

# Using Nano- and Micro-Particles of Silver in Lignin Analysis

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## ABSTRACT

Although there are numerous techniques available to study lignin in its various states, they all have limitations and to extract most amount of information, a number of analytical techniques have to be jointly used. We have recently started applying a new approach to lignin analysis - namely using nano- and micro-particles of silver for study of native and residual lignins by surface enhanced Raman spectroscopy (SERS). Silver particles were used for their unique electronic and optical properties (in this case, localized surface plasmon resonance). The SERS technique is highly sensitive and extremely selective. These attributes allow detection of small amount of lignin in presence of other constituents in a sample. For example, in wood and pulp samples, no signal from carbohydrates was detected and lignin was analyzed directly. Additionally, isolated milled-wood lignin was investigated with high sensitivity. This nano- and micro-particle based sensing could make a significant contribution to investigating lignin in difficult-to-analyze samples.

## INTRODUCTION

Wood and other lignocellulosics (agricultural residues, water plants, grasses, and other plant substances) are heterogeneous natural polymer composites with large variations in physical and chemical properties. These natural materials are composed of cellulose, lignin, and hemicelluloses. Lignin is the second most abundant organic material on earth; cellulose is the most abundant. Compared with cellulose and hemicelluloses, both of which have regular chemical structures, the structures of lignins are highly irregular [1] (Fig. 1 illustrates the molecular structure of softwood lignin). Because of this fact, even in their native states, the structures of lignins have proven to be difficult to study. The same holds for other modified states of lignins, such as in wood pulp where residual lignin poses similar characterization problem [2]. Because of their high molecular weight, lignin polymers cannot be isolated without significant structural modification. Lack of detailed information on lignin structures has hindered progress in a number of science and technology fields. Areas as diverse as lignin biosynthesis, plant cell wall lignification, forage digestibility, delignification in pulping and bleaching, biodegradation of agro-materials, genetic modification of plants, and biomass utilization are likely to benefit from an improved understanding of lignins at the molecular level. Surface enhanced Raman technique [3] is an ultrasensitive analytical method that has the capability to both detect single molecules [4, 5] and provide their detailed structural information. It is hoped that this ultrasensitive nature and Raman's *in situ* diagnostic capability will aid in investigating lignins in biomaterials and bioproducts, which would not only enhance the quality of products but also lead to more efficient processes for utilization of lignocellulosics.

Over the years, numerous techniques have been applied to study lignins [6, 7]. Whereas a large number of the techniques can be applied to study isolated lignins in solution state, only a few yield information when lignin is present in the solid state—either isolated or in the presence of carbohydrate polymers. Raman and infrared (IR) spectroscopy both belong to this latter category. Compared to IR, Raman investigations of lignins are relatively recent [8-20]. Nevertheless, this emerging methodology has the potential for obtaining much more informative data from lignin samples. As a solution-state technique for examining lignins, NMR has proven to be the most useful, although this technique suffers from the fact that lignin needs to be isolated first and isolated lignin represents only a small part of the total amount in the sample [21].

Surface Enhanced Raman Scattering (SERS) is a special phenomenon within the field of Raman spectroscopy and involves generation of enhanced Raman signal (by as much as  $10^8$ ) from analyte molecules adsorbed on roughened metal surfaces (usually nanoparticles of silver and gold) [22]. Although some enhancement is due to chemical contribution (electronic interaction between metal and adsorbate) to SERS, the main reason why SERS produces extraordinary enhancements is the electromagnetic property of nanostructures. Recent advances have made SERS a versatile technique having diverse fields of application, not only in analytical science but also in biomedicine, environmental monitoring, and artwork conservation. Detailed structural characterizations of various materials such

as single-wall carbon nanotubes, polymers, and self-assembled layers have been carried out. An additional advantage of SERS when applied to the study of fluorescent materials is that it effectively prevails over the fluorescence problem. We have only recently started developing the SERS technique to analyze lignocellulosics, and so far the preliminary results are very encouraging.

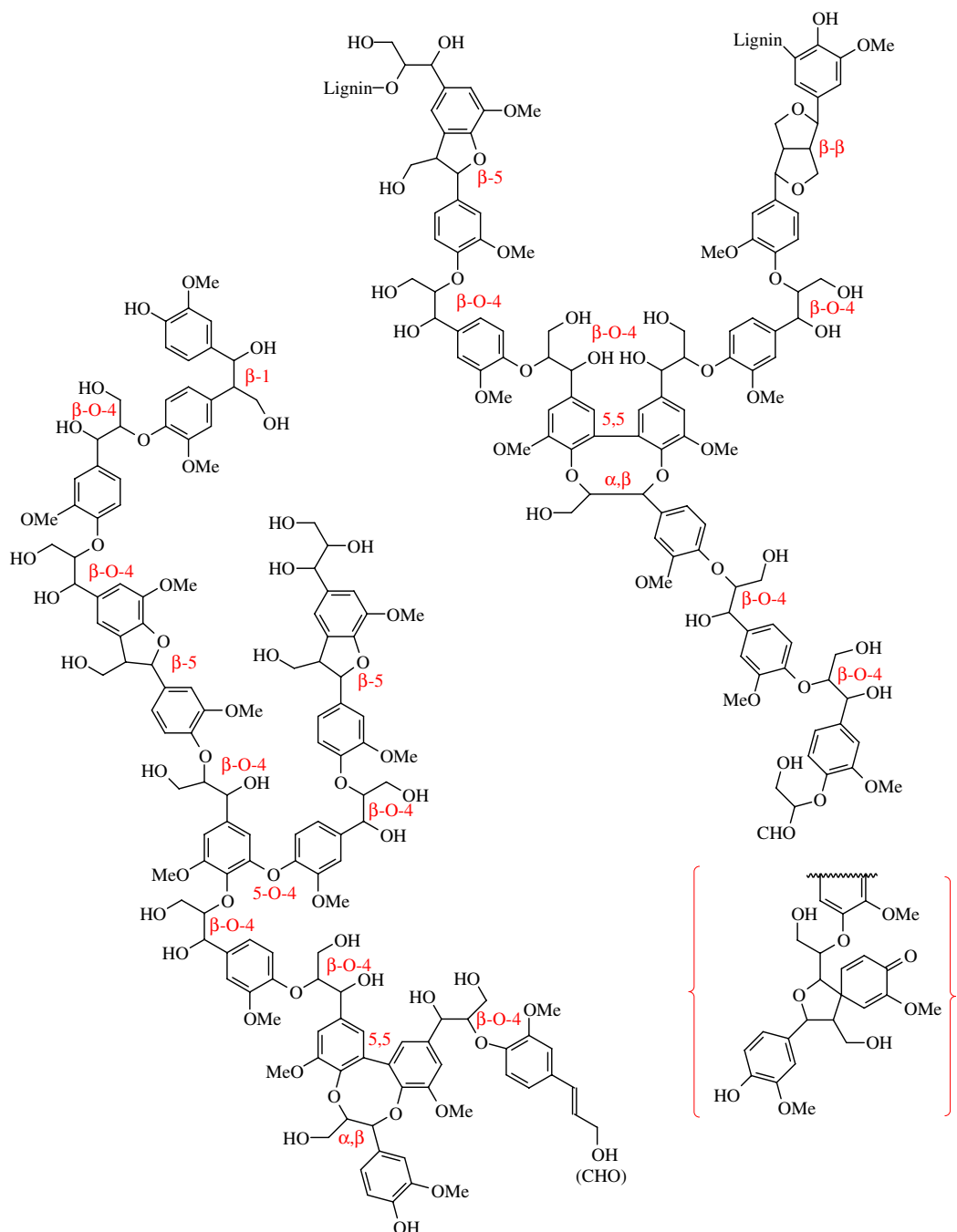


Fig. 1: Representation of molecular structure of softwood lignin. Although the lignin polymer is not a regular polymer with a particular constitutional repeat unit, the phenyl propane ( $C_9$ ) unit is considered a skeleton unit linked to other  $C_9$  units in 3D. The structure shown here incorporates recently discovered dibenzodioxocin and spirodienone units in lignin. Main interunit linkages in lignin are annotated.

## Description of SERS

SERS is a Raman spectroscopy technique based on unusual optical properties of noble metal nanoparticles. This enhancement of Raman scattering cross sections is shown by the nanoparticles of a number of noble metals but has been found to be the highest for silver [3]. SERS signal mostly arises from the localized surface plasmon resonance (LSPR) of the nanoparticles [22]. This resonance occurs when the appropriate wavelength of light strikes silver (Ag) or some other noble metal nanoparticle, causing the coherent oscillation of the free electrons in the conduction band. This oscillation is localized within the near surface region of the particle. There are two consequences of exciting the LSPR: selective photon absorption and generation of locally enhanced electromagnetic field at the nanoparticle surface. The LSPR for Ag nanoparticles, 20 to several hundred nanometers in size, occurs in the visible and IR region of the spectrum and can be measured by UV–visible–IR spectroscopy. Both size and shape of the particles influences the  $\lambda_{\text{max}}$  of the LSPR; increasing the particle aspect ratio shifts the maximum to longer wavelength.

### *Size-Dependent Nanoparticle Optics*

A number of methods have been used to produce a large variety of nanoparticles and 2D arrays [23-27]. The synthesis of different size noble metal particles in the 20–1000-nm range has allowed investigation of size-dependent optical behavior of nanoparticles. Specifically the effects of nanoparticle size, shape, interparticle interactions, and nanoparticle substrate interaction as well as those of solvent, dielectric over layers, and molecular adsorbates have all been investigated.

In this report initial results of applying SERS methodology to study wood and pulp using commercially available Ag particles are presented.

## **EXPERIMENTAL**

Unbleached mixed pine kraft pulp was obtained from a commercial source. Black spruce wood was same as used before [11]. Both samples were ball milled using the procedure outlined in reference [11]. Black spruce milled-wood lignin used was previously isolated using the procedure also described in reference [11].

Silver particles, sizes 100-150 nm and 2-3.5  $\mu\text{m}$ , were purchased from *Sigma-Aldrich Co.* Additionally, benzoic acid and t,t-muconic acid were also purchased from the same company. Ethanol, 200% proof, was from Aaper alcohol and chemical company, Shelbyville, KY. Cellulose powder CC31 was purchased from Whatman co.

For soluble samples, sample preparation involved using a few micrometers of the solution (low concentration) and mixing it with ~15-20 mg of Ag particles and mixing them well using a spatula in presence of absolute ethanol. For solids, small amounts (a few mgs) were mixed with Ag. Once the mixture was completely dry it was sampled in an Al well - a Raman sampling accessory.

A Bruker RFS-100 near-IR FT-Raman spectrometer was used to obtain spectra. The instrument has been described previously [11]. For most acquisitions, 660 mW of unfocused laser power was used and 1024 scans were accumulated.

Scanning electron micrographs (SEMs) of Ag particles and ball milled kraft pulp were obtained. For this purpose, samples were gold coated using a sputter coater and examined and photographed with a Zeiss EVO 40 SEM.

Diffuse reflectance UV-Vis and near-IR spectra were recorded using, respectively, Hitachi 3010 UV/Visible and Thermo Nicolet's Antaris systems.

## **RESULTS and DISCUSSION**

Benzoic acid and muconic acid were both used as target molecules to evaluate the suitability of commercially obtained Ag nano-substrates. Muconic acid was chosen because it is one of the by-products of lignin oxidation. In our laboratory, both these models produced good SERS spectra using commercial Ag particles (Figs. 3 and 4).

Benzoic acid is known to be SERS active [28] and this behavior is supported by the SERS spectrum in Fig. 2 which was produced using the commercially obtained 100-150 nm Ag particles (average particle size). Likewise, for muconic acid SERS spectra shown in Fig. 3 inexpensive 2-3.5  $\mu\text{m}$  Ag particles were used. In our preliminary work, it was observed that both the 100 nm and 2-3.5  $\mu\text{m}$  size Ag particles gave equally good SERS spectra with either of the target molecules.

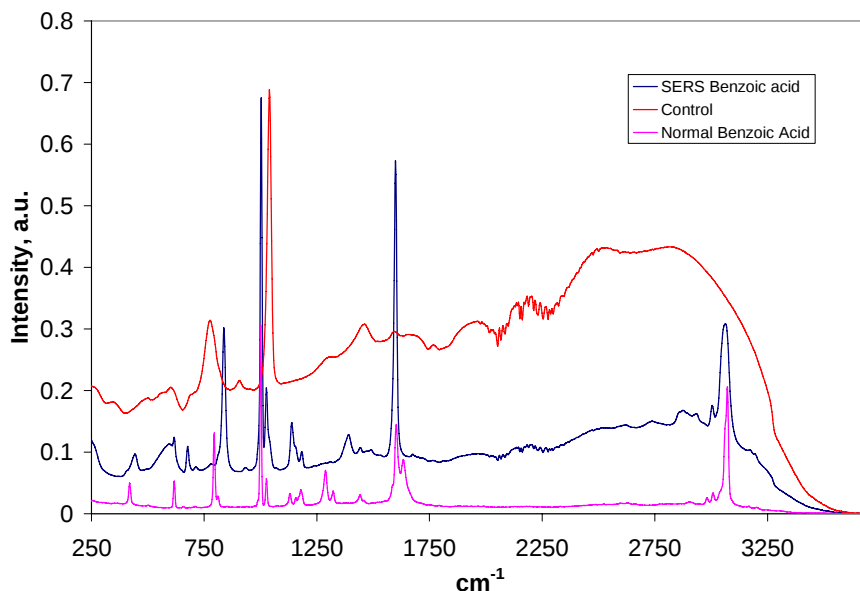


Fig. 2: Surface enhanced near-IR Raman spectrum (middle) of benzoic acid (50  $\mu\text{L}$  of 1 mg/mL acid solution on 15 mg of Ag (100-150 nm), only a few micrograms of the acid was sampled). Also shown are normal spectra of Ag particles (top) and solid benzoic acid (bottom).

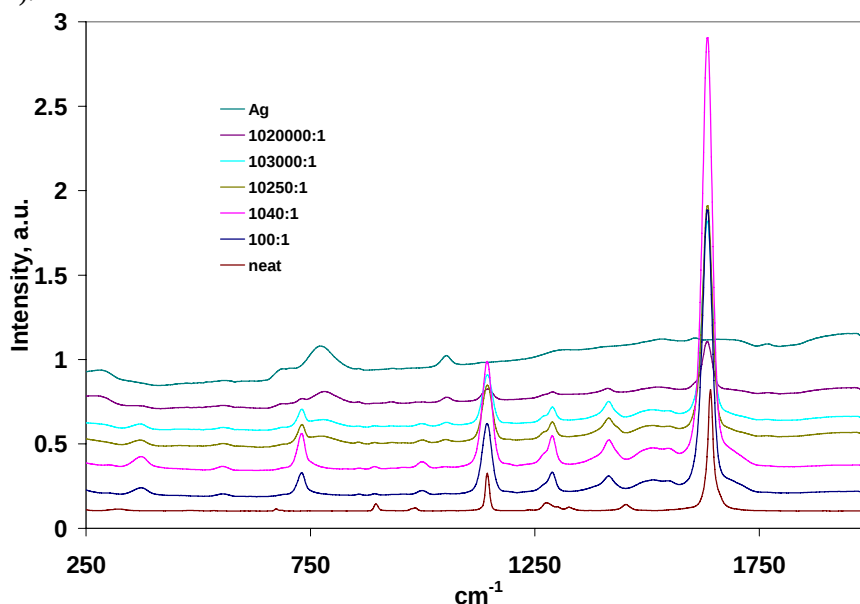


Fig. 3: Surface enhanced near-IR Raman spectra of t,t- muconic acid (at various Ag:Acid dilutions; from top to bottom — Ag particles to neat). All spectra were obtained using the 2-3.5  $\mu\text{m}$  Ag particles. Also shown are normal spectra of Ag particles and neat muconic acid.

Commercially obtained Ag particles clearly produced the SERS enhancement for both benzoic and muconic acids. The enhancement factor, calculated for the t,t- muconic acid spectra, showed that the scattering cross section for the SERS spectrum was enhanced by  $10^6$ , a value that is similar to other SERS enhancement factors reported in the literature [3, 22, 24].

To characterize the commercially obtained Ag particles, excitation spectra of 2-3.5  $\mu\text{m}$  particles were obtained in the UV-Vis and near-IR regions (Fig. 4). These spectra resemble those of interacting/aggregated Ag particles [3, 22, 24] and showed significant absorption at 1064 nm or  $9398\text{ cm}^{-1}$  (near-IR region).

Because our intent was to produce SERS in pulp samples, we used the nm and  $\mu\text{m}$  Ag particles to analyze unbleached kraft pulp (KP). Unbleached KP is difficult to study in visible and near-IR Raman spectroscopy because the sample fluorescence signal is strong and swamps the much weaker Raman scattering [15]. SEM images of Ag particles and ball-milled pulp are shown in Fig. 5 (respectively, on left- and right-hand sides).

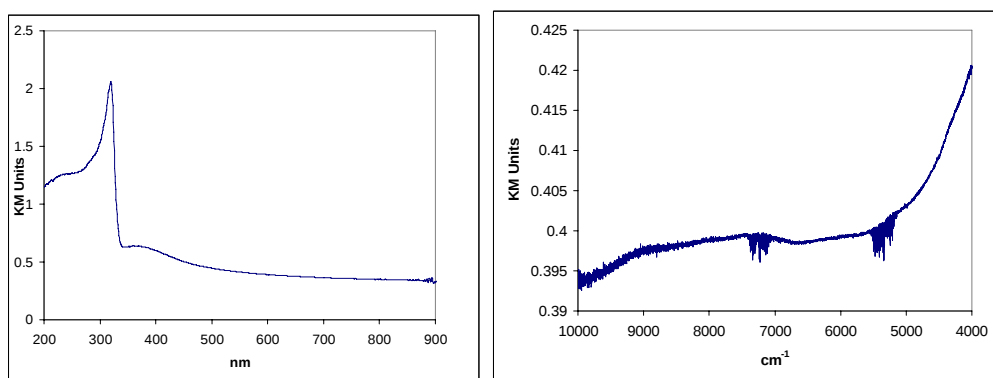


Fig. 4: Diffuse reflectance excitation spectra (UV-Vis and near-IR) of commercial 2-3.5  $\mu\text{m}$  silver particles/aggregates. Significant extinction present at  $9398\text{ cm}^{-1}$  (1064 nm) where near-IR Raman SERS measurements were made is likely to be responsible for the SERS effect.

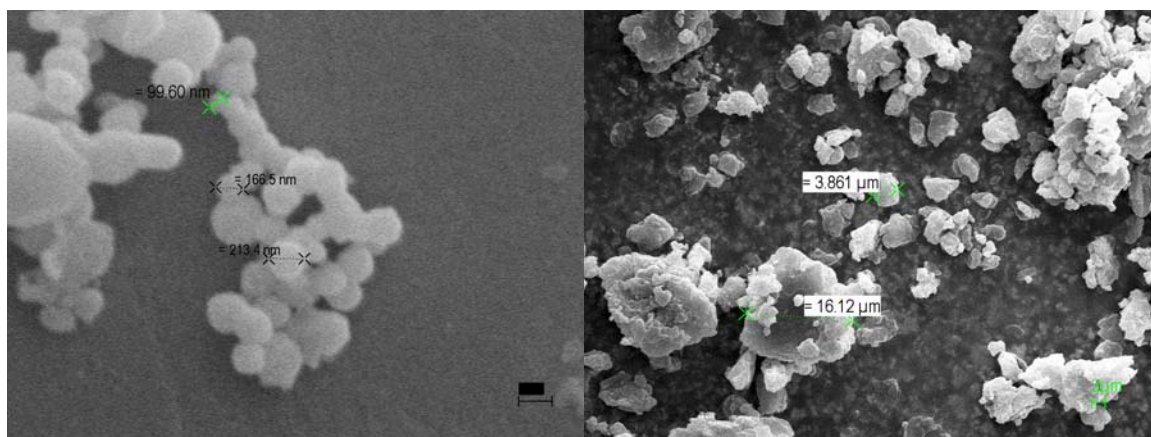


Fig. 5: Scanning electron micrographs (SEMs) of aggregated silver particles (left) and ball-milled kraft pulp (right). Particle sizes in both cases were widely distributed.

When a ball-milled unbleached kraft pulp (KP) sample was intimately mixed with silver particles, the Raman spectrum of the pulp was significantly enhanced and the fluorescence was suppressed (Fig. 6). The surprising result was that no carbohydrate signal was detected. The SERS spectrum produced from pulp is likely to be due to isolated residual lignin because bands in the aromatic stretch region were detected and bands in the carbohydrate regions were absent. It implied that in a multi-component pulp sample, SERS can be used to selectively investigate one

component—in the present case, residual lignin. This was found to be the case when additional samples were studied (see later).

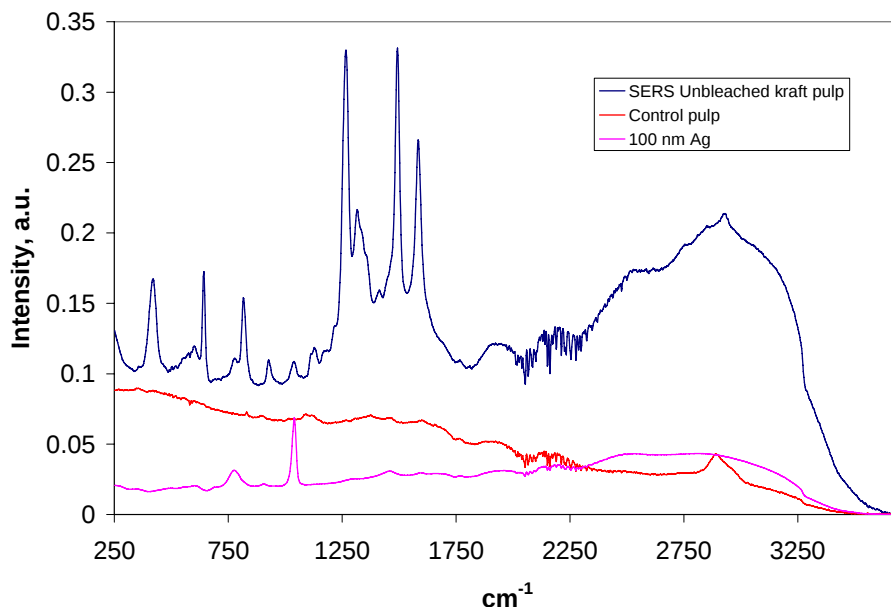


Fig. 6: Surface enhanced and normal near-IR Raman spectra of various samples: top, SERS of ball-milled unbleached KP (pulp-to-Ag weight ratio 1:1); middle, same pulp but without Ag; bottom, 100-150 nm Ag particles. Spectra have been vertically offset for improved visualization. In the pulp SERS spectrum, most enhanced bands were present between 250 and 1800  $\text{cm}^{-1}$ . In normal pulp spectrum (middle) hardly any peaks were detected due to high fluorescence.

To study the effect of Ag particle size, larger size particles (2-3.5  $\mu\text{m}$ ) of Ag were used. However, the SERS spectrum of the KP was similar with regards to both band positions and peak intensities (Fig. 7). The minor differences present were mostly due to Ag. This indicated that the SERS spectrum of unbleached KP was insensitive to Ag particle size, at least in the size range investigated.

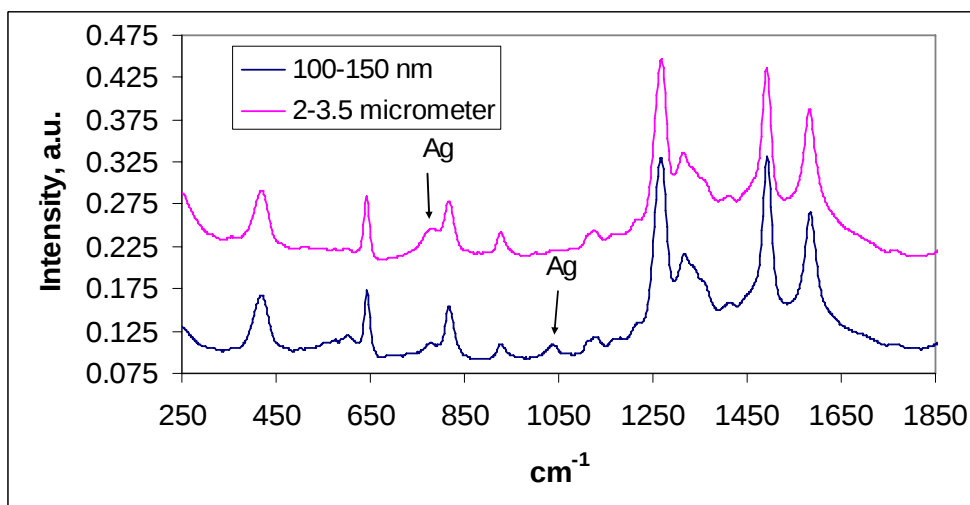


Fig. 7: Unbleached KP SERS spectra with different size Ag particles. Spectra are very similar except for the differences due to contributions of Ag particles.

To find out if any signals in the KP SERS spectrum were due to cellulose, a pure cellulose powder sample was analyzed using the SERS technique (Fig. 8). As expected, only bands due to Ag substrate were detected and no Raman signal from cellulose was seen. This indicated that nearly all peaks seen in the SERS spectrum of unbleached KP (Fig. 6) are likely to be due to lignin. The other small contributions were from Ag particles and can be easily identified by comparing with the spectrum of the pure Ag particles used in this work.

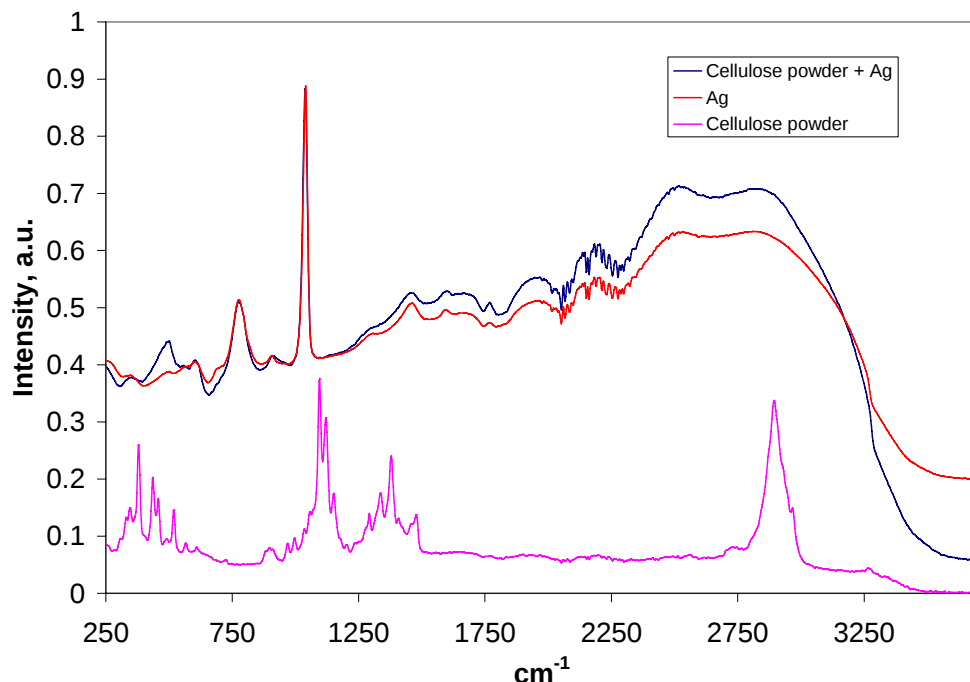


Fig. 8: No SERS spectrum was obtained from cellulose powder. Top, 100-150 nm Ag + cellulose powder (Whatman CC31); middle, 100-150 nm Ag; bottom, cellulose powder.

To determine how the spectral features in the SERS spectrum of unbleached KP (Fig. 6) were different from those of BS wood and MWL, the latter samples were analyzed using the SERS approach (Fig. 9). The SERS band positions for the 3 samples along with normal Raman frequencies (near-IR Raman) of BS wood are listed in Table 1. From the Table, a couple of observations can be made. First, there are great similarities between the 3 SERS spectra. Not only all the KP SERS bands (Table 1) are detected in the SERS spectra of wood and MWL but their relative intensity patterns are quite similar (Figs. 6 and 9). Next, both Table 1 and Fig. 9 clearly indicated absence of cellulose contributions (or that of any other carbohydrate) because all the KP SERS bands were detected in the SERS spectrum of MWL.

At first consideration it appears as if the SERS band positions and intensity patterns were similar for KP and MWL and that the SERS bands originated from that part of lignin structure that was common to both MWL and residual lignin. However this may be not completely accurate because when second derivative spectra were assessed there were significant differences. Although Raman bands with identical frequencies were still present, there was evidence for specific features that were present in only one sample. We believe that further analysis of the data, currently underway, will reveal more useful information.

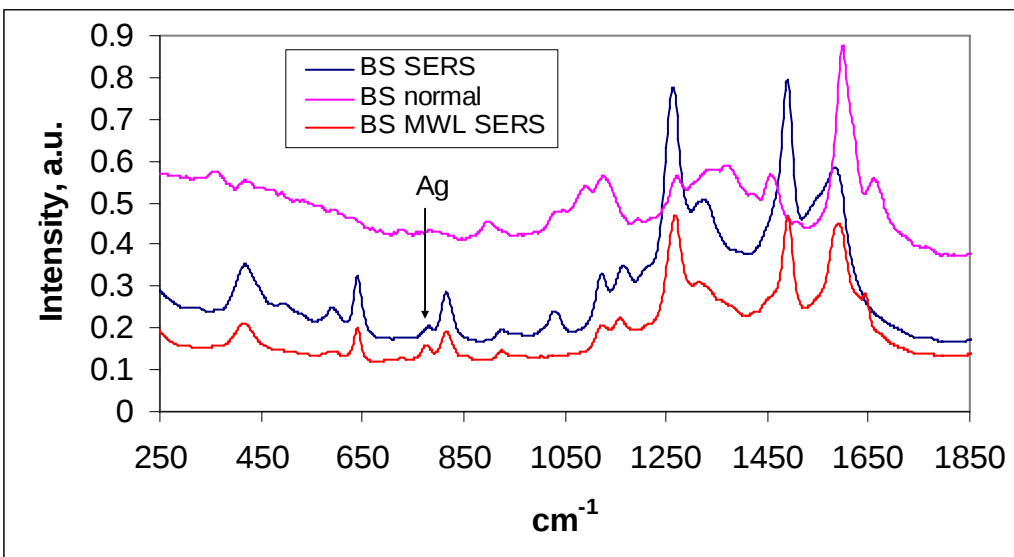


Fig. 9: SERS (BS and MWL) and normal (BS) spectra in the 250—1850  $\text{cm}^{-1}$  region. The band due to Ag particles is detected at 780  $\text{cm}^{-1}$ . For lignin bands common to both un-enhanced (normal, top) and SERS spectra (BS SERS, middle; BS MWL SERS, bottom) significant changes are detected in both band position and intensity. In SERS, several new bands were detected as well.

**Table 1: Comparison of Raman band positions ( $\text{cm}^{-1}$ ) in SERS and normal spectra (250—1800  $\text{cm}^{-1}$ )**

| Band ID | SERS, Unbleached kraft pulp (KP) | SERS, BS MWL | SERS, Ball-milled BS | Normal Raman, Ball-milled BS | Comment                                            |
|---------|----------------------------------|--------------|----------------------|------------------------------|----------------------------------------------------|
| 1       | ————                             | ————         | ————                 | 1658 m <sup>a</sup>          | Absent or shifted to 1643 $\text{cm}^{-1}$ in SERS |
| 2       | ————                             | 1645 m       | ————                 | ————                         | New band?                                          |
| 3       | ————                             | 1628 w, sh   | ————                 | 1620 sh                      | Not in SERS                                        |
| 4       | ————                             | 1595 vs      | ————                 | 1601 vs                      | Shifts to 1582-1589 $\text{cm}^{-1}$ ?             |
| 5       | 1582 vs                          | 1583 sh      | 1582 vs              | ————                         | New band?                                          |
|         | ————                             | 1546 w, sh   | 1551 w, sh           | ————                         |                                                    |
| 6       | ————                             | ————         | ————                 | 1508 vw                      | Shifts to 1489-1492 $\text{cm}^{-1}$ ?             |
| 7       | 1492 vs                          | 1489 vs      | 1489 vs              | ————                         | New band?                                          |
| 8       | ————                             | ————         | ————                 | 1456 m <sup>b</sup>          | In BS only                                         |
| 9       | 1443 vw, sh                      | 1449 br, sh  | ————                 | ————                         | In KP SERS                                         |
| 10      | ————                             | 1426 vw      | ————                 | 1425 sh                      | In BS only                                         |
| 11      | 1412 w                           | ————         | ————                 | ————                         | In KP SERS                                         |
| 12      | ————                             | 1380 w, sh   | ————                 | 1377 m <sup>b</sup>          | MWL SERS and BS                                    |
| 13      | 1359 m, sh                       | 1357 w, sh   | 1360 vw, sh          | ————                         | KP and MWL SERS                                    |
| 14      | 1333 sh                          | ————         | 1325 m               | 1338 m <sup>b</sup>          | Not in MWL SERS                                    |
| 15      | 1314 m-s                         | 1315 m       | ————                 | ————                         | In KP and MWL SERS                                 |
| 16      | ————                             | ————         | ————                 | 1298 sh <sup>b</sup>         | BS                                                 |
| 17      | 1267 vs                          | 1267 vs      | 1263 vs              | 1269 m                       | Intense in SERS                                    |
| 18      | 1215 w, sh                       | 1214 w, sh   | 1165 m               | ————                         | Not in BS                                          |
| 19      | ————                             | ————         | ————                 | 1191 w                       | In BS only                                         |
| 20      | 1180 vw, br                      | ————         | ————                 | ————                         | In KP SERS                                         |
| 21      | 1165 vw, br                      | 1159w, br    | ————                 | ————                         | In KP and MWL SERS                                 |

|    |            |         |           |                      |                           |
|----|------------|---------|-----------|----------------------|---------------------------|
| 22 | —————      | —————   | —————     | 1149 sh <sup>b</sup> | In BS only                |
| 23 | 1126 m     | 1125 m  | 1124 m    | 1123 s <sup>c</sup>  | In all                    |
| 24 | 1113 m, sh | —————   | —————     | —————                | In KP SERS                |
| 25 | —————      | —————   | —————     | 1095 s <sup>c</sup>  | In BS only                |
| 26 | —————      | —————   | —————     | 1073 sh <sup>b</sup> | In BS only                |
| 27 | —————      | —————   | —————     | 1063 sh <sup>b</sup> | In BS only                |
| 28 | —————      | —————   | 1029 m    | 1037 sh <sup>b</sup> | Not in KP and MWL SERS    |
| 29 | 1002 vw    | 1003 vw | —————     | 1000 vw <sup>b</sup> | Not in BS SERS            |
| 30 | —————      | —————   | —————     | 971 vw <sup>b</sup>  | In BS only                |
| 31 | 927 m      | 925 m   | 925 w     | 922 sh               | In all                    |
| 32 | —————      | —————   | —————     | 899 m <sup>b</sup>   | In BS only                |
| 33 | —————      | 855 m   | —————     | —————                | MWL SERS                  |
| 34 | 815 s      | 816 s   | 816 m-s   | —————                | Not in BS                 |
| 35 | —————      | —————   | —————     | 786 w                | In BS only                |
| 36 | 780 m      | 775 m-s | 781 w     | —————                | Ag (2 – 3.5 $\mu$ m) band |
| 37 | —————      | 727 w   | 728 vw    | 730 w                | Not in KP SERS            |
| 38 | —————      | 702 w   | —————     | —————                | MWL                       |
| 39 | 641 s      | 641 s   | 641 s     | —————                | New band?                 |
| 40 | —————      | —————   | —————     | 634 vw               | In BS only                |
| 41 | —————      | 596 m   | 589 m     | 595 w                | Not in KP SERS            |
| 42 | —————      | 587 m   | —————     | —————                | MWL SERS                  |
| 43 | —————      | —————   | —————     | 560 w                |                           |
| 44 | —————      | —————   | 526 w, sh | 520 m <sup>b</sup>   | BS and BS SERS            |
| 45 | —————      | —————   | 492 w     | 492 w <sup>d</sup>   | BS and BS SERS            |
| 46 | —————      | —————   | —————     | 458 m <sup>b</sup>   | In BS only                |
| 47 | —————      | —————   | —————     | 435 m <sup>b</sup>   | In BS only                |
| 48 | 420 s      | 416 s   | 419 s     | —————                | New band?                 |
| 49 | —————      | —————   | —————     | 406 vw <sup>b</sup>  | In BS only                |
| 50 | —————      | —————   | —————     | 380 m <sup>b</sup>   | In BS only                |
| 51 | 354vw      | —————   | —————     | 351 w <sup>b</sup>   | BS and KP SERS            |
| 52 | —————      | —————   | —————     | 330 sh <sup>b</sup>  | In BS only                |

<sup>a</sup>Band intensities are relative to other peaks in the spectrum

<sup>b</sup>Cellulose band [11]

<sup>c</sup>Band assigned to cellulose, xylan, and glucomannan [11]

<sup>d</sup>Band assigned to cellulose, xylan, and lignin [11]

## CONCLUSION

Based on preliminary examination, the SERS technique seems to be a useful method to study lignins in biomaterials. For unbleached kraft and black spruce wood, using commercially available Ag particles, lignin was selectively detected in presence of carbohydrates polymers. For kraft pulp, evidence supported existence of original MWL structures in residual lignin. Because numerous parameters (e.g., various morphologies of Ag particles and surfaces, excitation lasers wavelengths, and sampling alternatives) are likely to further improve the SERS method such factors need to be investigated and optimized. To assign bands in lignin SERS spectrum, future work using lignin models is expected to be of assistance.

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