

# Characterization of lignin

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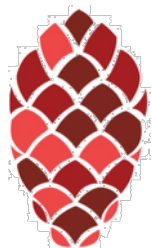
## Abstract

Lignin is a complex organic compound crucial to the structural tissues of vascular plants, such as trees. The cyclic structure and aromaticity of lignin give it significant potential to be used as a renewable and safe replacement for toxic aromatic compounds in chemical and industrial processes. The purpose of this experiment was to characterize lignin, specifically the particle diameter and zeta potential, using both the Zetasizer Nano ZSP and the ImageJ image processing software, and to compare the accuracy of both measurement methods. Due to the natural fluorescence of lignin, a fluorescence microscope is used to capture images of lignin particles. By having a known distance and the scale of measurement, with ImageJ it is possible to calculate distances, such as the diameters of particles in images. The Zetasizer Nano ZSP is a device capable of measuring both particle diameter and zeta potential, which is the electrical charge existing on particles suspended in a medium. Small amounts of lignin, in powder form, are placed into scintillation vials with different amounts of distilled water to create 3 different concentrations of sample to measure using the Zetasizer. Between 10-15mL of sample are placed into specialized measurement cells and put into the Zetasizer. Multiple measurements are conducted and averaged to achieve accurate results. The measured zeta potential value is indicative of the stability of the lignin. If the value of zeta potential is higher than 30mV, negative or positive, it has a high stability and low reactivity. The average values of zeta potential measured by the Zetasizer ranged from -27mV to -21mV. The particle diameter is important in characterization, as the smaller and more distributed the particles are, there is a larger surface area for reactions to occur. Average particle diameter measured by the Zetasizer ranged from 926-976 $\mu$ m. The results from the Zetasizer Nano ZSP are more accurate than those from the ImageJ software, as ImageJ allows for a substantial amount of human error to impact the results. The outcomes help direct future experiments using lignin and beneficial to future research concerning lignin and its potential.

## Key words:

lignin, characterization, zeta potential, particle diameter

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## Introduction

### Background

- Lignin is an organic compound found in the support tissues of trees, and it is similar to the more commonly known organic compound cellulose.
- Lignin's structure and aromaticity give it a lot of potential to replace other toxic aromatic compounds in industrial and chemical processes.
- Characterization is necessary for experiments being done with lignin. Knowing properties such as the zeta potential and particle diameter allow researchers to determine reactivity and other qualities of the compound.

### Purpose

- The purpose of this experiment was to characterize lignin particles and to compare the accuracy of the Zetasizer Nano ZSP and the Image J software.



Figure 1a: Extracted lignin in solid powdered form, the state in which it is used for our experiment.

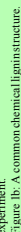


Figure 1b: A common chemical lignin structure.

## Methods

### Image J

- Image J is an image processing software.
- Lignin is naturally fluorescent, allowing us to use a fluorescence microscope to take images of the particles at the micrometer (µm) scale.
- Using ImageJ, fluorescent images can be analyzed to measure the diameter of individual particles.

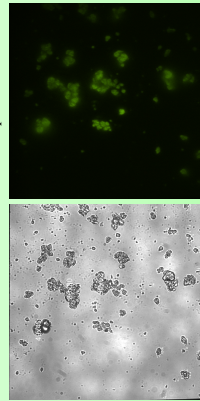


Figure 2: White (left) and green (right) fluorescence microscopy images of lignin particles.

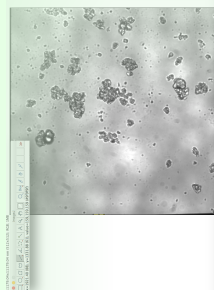


Figure 3: Screen capture of the ImageJ software, including a white fluorescence microscopy image, which is magnified 100x.

- To measure the diameter of particles with ImageJ, known distance and the measurement scale (mm, µm, etc.) are needed.

### Zetasizer Nano ZSP

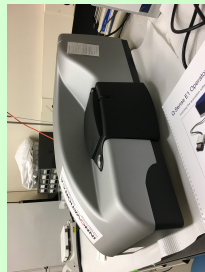


Figure 4: The Zetasizer Nano ZSP.

- The Zetasizer Nano ZSP is a device used to measure properties of particles. For this experiment, zeta potential and particle diameter were measured.
- Zeta potential is the value of electrical charge that exists on particles dispersed in a liquid. The higher (+/-) the zeta potential value, the more stable the particles are.
- The concentration of particles in the dispersant have a significant effect on the results the Zetasizer produces. The concentration can neither be too high or too low, or else the results become inaccurate.

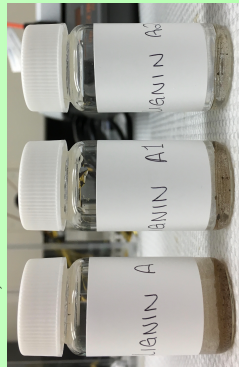


Figure 5: Various concentrations of lignin dispersed in water. The darker the color, the higher the concentration of lignin.

## Results

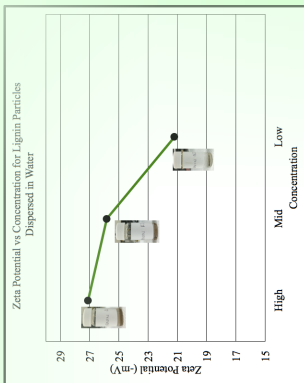


Figure 6: Graph relating the concentration of the lignin samples to their average zeta potential (mV) values.

- For each concentration, multiple zeta potential measurements were done with the "zeta" function of the Zetasizer and averaged to get one value.

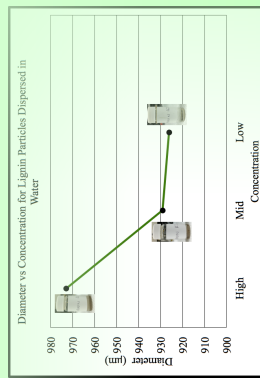


Figure 7: Graph relating concentration of the lignin samples to their average particle diameter (µm).

- Using the "size" function of the Zetasizer, the diameters of the particles for each concentration of lignin were measured. Multiple measurements were taken for each concentration and averaged to get one value.



Figure 8: The zeta potential (left) and size (right) cells used for measurement in the Zetasizer. The amount of sample in each cell can only be 10-15µL.

## Conclusion

- The characterization of lignin has much importance to biological and industrial studies.
- The viability of biofuel production would be greatly enhanced by the development of products derived from lignin.
- The use of fluorescence microscopy for imaging lignin content and composition is made easier by the natural fluorescence of lignin, as shown in Figure 2.
- When comparing the ImageJ processing software and the Zetasizer Nano ZSP, ImageJ is easier to use, however the results may not be accurate enough due to the possibility of human error.
- The Zetasizer Nano ZSP is the method that allows for the most accuracy in characterization, particle diameter and zeta potential.

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