



APPLICATION NOTE

ROUNDUP ABSORBANCE TESTING WITH VARIABLE PATH LENGTH DIP PROBE

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INTRO

BACKGROUND OF APPLICATION

The active ingredient in Roundup Weed and Grass Killer is a chemical called glyphosate. On the Roundup label, glyphosate, N-(phosphonomethyl)glycine, is in the form of its isopropylamine salt. In this form, glyphosate is easier to handle and more effective. Regular Roundup is 2% glyphosate, and when it is ready for use, the Roundup needs to be even more diluted with water.

In this experiment, we tested these low concentrations of glyphosate with a special variable path length dip probe with a narrow tip to measure the chemical's absorbance. We tested the dip probe with a path length of 10mm and 5mm to also measure the limit of detection for the lower concentrations. As pathlength is inversely correlated with detection limits in an absorbance measurement, we predicted that the 10mm path length would give more accurate and reliable results. We tested with the 5mm path length to see if the shorter path length still detected the glyphosate and if it provided similar results to the 10mm path length.

SPECTROSCOPY SYSTEM SETUP

We used Avantes' top-of-the-line UV/VIS spectrometer, the [AvaSpec-HERO](#) (Figure 1), for this experiment. The AvaSpec-HERO is built up around our High-Sensitivity, Compact (HSC), 100mm optical bench offering a numerical aperture (NA) of 0.13 and a cooled, back-thinned CCD detector (1024×58 pixels). Electronics-wise, the HERO uses our state-of-the-art AS7010 board, which includes a high-performance Analog to Digital converter with excellent noise performance and the ability to support high-speed communication through USB 3.0 and or gigabit ethernet. We used this spectrometer due to the need for high sensitivity in the ultraviolet range.



FIGURE 1 System including AvaSpec-HERO spectrometer, AvaLight-XE High Power Pulsed Xenon, and Variable path length dip probe

The light source used was the pulsed xenon [AvaLight-XE-HP](#). This source also works well in the ultraviolet region. The AvaLight-XE-HP connects to the spectrometer through a Y-cable and then receives TTL signals from the spectrometer to engage the pulsed source and ensure spectral measurements occur only when the source is flashing. The number of flashes per scan can be selected in the [AvaSoft software](#) so that readings are not saturated. Saturation occurs when there is too much light entering the spectrometer. We set the flashes to three for the 10mm path length trials and two for the 5mm path length trials.

The last component of the system was the narrow tip variable path length dip probe available only from Avantes North America. Avantes offers a complete line of [transmission dip probes](#) with fixed or variable path lengths for laboratory or industrial applications. The path length is the inner distance from the front to the back window and then back again. In this case, the window is the opening in the dip probe tip (Figure 2). The path length changes by loosening and or tightening the brass hardware. A longer path length is best for reading lower-concentration samples, while a shorter path length is best for reading higher-concentration samples. The longer distance allows more light to enter the sample exciting the particles. The 10mm path length trials should read the lower concentrations better than the 5mm path length trials.




FIGURE 2 Variable path length dip probe at the 10mm length

DESCRIPTION OF METHODOLOGY

The recommended dilution of Roundup with water to eradicate weeds and grasses is to mix 1.5 ounces (3 tablespoons) of herbicide with 1 gallon of water. The concentration of herbicide is about 1.2% in those samples. This concentration was the fixed amount based on the other three concentrations. The three samples were 25%, 50%, and 75% more diluted than the recommended concentration.

For the dilution, we used deionized water. So, the four concentrations were 1.2%, 0.88%, 0.59%, and 0.29% Roundup. Roundup is 2% glyphosate. In the four concentrations, the percentage of glyphosate is 0.024%, 0.018%, 0.012%, and 0.0058% (Figure 3). All four samples (Figure 4) were quite dilute, meaning that the 10mm path length trials would probably read better than the 5mm path length trials.



Samples	Concentration	% of RoundUp	% of Glyphosate
1	Recommended	1.2	0.024
2	25% more diluted	0.88	0.018
3	50% more diluted	0.59	0.012
4	75% more diluted	0.29	0.0058

FIGURE 3 Concentrations and percentages of the diluted Roundup samples



FIGURE 4: Four concentrations of Roundup and DI water

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TEST DATA AND RESULTS

Before testing the samples, we set the integration time and number of flashes on the AvaLight-XE-HP. We first tested the 10mm path length in the four samples. For this path length, the integration time was 30 milliseconds, and the number of flashes was three. We then took a dark reference in absorbance mode to eliminate unwanted noise from the instrumentation and a light reference with our solvent (distilled water). We began testing by dipping the probe in each sample. The dip probe was cleaned in distilled water and then air dried in between samples. Cleaning a dip probe between samples/referencing was vital to ensure no leftover residue was transferred to the next sample. The 10 mm absorbance measurements is shown in fig 5.

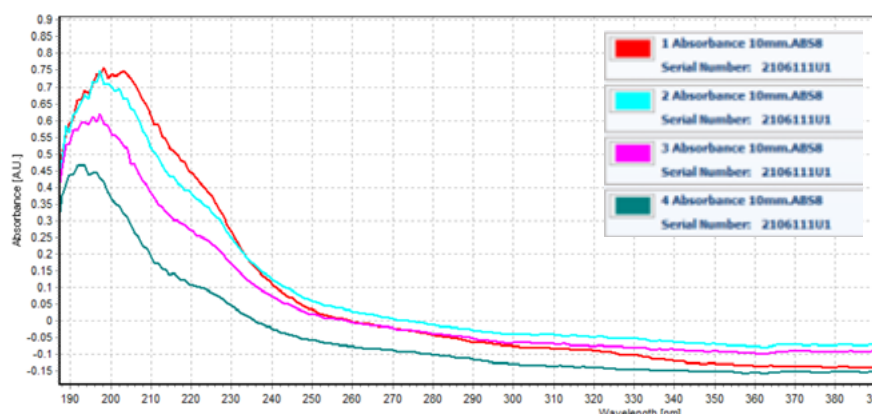


FIGURE 5: Four Absorbance reading from the 10mm path length trials

The absorbance peak of pure glyphosate is around 265 nm. The absorbance peak of glyphosate can range anywhere from 200 to 300 nm. The peaks that we received were around 200 nm. The shapes of our spectral measurements aligned with other researchers' spectral data. So, the 10 mm path length proved to measure the glyphosate fairly well in the four Roundup dilutions.

The second tests were with the 5mm path length (Figure 6). For this path length, the integration time was 20 milliseconds, and the number of flashes was two. We lowered the integration time and the number of flashes to avoid saturation. We again performed light and dark reference measurements and then began testing by dipping the probe in the samples. We continued to clean and dry the dip probe in between each sample to ensure no cross-contamination.

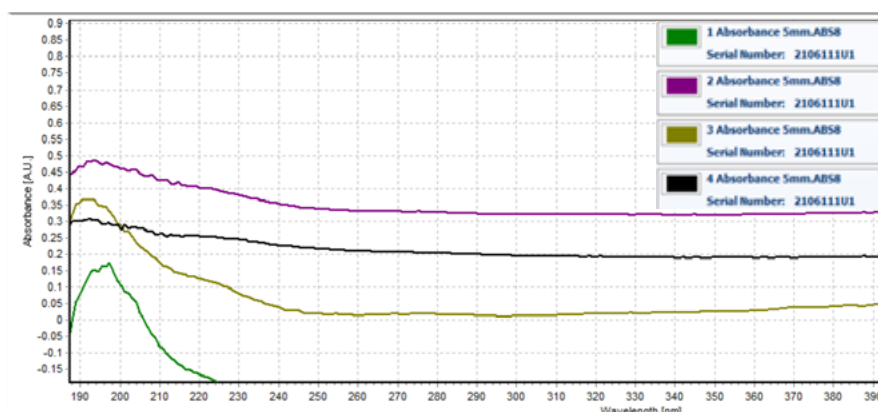


FIGURE 6: Four Absorbance reading from the 5mm path length trials

TEST DATA AND RESULTS

The 5mm path length spectral measurements were not as uniform as the 10mm trials. Sample 2 (25% more diluted) and sample 4 (75% more diluted) did not have the same shape as the other two samples. Instead of a peak that shallowed out reasonably quickly, sample 2 (purple) and sample 4 (black) had a shallow peak that lingered and never truly dropped. These results proved that the 5mm path length option does not provide as reliable as the 10mm path length option. This is consistent with our initial hypothesis because all four samples are highly diluted, so it makes sense that the 10mm path length provided better results.

The following figures (7, 8, 9, & 10) compare each sample's 10mm path length trial and the 5mm path length trial. In these spectral measurements, you will see that the 10mm path length reading provides more accurate and reliable spectral readings than the 5mm path length. The last figure (11) provides all the spectra of this experiment.

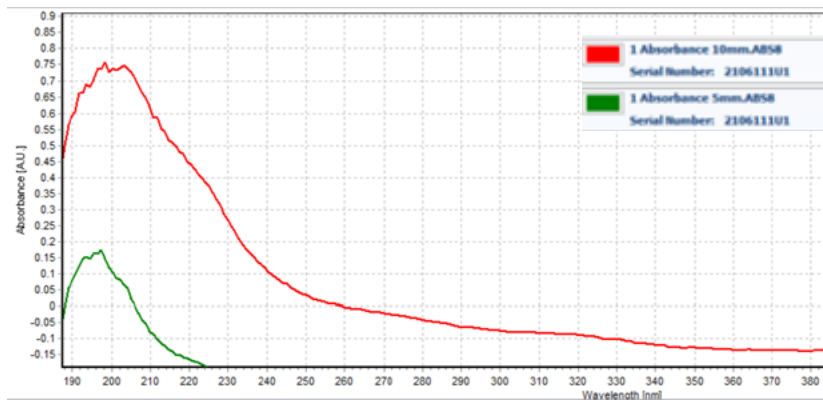


FIGURE 7: Recommended dilution concentration at 10mm and 5mm path length

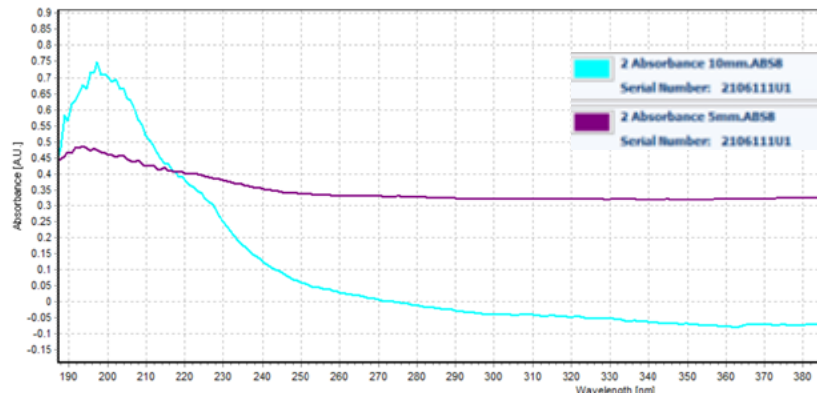


FIGURE 8: 25% more diluted concentration at 10mm and 5mm path length

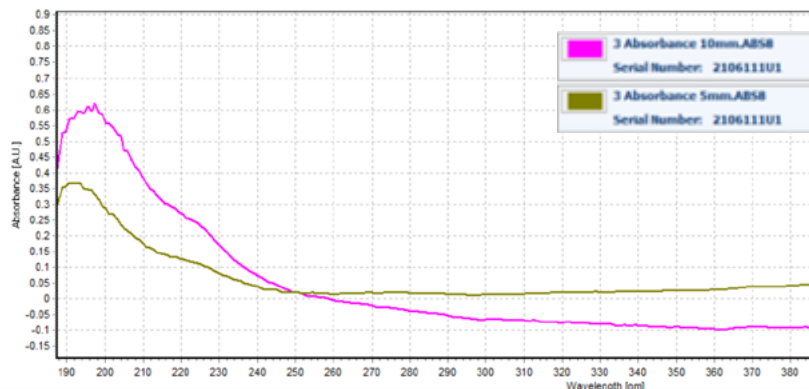


FIGURE 9: 50% more diluted concentration at 10mm and 5mm path length

TEST DATA AND RESULTS

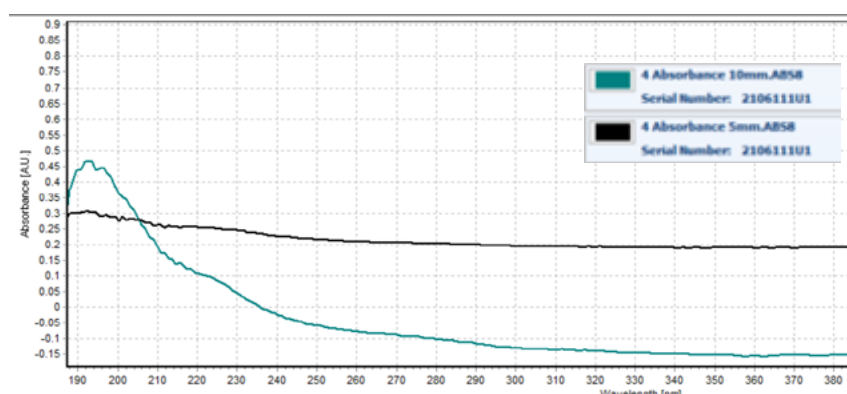


FIGURE 10: 50% more diluted concentration at 10mm and 5mm path length

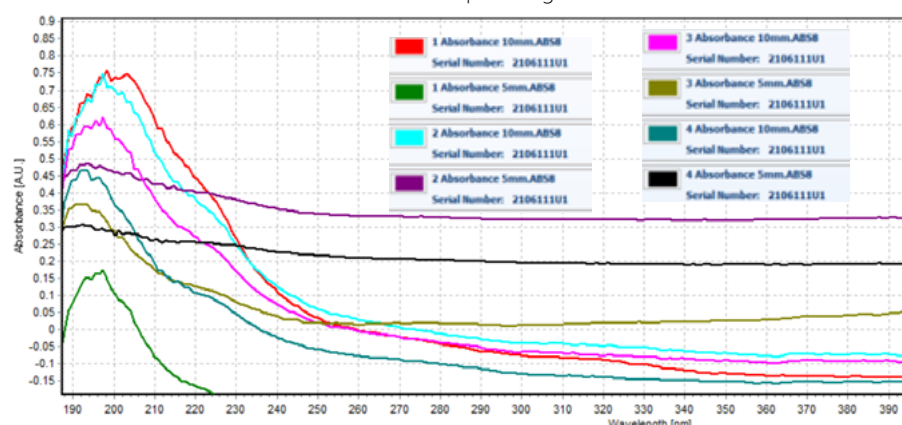


FIGURE 11: All absorbance spectra at 10mm and 5mm path length

CONCLUSION

As predicted, the 10mm path length on the variable dip probe provided better spectral results for identifying the absorbance peak of glyphosate. The 5mm path length trials still detected signals for the glyphosate, but the shape of the spectra was not as uniform as the 10mm. All four of these highly diluted samples proved to read better with the longer path length. With that said, if we decided to drastically increase the concentrations for another experiment, we would expect the 5mm path length to provide better results.

The variable path length dip probe proved that is very capable of picking up low concentrated absorbance measurements, as well as showing that it is diverse in the ranges that it can cover. This experiment provides good proof of the values of a variable pathlength transmission dip probe when variable concentrations are expected during a test procedure.

CONTACT

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