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1064 nm dispersive multichannel Raman spectroscopy for the analysis of plant lignin

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ABSTRACT

The mixed phenylpropanoid polymer lignin is one of the most abundant biopolymers on the planet and is used in the paper, pulp and biorenewable industries. For many downstream applications, the lignin monomeric composition is required, but traditional methods for performing this analysis do not necessarily represent the lignin composition as it existed in the plant. Herein, it is shown that Raman spectroscopy can be used to measure the lignin monomer composition. The use of 1064 nm excitation is needed for lignin analyses since high fluorescence backgrounds are measured at wavelengths as long as 785 nm. The instrument used for these measurements is a 1064 nm dispersive multichannel Raman spectrometer that is suitable for applications outside of the laboratory, for example in-field or in-line analyses and using remote sensing fiber optics. This spectrometer has the capability of acquiring toluene/acetonitrile spectra with 800 cm^{-1} spectral coverage, 6.5 cm^{-1} spectral resolution and 54 S/N ratio in 10 s using 280 mW incident laser powers. The 1135–1350 cm^{-1} and 1560–1650 cm^{-1} regions of the lignin spectrum can be used to distinguish among the three primary model lignin monomers: coumaric, ferulic and sinapic acids. Mixtures of the three model monomers and first derivative spectra or partial least squares analysis of the phenyl ring breathing modes around 1600 cm^{-1} are used to determine sugarcane lignin monomer composition. Lignin extracted from sugarcane is shown to have a predominant dimethoxylated and monomethoxylated phenylpropanoid content with a lesser amount of non-methoxylated phenol, which is consistent with sugarcane's classification as a non-woody angiosperm. The location of the phenyl ring breathing mode peaks do not shift in ethanol, methanol, isopropanol, 1,4 dioxane or acetone.

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1. Introduction

Lignin is one of three primary biopolymers in plant cell walls [1,2]. It is a heteropolymer composed of phenylpropanoid monomers with the aromatic portions: *p*-hydroxyphenyl, guaiacyl, and syringyl. These monomers are often referred to by the one letter designation H, G, and S, respectively. Lignin is classified as heterogeneous not only because of its complex composition, but also because lignin from different species, tissue types, plant age and growing conditions can be quite different. Lignin is classified as predominately G (gymnosperms), G/S (angiosperms) and G/S/H (non-woody angiosperms), although many exceptions to these classifications are known [3–5]. Lignin has many commercial uses in the pulp and paper industry and as the raw material for biorenewable fuels and commodity chemicals. For these applications it is desirable to know the lignin purity and composition. For example, the resulting complex chemical mixture generated from lignin pyrolysis (i.e., bio-oil) is dependent on the starting monomer composition [6]. Additionally, the degradation of lignin in the environment is slow, and the monomer composition is a biomarker that can be used to identify the source of sediments and soils containing decaying plant materials [6]. Reported lignin monomer compositions in the literature have large variations due to the commonly utilized extraction and analysis procedures, which can alter phenylpropanoid structure [5,7–9].

Raman spectroscopy has been used to measure chemical content in a wide-range of samples [10–14]. The appropriate selection of excitation wavelength is one of the most important factors for Raman spectroscopy experiments. Near infrared (commonly 785–1064 nm) excitation is often used for detecting biological materials that can exhibit fluorescence backgrounds using visible wavelengths [15]. The intensity of Raman scatter is proportional to the incident frequency to the fourth power. Using the same laser radiance, excitation with 785 nm will generate 3.8-times more scattered photons compared to 1064 nm excitation. Despite the low energy of the photons, and even with low fluorescence quantum

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yield samples, a fluorescence background may render the Raman spectrum undetectable or increase detection limits using 785 nm excitation [16]. In these cases, the lower background obtained with 1064 nm excitation often outweighs the reduced scattering intensity [17]; and higher laser radiances may be used to compensate for the lower Raman cross section above one micron. The use of up to 2.3 W incident 1064 nm laser powers have been reported in the literature for Raman measurements [16,18].

Reports of multichannel dispersive 1064 nm Raman spectrometers date back to 1986 [19], yet only a handful of references using a dispersive 1064 nm instrument are found in the recent literature. Fourier Transform (FT) Raman spectroscopy remains the primary instrument format for obtaining fluorescence-free Raman spectra. Yet, dispersive systems are better suited for many applications where robust, low cost instruments and remote sensing fibers are desirable [20–22]. Recently, portable Raman spectrometers have been made commercially available [23]. However, many of these systems are limited in performance characteristics, such as spectral range and spectral resolution.

The main limitation of 1064 nm dispersive Raman spectrometers is the availability of suitable low-noise detectors. Single channel detectors require minutes to hours to collect a 1064 nm Raman spectrum [18,24–26]. Multichannel dispersive Raman instruments with excitation wavelengths below 800 nm commonly utilize charge coupled devices (CCD). CCDs have a low response beyond 1.1 μm, limiting their use in 1064 nm Raman spectroscopy [27]. Hamaguchi *et al.* overcame this limitation using a transfer electron InP-InGaAs photocathode image intensifier CCD for dispersive 1064 nm Raman spectroscopy for the analysis of DNA and lung tissue [16,26,28]. This detector has a ~4% quantum efficiency between 1 and 1.4 microns (0–2250 cm^{-1} Raman shift) and required lengthy acquisition times. CCDs have also been used to collect dispersive 1064 nm excitation anti-stokes Raman spectra [29].

Indium gallium arsenide (InGaAs) detectors with quantum efficiencies above 80% have high sensitivity between 900 and 1,700 nm. This enables detection out to the 3,100 cm^{-1} C–H stretching region using 1064 nm excitation. These quantum efficiencies are substantially higher than those of the competing germanium detectors [30]. Dispersive multichannel 1064 nm Raman spectrometers equipped with 128 or 256 element Ge or InGaAs arrays have been used to measure strong Raman scattering solids and liquids (e.g., toluene, anthracene) [26,31–33]. These multichannel InGaAs detectors had high dark and read noise and provide limited 100–500 cm^{-1} spectral coverage in a single acquisition. The development of improved multichannel InGaAs detectors with 1024 or more elements and low read noise has opened the possibility of improved 1064 nm dispersive multichannel Raman instruments with wide spectral coverage that compete with FT Raman instruments.

Raman spectroscopy is an ideal non-invasive screening method to measure lignin composition. Lignin has been previously analyzed by Kerr gated resonance Raman spectroscopy using a benzoquinone/laccase/mediator treatment to generate a charge transfer state that extends lignin's absorption out to 500 nm [34]. Ultraviolet RR spectroscopy has also been used to measure lignin since the fluorescence signal is spectrally separated from the Raman spectrum at these wavelengths [35]. FT Raman and surface enhance FT Raman spectroscopies using a 600 mW 1064 nm laser have been used to measure and assign lignin spectral peaks [36]. The presence of lignin in plant tissues has also been measured by Raman spectroscopy [37,38]. Finally, the spectral differences of three model lignin monomers were identified experimentally and using DFT calculations [39], but were not extended to real plant lignin samples or monomer mixtures. The reported differences in the monomer spectra reveal the possibility of measuring lignin compositions using Raman spectroscopy.

Herein, we describe a dispersive 1024-multichannel 1064 nm Raman spectrometer with wide spectral coverage, S/N values that exceed those of a FT Raman instrument for most acquisition times, and background values orders of magnitude lower than a dispersive 785 nm Raman instrument for the analysis of lignin and model lignin monomers. The results show that the new generation multichannel InGaAs array detectors produce high quality spectra with ~280 mW laser radiance using relatively fast acquisition times, and will enable facile measurements in laboratory and other settings. It is demonstrated that Raman spectroscopy can be used to quantitatively measure the lignin monomer composition using partial least squares analysis and qualitatively using first derivative spectra.

2. Experimental

2.1. Materials

All chemicals were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO). Hydrolytic lignin (Aldrich, CAS 8072-93-3) was extracted from sugarcane. Aqueous solutions were prepared using deionized water (18.2 MΩ cm^{-1}) from an Ultrapure II water system (Thermo Scientific, Waltham, MA).

2.2. Dispersive multichannel 1064 nm instrumentation

A home-built 1064 nm dispersive multichannel Raman instrument is shown in Fig. 1. A 1064 nm Nd:YVO_4 solid state laser (Blue Sky Research, Milpitas, CA) with a maximum output power of 1 W was used as the excitation source. The beam was focused at the sample with a plano-convex lens with a focal length of 250 mm (Newport Optics, Irvine, CA). Liquid samples were placed in a nuclear magnetic resonance tube (Sigma–Aldrich), and mounted on a home-built sample stage. Raman scatter was collected in a 90° collection geometry. The light was collected with a plano-convex lens with a focal length of 60 mm (*f*/1.2). The light was focused with a plano-convex lens with a focal length of 200 mm (*f*/3.9). The optical system was designed to slightly overfill the 150 μm entrance slit of the spectrometer to maximize signal. A holographic super notch filter (HNF) centered at 1064 nm (Kaiser Optical Systems, Ann Arbor, MI) was placed before the spectrometer. To aid in

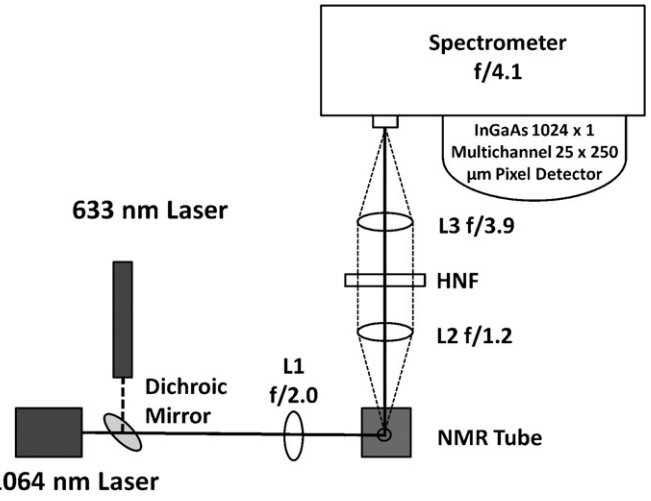

Fig. 1. Instrument schematic of the 1064 nm dispersive multichannel Raman spectrometer. The 1064 nm laser is focused with a plano-convex lens (L1) onto a sample. The Raman scatter is then collected with a plano-convex lens (L2) and focused onto the spectrometer slit with a plano-convex lens (L3). A holographic notch filter (HNF) was used to filter out Rayleigh scattering. The spectrometer is equipped with a 1024-multichannel InGaAs detector. The helium–neon laser is aligned co-linearly with the 1064 nm laser using a dichroic mirror to aid in instrument alignment.