

SPECTRA OF THE MONTH **MEASUREMENT OF UV/VIS SPECTROSCOPY ON VITAMINS** CONDUCTED BY: KURT AMEKU



### INTRO BACKGROUND OF APPLICATION

As the new year begins, many set goals for self-improvement, often focusing on health. Taking vitamins is a common step, as many people lack essential nutrients in their diet. Choosing supplements requires considering dietary needs, such as iron for vegans or vitamin B12 and D3 for specific groups. Beyond active ingredients, marketing claims like 'organic' or 'gluten-free' can drive up costs without added benefits. In this study, we plan to demonstrate that the difference in cost does not necessarily correlate with a difference in quality, at least in terms of active ingredient, through the use of absorbance spectroscopy.

This experiment aims to measure the absorbance spectra of two different vitamin types, B12 and D3, both at two different price points, to determine if there are any differences that can be determined spectroscopically between them based on cost. One inexpensive, generic brand of vitamin and one more expensive option of vitamin were chosen (Figure 1) and measured by dissolving them individually in warm water. Measurements were taken with a dip probe after each sample was sufficiently dissolved in the water solution.



**FIGURE #1:** Spectra results - put as many images here as there were samples tested on Vitamin samples used for this experiment. (from left to right: inexpensive B12, expensive B12, inexpensive D3, and expensive D3).

## DESCRIPTION OF SPECTROSCOPY SETUP

The setup for this experiment (Figure 2) was based around our AvaSpec-NXS2048CL compact spectrometer, commonly known as the Nexos. This compact instrument is our next-generation photonics backbone spectrometer, designed to empower a wide range of applications in various industries. This device is built using our new semi-automated manufacturing technique that ensures higher levels of consistency and reproducibility unit-to-unit. The Nexos offers USB2.0 communication as well as RS232 and SPI communication protocols, a CMOS linear array detector, ultra-low stray light as low as 0.1%, and a signal/noise ratio of 375:1. Furthermore, this spectrometer can be customized with a wide range of gratings (13 total available) and the replaceable slit option is now standard for non-OEM units, which provides even more flexibility for a variety of application needs. The unit used in this experiment was optimized for the UV and visible range needed to measure the absorbance peaks of the vitamins selected for this experiment, with a 200-850 nm wavelength range and a 25-micron slit installed.

The light source used for this experiment was the AvaLight-DHc, which features both a deuterium light source and halogen light source, giving the user the best of both worlds. The deuterium light provides a UV source between 200-550 nm, and the halogen light provides visible and near infrared light up to 2500 nm. The AvaLight-DHc also features an internal TTL shutter that is controllable from an AvaSpec spectrometer.

Other accessories used for this experiment included a 600 mL beaker, a custom FDP-2UVIR600-1-BX variable path length dip probe, and our U.S. Transmission-Reflection (TR) Stage. A 10 mm dip probe path length was used for this experiment.



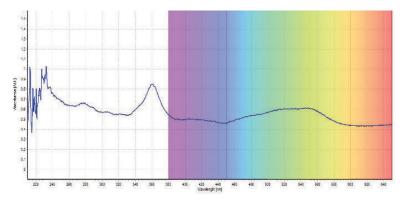
**FIGURE #2** Experimental setup for the absorbance measurements. The spectrometer and light source are attached to the dip probe. The dip probe is mounted in the Transmission-Reflection (TR) Stage and immersed in the vitamin solution (vitamin B12 solution shown here) to perform a measurement.

## DESCRIPTION OF METHODOLOGY

Vitamin samples for this experiment were purchased from a local grocery store. The expensive options for B12 and D3 were name-brand supplements, while the inexpensive alternatives were store-brand versions, costing roughly half as much per pill. Each vitamin sample consisted of dissolving 3 vitamins into approximately 150 mL of warm tap water. This was done to both ensure enough vitamin was dissolved in the solution to be detected and to minimize the effect of variance of water to the solution, all while minimizing the volume of solution needed to be made per sample. Warm tap water was used to increase the speed of the dissolution, as room temperature tap water was initially used but determined to be too slow of a reaction time. Each dissolution was hand-stirred and took approximately 5 minutes to sufficiently dissolve. The D3 vitamins contained a significant amount of oil, which resulted in more of a suspension, though sufficient measurements were obtained. After each vitamin measurement, the dip probe was cleaned with a task wipe, rinsed in a separate water beaker, then cleaned with a new task wipe to minimize contamination.

For data analysis, we used Absorbance mode in AvaSoft, our custom software package. As the name suggests, Absorbance mode is designed for absorbance applications, where the reference measurement will report 0 A.U. (absorbance units) and 5 A.U. for the dark measurement. In this experiment, the tap water with no solute with the light on was used as the reference and the tap water with the light off was used as the dark. We used an integration time of 200 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. We set averaging to 5, meaning 5 values were averaged together to provide more consistent spectra results.

### TEST DATA AND RESULTS





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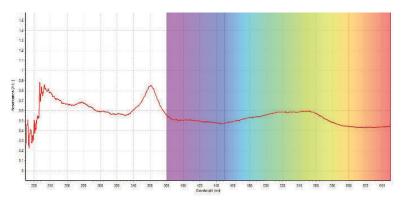


FIGURE #4: Absorbance spectrum of inexpensive vitamin B12.

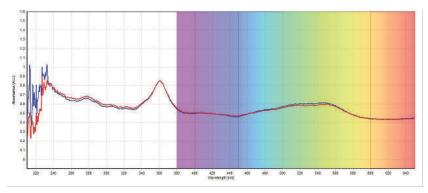
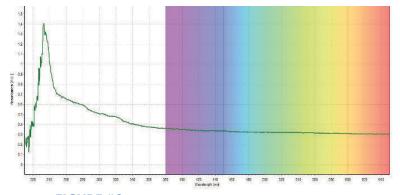


FIGURE #5: Absorbance spectrum of expensive vitamin B12 (blue) and inexpensive vitamin B12 (red), shown together for comparison.





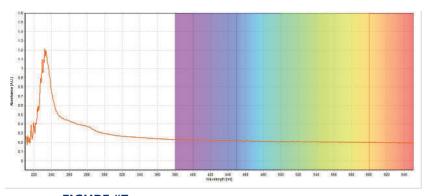


FIGURE #7: Absorbance spectrum of inexpensive vitamin D3.

### TEST DATA AND RESULTS

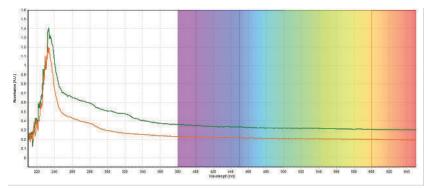


FIGURE #8: Absorbance spectrum of expensive vitamin D3 (orange) and inexpensive vitamin D3 (green), shown together for comparison.

## ANALYSIS

The two vitamin B12 samples showed similar absorbance peaks throughout the measured spectral range (Figures 3-6), with the most prominent peaks around 278 nm, 361 nm, and 550 nm. These align well with literature, where the most intense peak at 361 nm corresponds to the highest measured peak. The common UV absorption peaks for vitamin D3 are 228 nm and 265 nm. A peak around 230 nm is observed, matching the reported 228 nm peak. A small hump around 280 nm is also seen, though it may be an absorbance peak from an inactive ingredient in the vitamin D3 pills, such as one of the oils.

Comparing between the expensive versus inexpensive pills, it is interesting to see virtually no difference between the vitamin B12 pills. If nothing else, one might think that the size difference would affect the amount of inactive ingredients in the solution such as the coloring of the pills, though perhaps this is just more concentrated in the inexpensive option. Regardless, the known characteristic absorbance peaks of vitamin B12 being the same magnitude is not surprising and reassuring considering the two options are reported to have the same quantity of the vitamin per pill. Comparing between the two vitamin D3 samples, it is interesting to see such an offset in absorbance across the whole spectral range. This could be attributed to a couple factors. One, the expensive options does look slightly darker in person. Two, the expensive brand also has a larger volume per pill, which would increase the amount of oil and therefore the absorbance value. Despite this, even if the spectra are normalized based on the values at 650 nm of each respective absorbance spectra, the expensive option does still have a higher absorbance peak around 230 nm, though this could also be attributed to noise in the spectrum. The last point of note is the small peak around 320 nm in the expensive option for vitamin D3. One difference between the two vitamin D3 options is the type of oil used for the inactive ingredient. For the inexpensive option, the primary oil used is soybean oil with a small amount of corn oil. For the expensive option, flaxseed oil is used. While soybean oil does not have a significant amount of UV absorbance, flaxseed oil has UV absorption bands deep in the UV-C range as well as some absorption around 320 nm. This correlates well with the small peak seen in the expensive vitamin D3 sample.

## CONCLUSION

In conclusion, the present experiment highlights the use of our spectrometers to measure the contents of vitamins in a solution and compare different vitamins to determine if the cost has any effect on what is seen spectroscopically. Unsurprisingly, this is not the case, as mainly only the active ingredient was seen in the spectrum, though other factors in cost such as manufacturing processes and cost of different ingredients cannot be factored in these measurements.

Other spectroscopic measurement techniques could potentially be implemented to detect and quantify some of the other inactive ingredients in the vitamins for more in-depth analysis. Despite this, the implemented measurement still correctly identified known absorbance peaks for both vitamin B12 and vitamin D3, as well as an absorbance peak for flaxseed oil. The AvaSpec-NXS2048CL is an ideal solution for OEM applications or any other cases where compact form factor is critical. AvaLight-DHc is suited for nearly all absorbance applications, with a broad wavelength range from 200 to 2500 nm thanks to its deuterium and halogen bulbs. Please contact Avantes for more information on the configuration that is best suited for your data collection.



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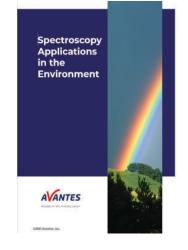
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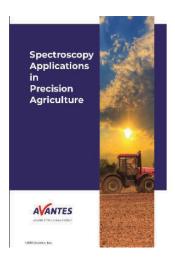
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