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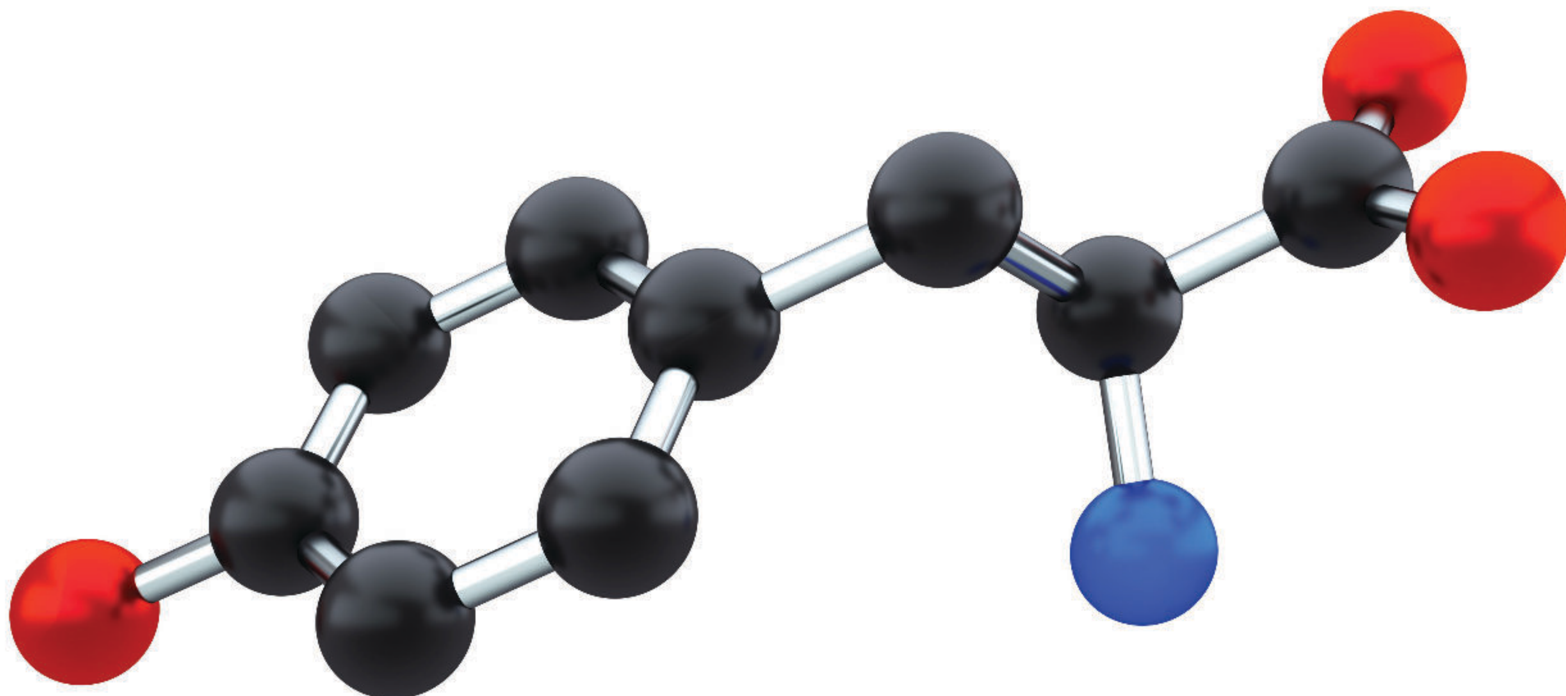
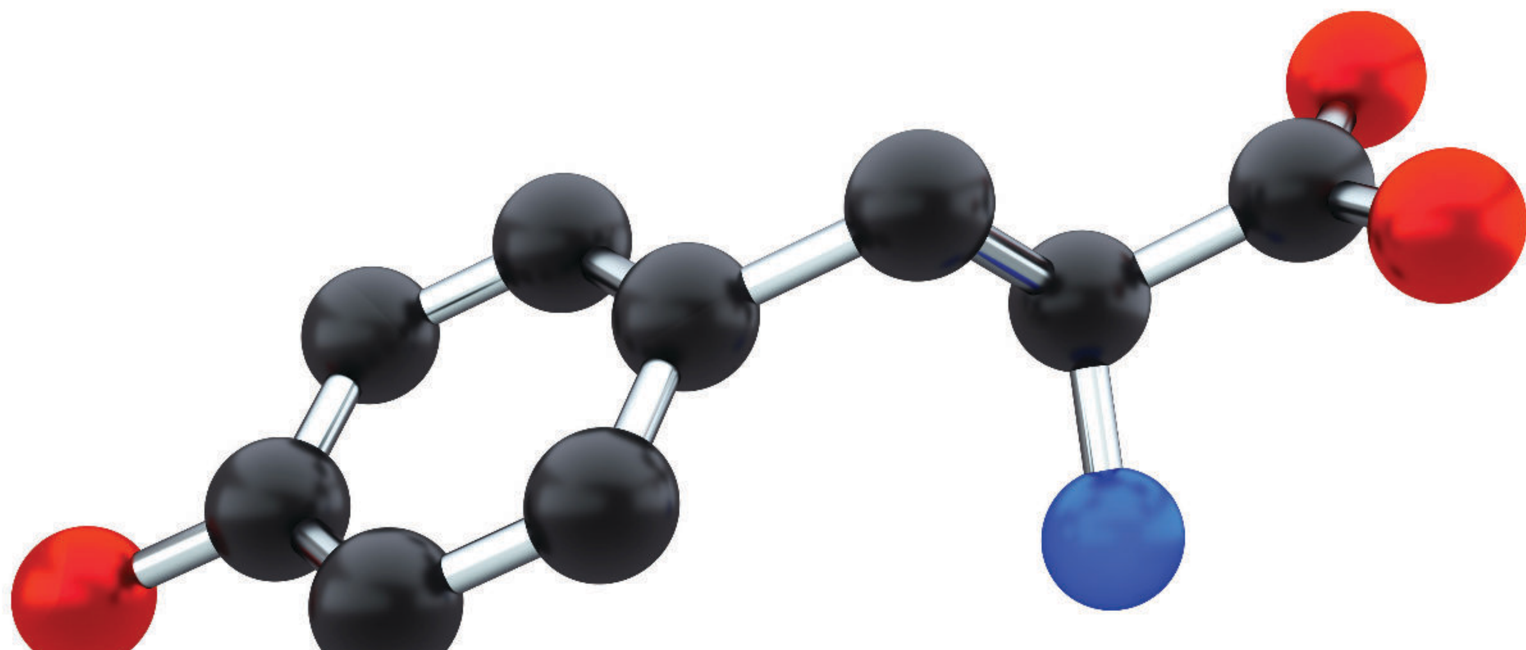


SPECTRA OF THE MONTH

# DETECTING AMINO ACID DILUTION WITH FLOW CELL ABSORBANCE SPECTROSCOPY

CONDUCTED BY: KURT AMEKU AND EVELYN ROMINE



## INTRO

# BACKGROUND OF APPLICATION

This experiment aims to monitor the absorbance peaks of amino acids in separate solutions and measure the magnitudes of any present peaks over time while the solution is slowly diluted. The amino acids used in the experiment were L-tyrosine and L-tryptophan, both of which were diluted in deionized water (Figure 1). To ensure continuous measurement capabilities, a flow cell and peristaltic pump were utilized.

L-tyrosine and L-tryptophan were selected for this experiment because they are classified as aromatic amino acids. In this context, the term "aromatic" does not refer to smell but rather indicates organic compounds that can be characterized by planar rings of atoms linked by covalent bonds. Thus, these amino acids can often be identified by distinctive rings of double bonds. This group is interesting spectroscopically because, although amino acids don't absorb light in the visible range, aromatic animo acids do absorb light in the ultraviolet range.

To measure L-tyrosine and L-tryptophan, a flow cell and peristaltic pump were used. The peristaltic pump was named after the principle of peristalsis, a series of muscle contractions that push food through the digestive system. The pump aims to mimic this by pushing solution through a flexible tube, squeezing the tube with rollers that transfer the liquid at a consistent rate.The flow cell facilitates the passage of solution by fixing the light source and spectrometer at a specific distance. This configuration is advantageous for a multitude of applications, one being blood analysis.,

**FIGURE #1:** Solution samples measured for this experiment, including a solution of L-tyrosine (left) and a solution of L-tryptophan (right).

# DESCRIPTION OF SPECTROSCOPY SETUP

The setup for this experiment (Figure 1) utilized our SensLine ULS2048x64-EVO. Alongside our cooled AvaSpec-ULS2048x64TEC-EVO, the ULS2048x64-EVO offers the same 2048x64 back-thinned CCD detector in an uncooled device for less demanding applications in the UV and NIR range. While cooling offers a more stable signal, this is often unnecessary for applications that use integration times shorter than 2 seconds. Like all of our EVO instruments, the ULS2048x64-EVO uses the AS-7010 electronics board, offering USB3.0 and gigabit ethernet communication along with better signal processing. Additional options include an order-sorting filter to reduce second-order effects and purge ports for deep-UV measurements. This unit comes with a wide range of slit sizes and gratings and can be configured with SMA or FC/PC fiber optic entrance connectors. The unit used in this experiment was optimized for the UV range with a 200-450 nm wavelength range and had a 50-micron slit installed.

The light source used for this experiment was the AvaLight-XE-HP, a high-powered pulsed xenon light source. This light source comes in a compact housing, making it well-suited for integration into customer systems. Compared to the AvaLight-XE, which has a maximum power of 2 W, the AvaLight-XE-HP provides significantly more power (6 W). When connected to an AvaSpec spectrometer via our custom interface cable, the number of flashes per scan can be set in our AvaSoft software, and the flashes are synchronized with the data collected by the spectrometer.

Other accessories included in this specific bundle for this experiment included a Flowcell-Z-10-PEEK , 10 mm pathlength PEEK flow cell, small diameter tubing to connect the flow cell to the pump, two 200-micron core fiber optic cables (FC-UVIR200-1-MS) with waterproof silicone-coated stainless steel jacketing, and a custom interface cable to connect the spectrometer and xenon light source (not pictured).

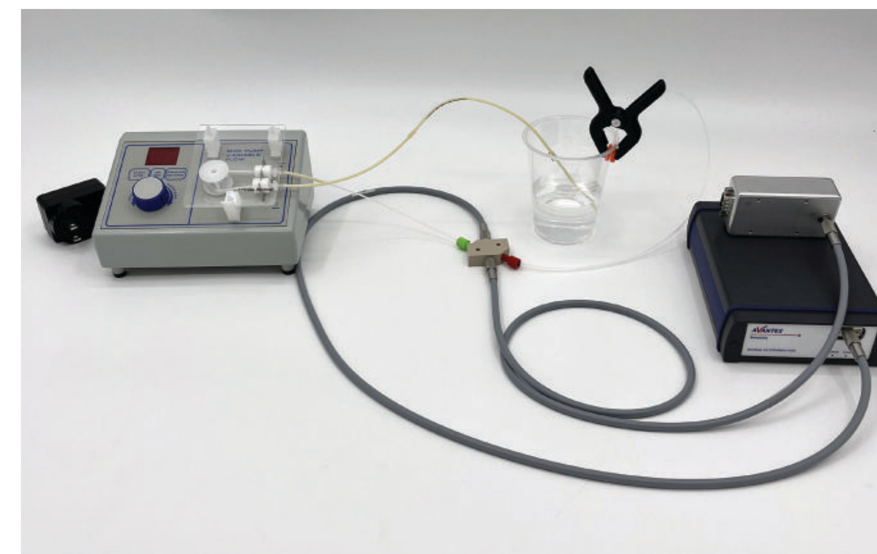


**FIGURE #2:** Experimental setup for solution measurements. The flow cell is connected to the peristaltic pump via tubing, and the pump is connected to the solution sample with similar tubing. The outlet of the flow cell has tubing back to the solution. The spectrometer and light source are connected to the flow cell in the perpendicular direction to the flow inlet and outlet via fiber optic cables. An interface cable (not shown) connected the light source to the spectrometer to control the light source pulse rate.

# DESCRIPTION OF METHODOLOGY

The solution samples used for this experiment were two amino acids, L-tyrosine and L-tryptophan, used as individual solutes, with both dissolved into deionized (DI) water. 10 mg of each amino acid were dissolved in individual DI water solutions that resulted in clear absorbance peaks. The 10 mg of L-tyrosine was diluted into 25 mL of DI water, while the 10 mg of L-tryptophan was diluted into 125 mL of water. Continuous measurements were taken of the solutions by flowing each solution through the flow cell and pump. During this time, dilution steps were taken at 1-minute intervals, with an initial 1-minute interval used to measure the solutions before dilution. For the L-tyrosine solution, each dilution step added 0.5 mL of DI water to the solution and 7 total dilution steps were taken, while 5 mL of DI water were added to each dilution step for the L-tryptophan solution and 14 total dilution steps were taken. The additional steps were taken for the L-tryptophan solution to determine if more dilutions would lead to different changes in absorbance peaks at later steps. No additional mixing was performed.

For data analysis, we used Absorbance mode and the TimeSeries module in AvaSoft, our exclusive custom software package. Absorbance mode, as the name suggests, is designed for absorbance applications, where the reference measurement will report 0 A.U. (absorbance units) and 5 A.U. when the light source is turned off. In this experiment, pure DI water was flowed through the pump and flow cell and was used as the reference. The TimeSeries module was used to record the magnitude of the observed absorbance peaks for each solution over the whole duration of measurements. This module can also track the peak wavelength in a certain range or the integral of a wavelength range over time, among other functions. Data from this module was exported as a .dat file and plotted in Octave, an open-source coding software. For the L-tyrosine solution, we used an integration time of 150 ms, which can be adjusted to increase or decrease the amount of light being measured at one time and affects the overall magnitude of the reported spectrum. For the L-tryptophan solution, an integration time of 120 ms was used. The number of flashes per scan for the light source has a maximum frequency of 100 Hz (or 1 flash per 10 ms), so the number of flashes per scan for the L-tyrosine and L-tryptophan solutions was set to 12 and 15, respectively. We set averaging for both solutions to 10, meaning 10 measurements were averaged together to account for the pulse-to-pulse variation of the xenon light source and provide more consistent results in the spectra. These total measurement times allowed for multiple measurements to occur between each dilution step to monitor how quickly the absorbance peaks changed.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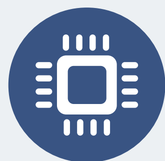

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# TEST DATA AND RESULTS

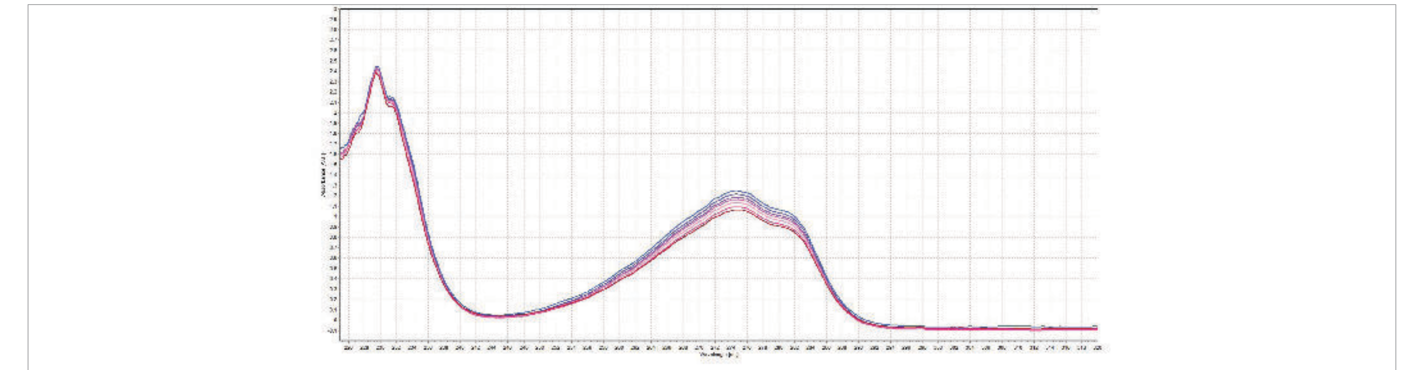

**FIGURE #3:** Absorbance spectra of L-tyrosine solution over 8 minutes (shown as blue-to-red gradient).

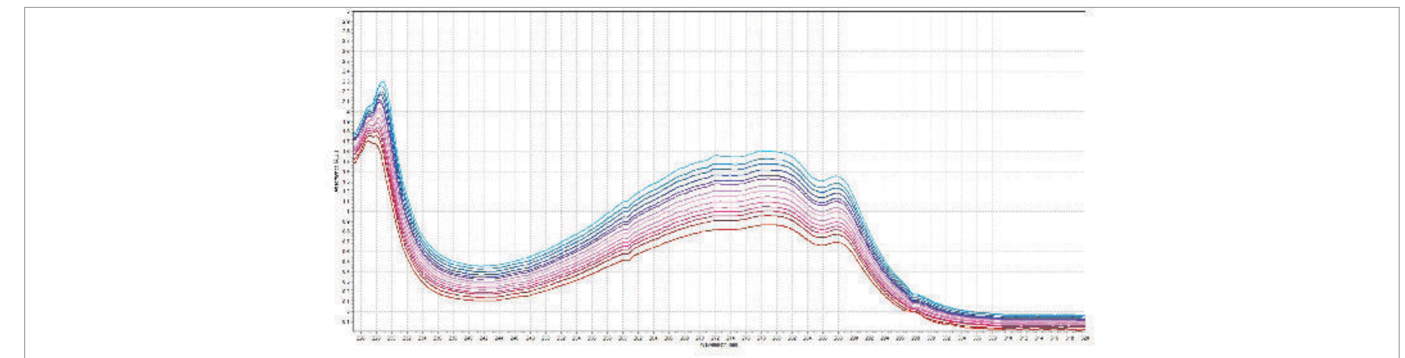

**FIGURE #4:** Absorbance spectra of L-tyrosine solution over 15 minutes (shown as blue-to-red gradient).

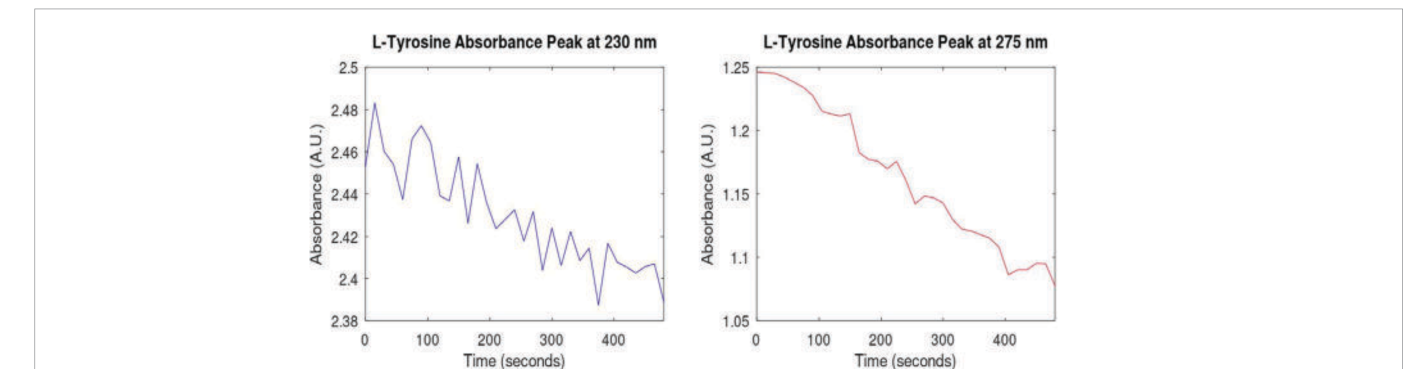


**FIGURE #5:** Magnitude of L-tyrosine absorbance peaks over measurement duration.

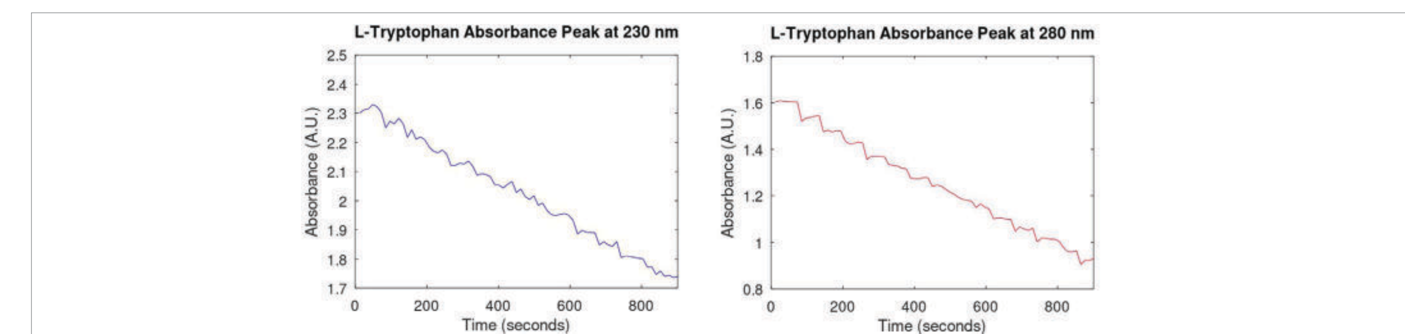


**FIGURE #6:** Magnitude of L-tryptophan absorbance peaks over measurement duration.

# ANALYSIS

Both amino acid solutions displayed two prominent absorbance peaks, with the L-tyrosine solution having peaks around 230 nm and 275 nm (Figure 3) and the L-tryptophan solution having peaks around 230 nm and 280 nm (Figure 4). These peaks correspond well to reported absorbance peaks in literature for these amino acids, demonstrating accurate measurement of each solute in their respective solutions. The dilution steps resulted in consistent decreases in the absorbance peaks for both solutions as expected, though the L-tyrosine peak decrease appeared much less linear (Figure 5) than the L-tryptophan peak decrease (Figure 6). This could be due to nonhomogeneous mixing of the solution with such a small liquid volume, disturbances in the pump flow during dilution steps, or just an artifact of only measuring for 7 dilution steps. Indeed, the L-tryptophan absorbance peaks may appear similarly nonlinear if half the dilution steps are observed.

Comparing the absorbance peak changes over time, there also seems to be much more noticeable sharp decreases in the longer wavelength absorbance peaks (275 nm for L-tyrosine and 280 nm for L-tryptophan) compared to the shorter wavelength absorbance peaks. This may indicate that these longer wavelength peaks are better representations of the change in solution concentration. On the other hand, the shorter wavelength peaks are in a region that shows significant noise due to being in the lower UV range, which could affect the overall magnitudes measured.

# CONCLUSION

In conclusion, the present experiment highlights the ability of our spectrometers and light sources, in conjunction with a third-party flow cell and peristaltic pump, to dynamically measure changes in a flowing amino acid solution as it is being diluted. Both the L-tyrosine and L-tryptophan solutions showed distinct absorbance peaks that correspond to reported peaks in literature. The peaks decreased in magnitude at each dilution step as expected, though the decrease was not as linear as expected for the L-tyrosine solution. The AvaSpec-ULS2048x64 provides the same high-performance capabilities as its thermally-cooled counterpart in applications where shorter integration times do not require the added stabilization of a cooled instrument. The AvaLight-XE-HP is a xenon light source that is well-suited for applications where high power is needed or compact form factor is a must. The custom interface cable highlights the capabilities of our engineering team to provide custom assemblies and solutions for customer needs.

Please contact Avantes for more information on the configuration that is best suited for your data collection.

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