysis of lignin and hemicellulose structures in plant cell wall constituents.

- **2D HSQC spectra**
  - DRX 500 MHz NMR spectrometer
  - At 333K
  - Scans: 256

- **Synthesis of perdeuterated pyridinium chloride**
  - Methanol-d4 and pyridine-d5 (1:1) in anhydrous ethyl ether
  - Stirred in ice water for 2 min
  - Acetyl chloride (1 equivalent) in anhydrous ethyl ether was added drop wise
  - The reaction mixture was stirred for 30 min
  - Filtered to get a white solid, dried under vacuum
  - Ionic liquid pyridinium chloride-d6 (90%)

- **Plant cell wall preparation for NMR analysis**
  - Poplar extracted successively (24 h each) with ethanol-benzene mixture and ethanol
  - The extractive free, dry sample was placed in a mixer mill
  - Equipped with two 10-ML stainless steel jars each with one stainless steel ball bearing
  - Grind for 1 hour at 25 Hz.

- **Biomass dissolution**
  - DMSO-d6: Perdeuterated pyridinium chloride 1:3 (w/w)
  - In a 5 mL stopped vial 60 mg milled biomass sample
  - 0.60 g perdeuterated pyridinium chloride-DMSO-d6 mixture was added
  - Stirred at 60°C for 2h.
  - Viscous solution was transferred directly into a 5 mm NMR tube

- **Results and discussion**

- **Table 1:** Various Pretreatment conditions on biomass-poplar NRELHPT000003

- **Table 2:** Semi-quantitative estimation of various lignin and hemicellulose units in Poplar

- **Conclusions**
  - Developed an efficient perdeuterated solvent system for biomass dissolution.
  - Cellulose, hemicelluloses and lignin were characterized from HSQC spectra of biomass.
  - Semi-quantitatively estimated the recalcitrance changes after pretreatment.

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