UV-VIS Spectroscopy of Tannins and Phenols in Red Wine Using the Short Path SpecVette™ Cuvette

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Abstract

Measurement of wine color and tannins involves spectroscopic analysis in the UV and visible region. The test protocols are complex and are hampered by the need make multiple wavelength measurements. A typical spectrophotometer can only make one wavelength measurement at a time. In addition, the assessment of total phenols requires measurement of the sample at 280 nm requiring the use of an expensive, difficult to clean and easy to break 1 mm path length quartz cuvette. Even with a 1mm pathlength, most red wines still require 5 to 10 fold dilution in order to keep the absorption at 280 nm near 2.0 OD, further complicating the test protocol.

In this application note, we report the performance of a new short path cuvette, the SpecVette™ with an Ocean Optics HR4000 spectrometer using a balanced Deuterium-Halogen light source to measure the absorbance of undiluted red wine samples from 250 to 650 nm. A DOS routine was written to automatically collect and transfer ten sets of data from the SpectraSuite software into and Excel™ spreadsheet. Data was collected from either ten different cuvettes (inter-cuvette measurements) or from a single cuvette that was repeatedly measured after removal and re-insertion into the cuvette holder (intra-cuvette measurements). The ten sets of data transferred into the spreadsheet were averaged at each 0.25 nm interval across the entire spectrum and the standard deviation and % error calculated. The standard deviation of the measurement at each 0.25 nm from 250 to 600 nm was plotted. The % error across the same range was plotted separately below the spectrum. Results show that with a 0.25 mm path length cuvette, the absorbance at 280 nm was 2.0 +/- 0.1 OD with an error of 1.0 to 2.5% across the wavelength regions of 250 to 450 nm for intra-cuvette measurements. The % error for the inter-cuvette measurements was 1.5 to 5.0% between 250 and 450 nm.

Introduction

Phenolic compounds in young red wines display complex chemistry which greatly contributes to the color, astringency, bitterness, and hence the quality of the wine as it ages. In general, the anthocyanins are responsible for the color of the wine, while the tannins contribute greatly to the mouth feel (astringency) and bitterness of the wine. Quantitation of the total phenols and methods to evaluate the ratios of the various classes of phenols have been developed by researchers at the University of California, Davis[1-3]. The methods involve spectroscopic analysis of wine samples standardized to pH 3.6 and treated with various reagents to determine the contribution from different classes of phenols including free anthocyanins, co-pigments of anthocyanins, tannins, and polyphenolic pigments formed by the reaction of anthocyanins with tannins as the wine ages. Collection of large sets of data over time in combination with traditional assessments of wine quality by

master sommeliers are used as a guide to optimize the ratios of these various phenols in young red wines before bottling.

Method:

Wine samples were taken from a freshly opened bottle of Pinot Noir. A small volume, less than 1 mL was required for all measurements. The rest was sampled by the author with cheese and crackers as an accompaniment. The wine was determined to be palatable.

A SpecVette shown in Figure 1 with the adaptor, and was filled using a pipetter as shown in Figure 2.

Figure 1: ALine's SpecVette

Figure 2: Filling the SpecVette

The SpecVette is fabricated from a stack up of a UV transparent film material separated by a spacer layer that is 0.25 mm thick which bonds the window layers to the spacer layer through a pressure sensitive adhesive. The edges of the spacer are outside of the light path and do not contribute to light scatter in the measurement. Inlets and outlets are fabricated on one side of the cuvette and then mounted onto the black delrin body of the SpecVette which has access ports to the spacer layer to provide easy sample introduction and support for the thin cell. The error in the thickness of the cell is expected to be around 10%, based measurements of the variability of the film stock used to fabricate the cuvette. The films that make up the windows of the SpecVette are plasma treated to allow the device to easily fill by capillary action. The 0.25 mm pathlength cuvette contains a volume of about 10 uL. In these experiments, the SpecVette sits at 45 degrees to the light path. Each SpecVette accommodates two samples that are labeled "A" and "B".

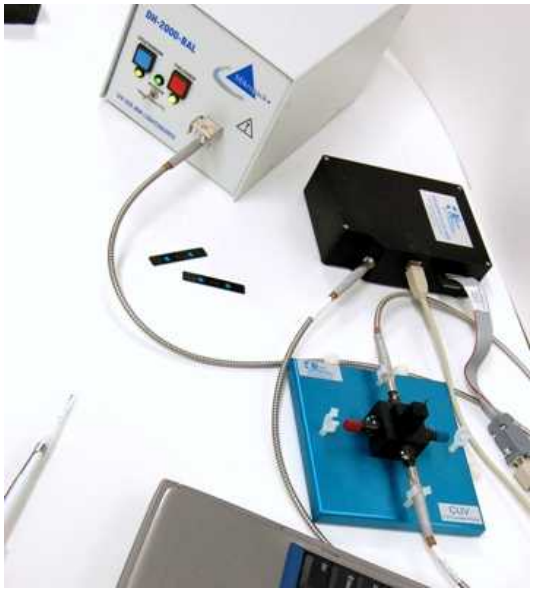

Figure 3 Ocean Optics HG4000 Spectrometer

The Spectrometer, shown in Figure 3 is an Ocean Optics high resolution HR4000 USB spectrometer with an extended wavelength range from 190 to 1100 nm. The DH-2000-BAL light source, with both a deuterium and halogen lamp, was balanced to give about 12,000 counts at 280 nm region, 6000 in the 450 to 550 nm region, and 10,000 counts near 600 nm as shown in Figure 4. The integration time was set to 100 ms, with a boxcar width of 4 and an average of three scans and the non-linearity enabled. The CUV ALL cuvette holder was used to couple the light source and the spectrometer to the sample using the extreme solarization resistant fiber optic cables that permit good light transmission to 180 nm. At the beginning of each experiment the dark and background spectra were captured and saved in the SpectraSuite software.

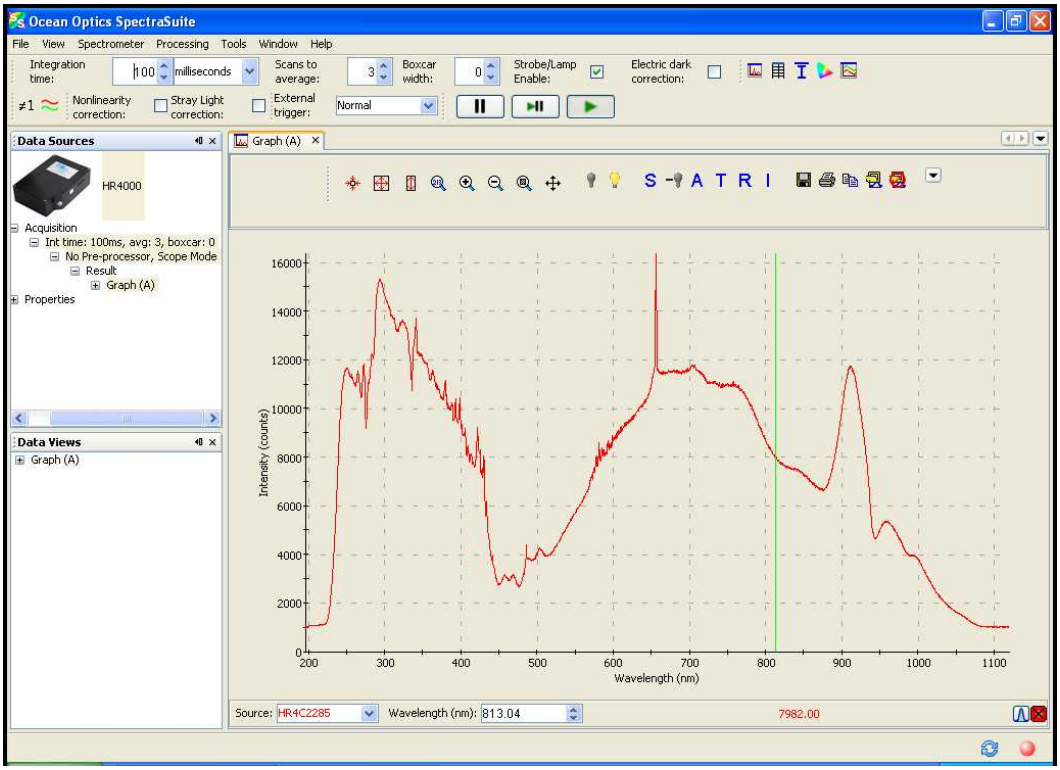

Figure 4 shows a plot of the intensity for a SpecVette filled with DI water from 200 to 1100 nm as displayed by the SpectraSuite software. The intensity is for both the deuterium lamp, which has most of its spectral intensity in the UV, and the halogen lamp, which has most of its intensity in the visible region. The Deuterium lamp output was adjusted to give the same intensity as the brightest region of the halogen lamp. Neither lamp has strong emission between about 450 and 550 nm.

Intra-cuvette determination of the error of measurement

Using a single SpecVette™ with a .25 mm pathlength, sample window "A" was filled with DI water for the blank or background measurement, and "B" was filled with the wine sample. The background was measured and saved in the SpectraSuite software and was automatically subtracted from each subsequent measurement made. The wine sample in "B" was measured ten successive times after removing and replacing the SpecVette in the holder. The holder was secured with the positioning screw provided in the CUV ALL to hold it snug during the measurements. Each of ten spectra were transferred to an excel spreadsheet and averaged with the standard deviation, the standard error and a measure of the outliers was determined with .5 nm wavelength intervals across the 250 to 650 nm region. Figure 5 shows a plot of the standard deviation at each wavelength, with the % error at each wavelength reported below the spectrum.

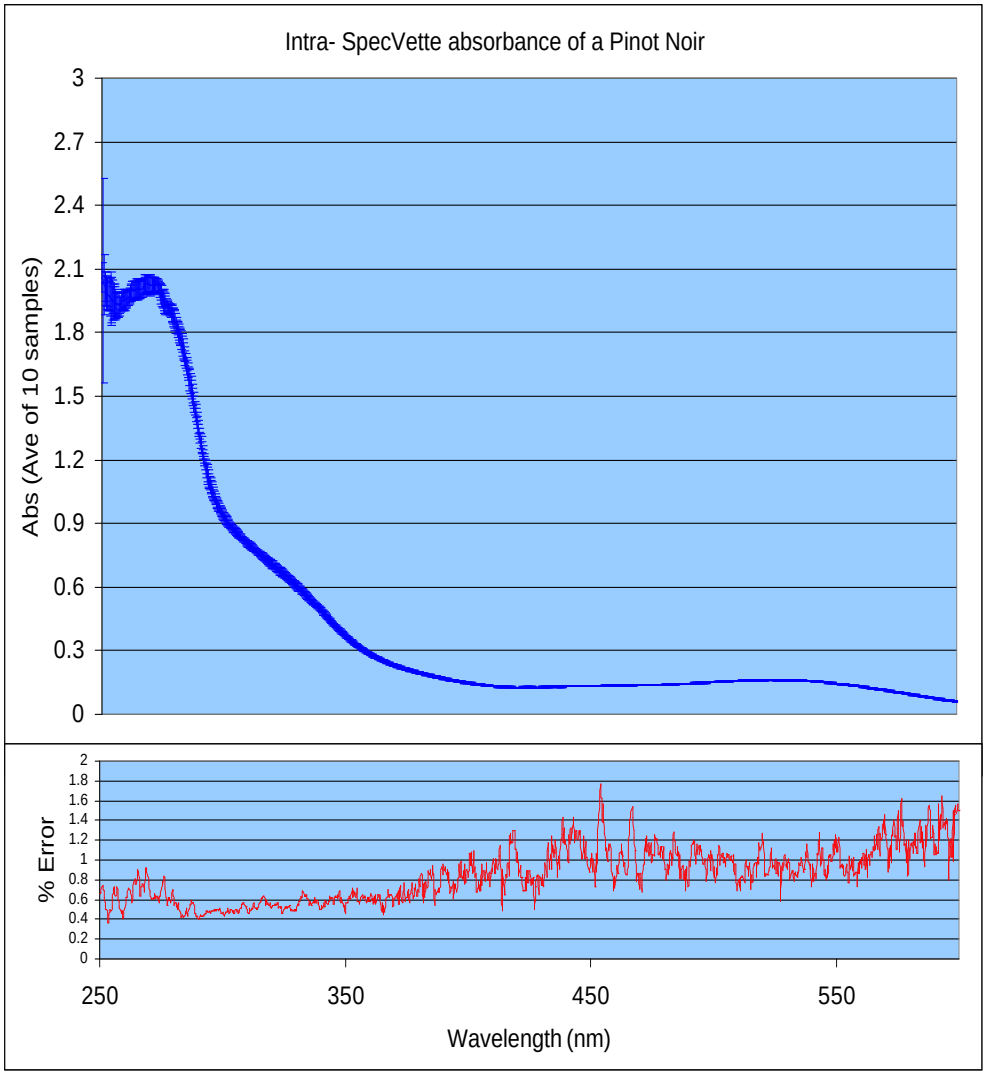

Figure 5 The spectrum from 250 to 600 nm was collected for ten different measurements of a single SpecVette removed and then re-inserted into the holder. The standard deviation of the average of ten is plotted in blue. The % error, which was calculated as the standard deviation divided by the average absorbance at each wavelength, is plotted below in red.

Inter- SpecVette determination of the error of measurement

Using a set of six different SpecVette™ cuvettes, two samples per cuvette, with a .25 mm pathlength, background and ten wine spectra were measured and stored. Five different cuvettes were filled with two samples of Pinot Noir and measured sequentially and saved and evaluated as above. The results are shown below in Figure 6.

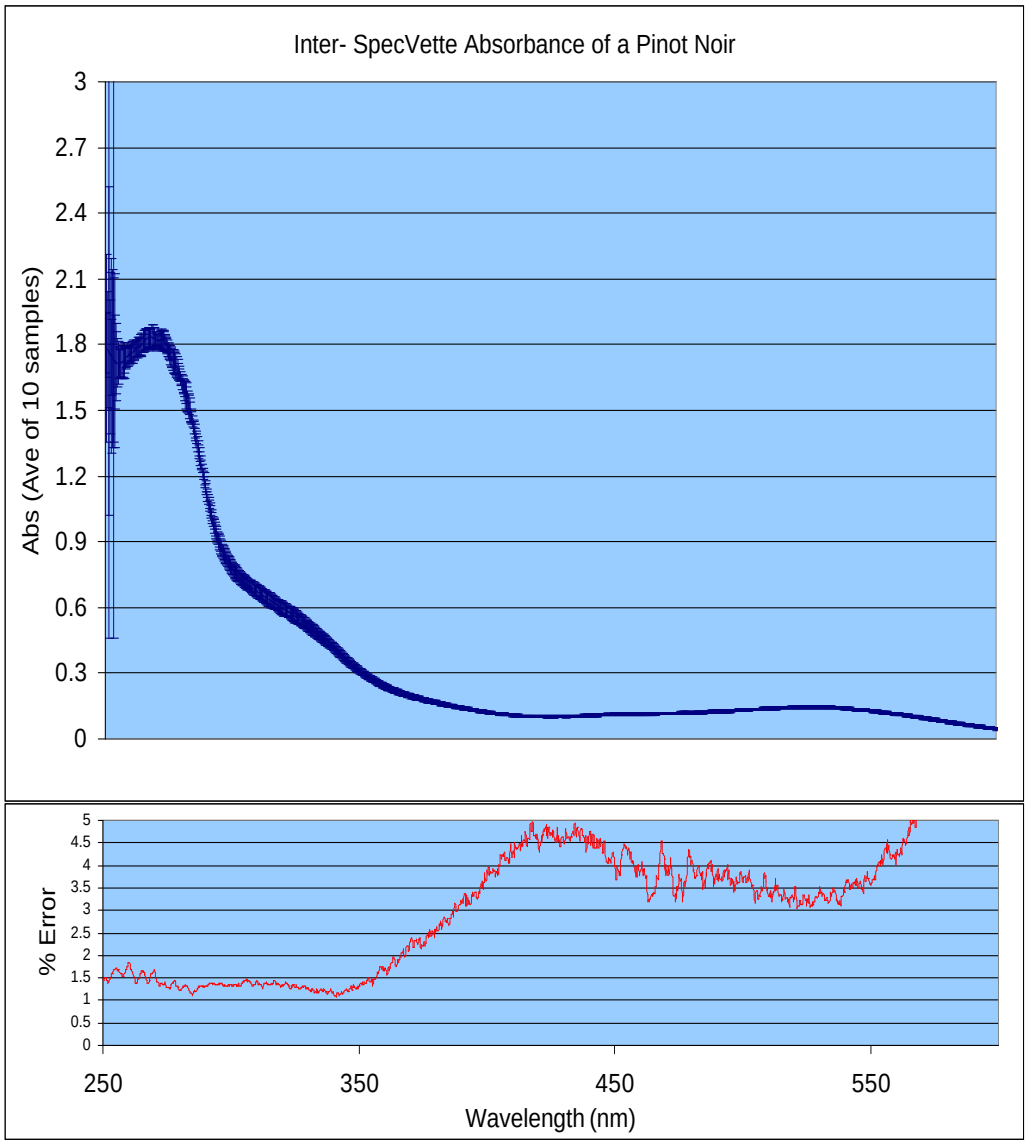

Figure 6 The spectrum from 250 to 600 nm was collected for ten different measurements of ten different samples of the same wine using five different SpecVettes. The standard deviation of the average of the ten measurements is plotted in blue. The % error, which was calculated as the standard deviation divided by the average absorbance at each wavelength, is plotted below in red. The % error increases in the region where the absorbance is less than 0.1 OD

Results

In the UV portion of the spectrum, between 250 and 450 nm, the maximum absorbance for an aged Pinot Noir was near 2.0 OD at 280 nm using the 0.25 mm pathlength SpecVette. The absorbance at wavelengths greater than 450 nm was about 0.10 OD. As the OD drops to .1 or less the % error in the measurements climbs rapidly to greater than 5% for the *Inter*-SpecVette data set.

Comparison of data collected for the *Intra*-SpecVette measurements show that with proper adjustment of the adaptor, the error in the measurement reflects the instrument noise, with the cuvette, holder and the experimenter contributing very little to the measurement error. For the Inter-SpecVette data, the error in the data increases to about 5% as the measured absorbance approaches drops below 0.1 OD. This suggests that there are slight variations between the cuvettes, but if the absorbance is above 0.1 its contribution to the total error is insignificant.

Conclusion

The SpecVette is a reliable and reproducible cuvette that can be used to determine the absorbance of most red wines from 250 to 600 nm without the need for dilution. Using the Ocean Optics HG4000 spectrometer and the DH-2000-BAL light source, the entire region of interest can be measured and analyzed in an excel spreadsheet. Using a data capture and transfer routine written in DOS, ten sets of data were collected in the SpectraSuite software and transferred automatically into an excel spreadsheet where all the calculations were performed and the data graphed as shown in Figures 4 and 5.

References:

1. Chemistry of Winemaking, A.D. Webb ed., American Chemical Society, Washington, DC, (1974) pp 184-211.
2. Boulton, R. “The co-pigmentation fo anthocyanins and its role in the color of red wine: A critical review.” American Jouranl of Enology and Viticulture (2001), Vol 52, pp 67-87.
3. Harbertson, J., Picciotto, E., Adams, D. “ Phenolic And Anthcyanin assay for us with Spectrophotometer”. http://wineserver.ucdavis.edu/adams/tannin/index.htm.

For further information on the spectrometer system, the data capture and transfer routine and the SpecVette, please contact Dr. Leanna Levine at 310-707-8575 or by e-mail at llevine@alineinc.com, or visit the ALine, Inc. website at www.alineinc.com.