SURFACE ENHANCED RAMAN SPECTROSCOPY FOR LIGNIN ANALYSIS

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ABSTRACT

Near-IR surface enhanced Raman scattering (SERS) spectra of spruce Wiley milled-wood (WMW) and milled-wood lignin (MWL) adsorbed on the silver nano- and micro-particles were obtained recently in our laboratory. When the SERS spectra of wood and MWL were compared they were found to be almost identical which suggested that the signals from the carbohydrate components were absent in the WMW SERS spectrum. This was also supported by the fact that no SERS spectrum of cellulose could be obtained under these conditions. Therefore, lignin could be selectively studied in the presence of other wood constituents without separation. Some general characteristics of the lignin SERS spectrum are described. The SERS technique was also found to be highly sensitive because only a very small amount of the sample is needed. For MWL, SERS and normal Raman spectra were compared and it was determined that significant spectral changes were present. Raman spectral changes and x-ray photoelectron spectral analysis of the MWL SERS sample provided evidence that showed that lignin is strongly adsorbed on silver. This nano- and micro-particle based lignin analysis technique is expected to make a significant contribution to investigating lignin in various lignocellulosic materials.

BACKGROUND

Because native lignin structures are highly complex, they have proven to be difficult to study. The same holds true for other modified states of lignin, such as in wood pulp where residual lignin poses a similar characterization problem [1]. Given their high molecular weight and complex structure, lignin polymers cannot be isolated without some 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 (e.g., biofuels) are likely to benefit from an improved understanding of lignins at the molecular level.

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) [2]. SERS technique, attributed to large electromagnetic fields near nanostructured metallic surfaces and short range chemical effect, has the capability to detect a single molecule and provide information on molecular structure. Although some enhancement in SERS is due to chemical contribution (electronic interaction between metal and adsorbate), the main reason why SERS produces extraordinary enhancements is surface plasmons - the electromagnetic property of nanostructures.

In our laboratory, earlier efforts to induce SERS in wood and its component polymers (cellulose, hemicellulose, and lignin) have not succeeded. In the case of wood, such attempts consisted of precipitating silver in the cell wall and applying gold and/or silver coating, using a sputter coater, to 30 μm sections of wood. The cell-wall components cellulose, hemicellulose, and lignin when analyzed in presence of silver sols, prepared using the literature-based methods, failed to bring about the surface enhancement effect. Nevertheless, when the commercially obtained larger size particles of silver were used, the SERS effect was induced in the samples of MWL and WMW, indicating that these materials were spontaneously adsorbed to interacting metallic silver particle surfaces. In this report, results of applying SERS to study lignin, and wood using commercially available Ag particles are presented.

EXPERIMENTAL

Black spruce wood was used with previously described procedures to obtain samples of WMW and MWL [3]. The isolated MWL represented ~15% of the total lignin present in WMW [3]. As described in reference [3], WMW was extracted overnight by acetone/water (9: 1) solvent mixture and was filtered. The extraction process was repeated four additional times.

Silver particles (average sizes, 100-150 nm and 2-3.5 μm; purity, >99 and 99.9+%, respectively), benzoic acid (≥99.5%), and t,t-muconic acids (98%) were purchased from Sigma- Aldrich, Milwaukee, WI. Ethanol (200% proof) was from Aaper Alcohol and Chemical Company, Shelbyville, KY. Cellulose powder CC31 was purchased from Whatman Ltd., Maidstone, Kent, England.

For benzoic and muconic acids, SERS sample preparation involved adding 50 μl of $8x10^{-3}$ M ethanol solution to 15 mg of Ag particles and vigorously mixing them together using a spatula. Additionally, in the case of muconic acid, various Ag:acid dilutions in the range of 100: 1 to 1,020,000:1 were prepared and analyzed using 2-3.5 μm Ag powder. For MWL, WMW, and Whatman CC31 cellulose, the samples were prepared by mixing, in the presence of ethanol, a small amount (a few mg) of the solid sample with 15-20 mg Ag substrate. If needed for dispersion, a few drops of ethanol were further added. Once the mixture was completely dry, it was sampled in an "aluminum well," a Raman sampling accessory. Various dilutions of Ag:MWL samples were also prepared in a similar manner. A MWL solution (0.0281 M) was made by dissolving 5.2 mg lignin in 1 ml of dioxane:water (9:1). Further dilutions were made as needed.

A Bruker RFS-100 near-IR FT-Raman spectrometer (Bruker Instruments Inc., Billerica, MA) was used to obtain spectra. The instrument has been described previously [3]. For most acquisitions, samples were pressed into the "aluminum well," and spectra were acquired using 600 mW of unfocused laser power; 1024 scans were accumulated for each spectrum. Normal Raman spectrum of WMW was obtained by pressing it into a pellet. Bruker's OPUS software was used to find peak positions and manipulate spectra. Transmission IR spectrum on MWL, as KBr pellet, was recorded on a Galaxy 5000 spectrometer. The Perkin Elmer 5400 X-ray Photoelectron Spectrometer (XPS) at the Material Science Center, UW-Madison, WI was used for surface analysis. An Mg K_α x-ray source was operated at 300 watts and its window was about 1 cm above the specimens. Samples for XPS analysis were prepared using only the 2-3.5 μm particles of Ag. High-resolution spectra were obtained using a hemispherical electron analyzer operated with a pass energy of 35 eV. The spectra were recorded in 0.05 eV steps. The XPS data were manipulated, including curve fitting, using AugerScan2 software.

RESULTS AND DISCUSSION

Target compounds

Benzoic and muconic acids were used as target molecules to evaluate the SERS suitability of commercially obtained Ag substrates. Muconic acid was chosen because these kinds of structures are one type of by-product in oxidized lignin. In our laboratory, both these compounds produced good SERS spectra using commercial Ag particles (Figs. 1 and 2). For either acid, under identical experimental conditions, no Raman signal was detected from the ethanol solution. Spectra of neat solid samples are included in the Figures for comparison purposes.

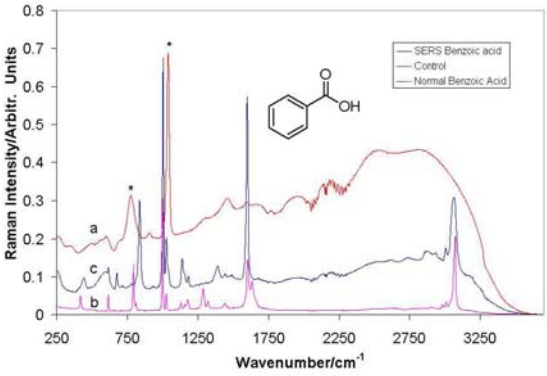

Figure 1: Near-IR spectra of (a) 100–150 nm Ag particles, (b) solid benzoic acid, and (c) Ag:benzoic acid. In (a) silver substrate, bands are marked with an *.

Commercially obtained Ag particles clearly produced the SERS enhancement for both benzoic and muconic acids. The enhancement factor calculated for the muconic acid spectra showed that the band at 1636 cm^{-1} was enhanced by 10^6, a value similar to other SERS enhancement factors reported in the literature [2]. Also noteworthy is that at higher Ag:acid dilutions, peaks caused by Ag were more prominently seen in Fig. 2.

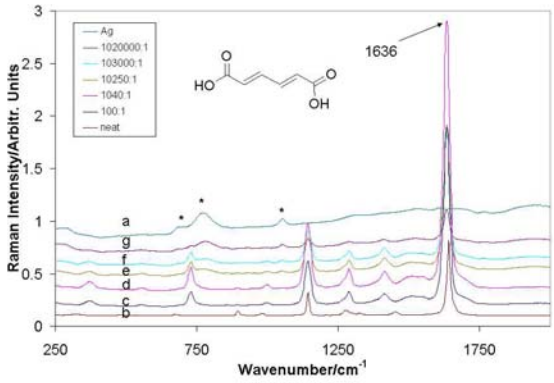

Figure 2: Near-IR SERS spectra of t,t- muconic acid at various Ag:acid dilutions; (a) Ag 2–3.5 μm, (b) neat acid, (c) 100:1, (d) 1,040:1, (e) 10,250:1, (f) 103,000:1, and (g) 1,020,000:1. Peaks due to Ag particles are marked with * in spectrum (a).

Spruce milled-wood lignin

When a sample of MWL thoroughly mixed in presence of ethanol with the silver particles was analyzed, the Raman spectrum of the MWL was found to be surface enhanced (Fig. 3b) when compared with the normal near-IR FT-Raman spectrum of MWL [3] (Fig. 3a). As expected in SERS [2], C-H stretches were weak and the normal Raman selection rules seem to have been relaxed (modes usually not seen in Raman are detected). A number of peaks in the normal Raman became stronger and were found to have shifted in frequency. The latter suggested the likelihood of silver-lignin complex formation. The nature of the mixing solvent did not influence the SERS spectrum in any manner. The vibrational assignments are expected to be similar to that of normal lignin spectrum but additional insights will result from the SERS studies of lignin models that are currently ongoing in our laboratory.

Spectral characteristics

To determine how the spectral features in MWL SERS spectrum (Fig. 3b) were different from those of normal Raman (Fig. 3a), the two spectra were compared along with the normal Raman (near-IR) and FTIR (not shown) spectra. A number of observations can be made. The composite coniferaldehyde C=O and ethylenic C=C band in normal Raman is shifted from 1662 to 1645 cm^{-1}. A shift of 17 cm^{-1} is significant and suggests existence of an altered chemical environment around these groups. One of the strongest bands in the spectra corresponds to the aromatic ring stretch mode, observed at 1597 and 1589 cm^{-1} for normal and SERS Raman, respectively. A lowering of frequency by 8 cm^{-1} in SERS is noted for this mode.

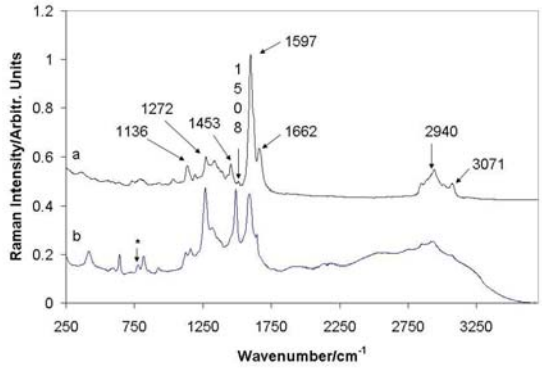

Figure 3: Near-IR spectra - (a) normal, (b) surface enhanced spruce MWL (Ag:MWL = 4.36). In the lignin SERS spectrum, most enhanced bands were present between 250 and 1800 cm^{-1}. * indicates Ag peak.

Additionally in the SERS spectrum, considerable change of intensity for many other bands can be noted from Fig. 3. The very weak 1508 cm^{-1} band present in the normal spectrum of lignin (Fig. 3a) seems to have shifted in SERS to a very strong 1491 cm^{-1} band (Fig. 3b), and in IR corresponded to the band at 1513 cm^{-1}. Another lignin SERS band belonging to this category that has a strong IR intensity is the band at 1267 cm^{-1} (Fig. 3b), although in SERS its frequency shifted by a lesser amount (5 cm^{-1}). Other strong lignin SERS bands were present at 816, 641, and 416 cm^{-1}. These were bands that had no corresponding peak in normal Raman and IR spectra of MWL and therefore, are new. Nevertheless, these bands have been observed in the SERS spectra of thiophenol, vanillic acid, and variously substituted monomeric lignin model compounds [authors' unpublished results].

From such observations, it is clear that these modes belong to the aromatic ring in lignin. Comparing normal Raman and SERS MWL spectra, it is interesting to note that a number of bands have lowered frequencies and enhanced relative intensities, indicating that these bands are sensitive to the complexation of lignin with the Ag particles.

Wiley milled wood

Because isolation procedures modify lignin chemically to some degree it is of great interest to develop methods that are capable of studying lignin in its native environment. Therefore, it would be advantageous if the SERS of lignin and other wood components could be obtained *in situ*. For this purpose, SERS samples of WMW were prepared and analyzed.

Normal (peak positions annotated) and SERS spectra of the WMW are shown in Fig. 4a and Fig. 4b, respectively. The latter is very similar to the SERS spectrum of MWL (Fig. 4c). Nevertheless, a detailed comparison of the two spectra indicated some minor differences. For example, in the MWL spectrum, subtle features at 1645, 1628, and 1450-1380 cm^{-1} were different. But these differences disappeared in the higher Ag:MWL ratio spectrum (not shown), indicating that the dissimilarities were concentration dependent. The only extra features present in the WMW SERS were a stronger peak at 1216 and a shoulder at 1114 cm^{-1}, which may have something to do with the undisturbed structure of lignin in wood. Compared to MWL, lignin in WMW is not degraded and is therefore representative of whole wood lignin.

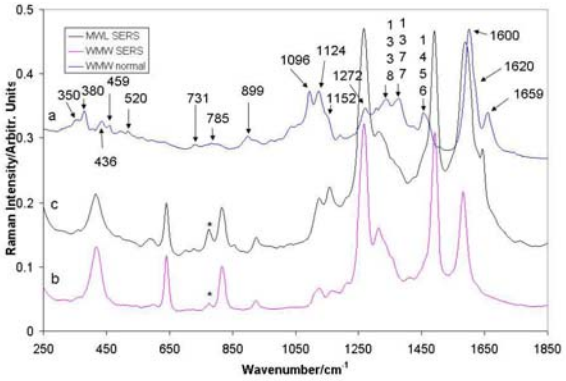

Figure 4: Raman spectra – (a) Wiley-milled wood, normal; (b) Wiley-milled wood, SERS (Ag:wood ratio 1.07); and (c) MWL, SERS (Ag:MWL ratio 4.36). In (b) and (c) * indicates Ag peak. Most peaks in the SERS spectra of wood and lignin are same indicating that cellulose contributions were not detected.

The majority of bands in the WMW SERS spectrum belonged to lignin but surprisingly no features in the carbohydrate regions [31 were detected. This implied that SERS can be used to selectively investigate lignin in a multicomponent lignocellulose sample. This was found to be the case when samples of kraft pulp (another type of lignocellulose) were studied using the SERS technique [4].

To find out if indeed cellulose would not produce a SERS spectrum under these conditions, an Ag-adsorbed cellulose sample (Whatman CC31) was analyzed (Fig. 5). As expected, only bands caused by the Ag substrate were detected and no Raman signal from cellulose was seen. This supported the above-mentioned observation that peaks seen in the SERS spectrum of WMW (Fig. 4b) are due to lignin. Inability of the cellulose molecule to produce SERS may have to do with a lack of lone pair or π electrons because molecules with the latter trait are known to show the strongest SERS (e.g., carboxylic acids and phenols). Moreover, lack of such electrons is likely to be responsible for cellulose's inability to experience chemical .enhancement because of adsorption on the rough silver surfaces.

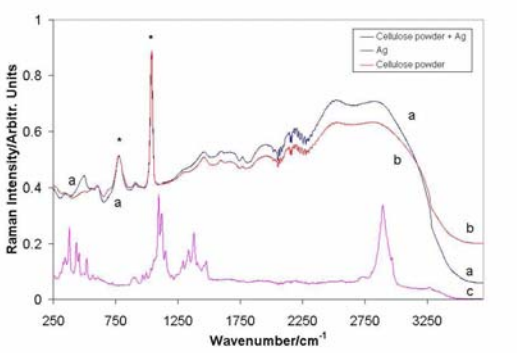

Figure 5: Raman spectra - (a) sample containing cellulose and silver, (b) silver, and (c) cellulose powder Whatman CC31. In (a) and (b) * indicates Ag peak (783 and 1040 cm-1).

XPS

Using pressed disks from powders of Ag, MWL, and Ag:MWL (Ag-to-MWL ratio 4.85) samples were analyzed by XPS (also known as ESCA) to determine if there was chemical adsorption/interaction that could possibly lead to the "chemical enhancement" contribution. The magnitude of this SERS factor usually does not exceed 10 [2] and can be rationalized in terms of change in the Raman polarizability tensor of the molecule due to the formation of the complex between silver and molecule.

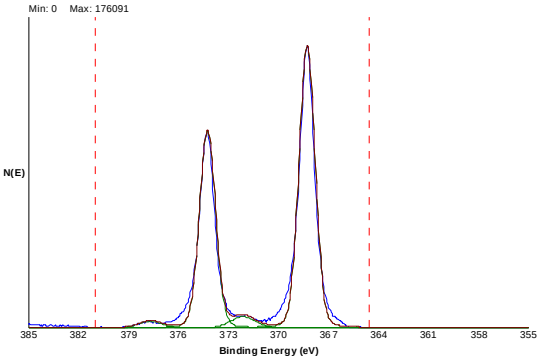

Figure 6: 3d 3/2 and 3d 5/2 electron emission from silver.

In the silver 3d XPS spectrum, in addition to the two peaks (at 3d binding energies of 374.2 and 368.2 eV for $3d_{3/2}$ and $3d_{5/2}$, respectively), two small components separated by 3.6 eV were detected (Fig. 6). These components were interpreted as phonon satellites to the major peaks.

A somewhat different Ag 3d spectrum was obtained from the MWL:Ag sample (Fig. 7). In this case the satellite peaks are more intense and shifted to 4.0 eV. Most of the silver can be expected to be in the metallic state since there is much more Ag powder than lignin and any interaction is confined to the surface of the silver. Furthermore, the chemical shifts of the Ag 3d are rather small. These satellite peaks are characteristic of Ag (II) [5].

The satellite peaks in Fig. 7 are about three times the intensity of those in Fig. 6. As in Fig. 6, some of the contribution in Fig. 7 is due to excitation of phonon states in the Ag metal. However, the presence of Ag (II) contribution is clear evidence of the formation of chemical bond between lignin and silver.

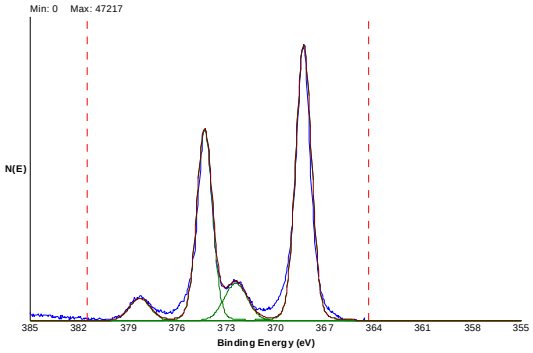

Figure 7: 3d 3/2 and 3d 5/2 electron emission from MWL:silver sample

Further evidence for such bond formation was found in the oxygen and carbon photoemission spectra of the MWL:Ag powder sample. However, such evidence could not be presented here due to the restriction on the length of the manuscript.

CONCLUSIONS

Using commercially available Ag particles, SERS spectra of MWL and WMW were obtained. Based on the relative changes in Raman intensities and frequencies and XPS results, it appears that the lignin and silver are involved in complex formation. In the WMW SERS spectrum, lignin was selectively detected in presence of carbohydrate polymers, and no cellulose features were detected. Based on the initial examination, the SERS technique seems to be a useful method for selectively investigating lignin in unmodified state of the native environment of various lignocellulose biomaterials.

REFERENCES

1. Tamminen,T.L., Hortling, B.R. (1999) "Isolation and characterization of residual lignin" in *Advances in Lignocellulosics Characterization*, D.S. Argyropoulos, (Ed.) TAPPI Press, Atlanta, 1-42
2. Aroca, R. (2006) Surface-Enhanced Vibrational Spectroscopy, John Wiley & Sons, Chichester, UK, 2006, pp. 73; and references therein.
3. Agarwal, U.P., Ralph, S.A. (1997) "FT Raman spectroscopy of wood", *Applied Spectro.* **51**:1648-1655
4. Bujanovic, B., Reiner, R.S., Ralph, S.A., Agarwal, U.P., Atalla, R.H. *Proc. 2005 TAPPI Engineering, Pulping, and Environmental Conference*, TAPPI, Atlanta, 2005; Book 2: paper 30-3
5. Carlson, T. A. (1975) "Photoelectron and Auger spectroscopy", Plenum Press, New York

In: Proceedings of the 15th International Symposium on Wood, Fiber and Pulping Chemistry, Oslo, Norway (June 15-18, 2009). Paper number: O-003_ISWFPC_SERS. Oslo, Norway : Congress-Conference AS, 2009. 1 flash drive.