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Spectral Analysis for Small/Medium Wine Laboratories

Richard Carey



OVER THE LAST DECADE or so, the functionality of UV-VIS spectrophotometers has not changed much in terms of the sample's accuracy of measurement. What has changed is that the companies which produce these instruments have increased the ease of sample handling, from manual insertion of one sample at a time at each step of the analytical process to fully automated handling of samples. In addition, they have increased the use of software manipulation of data. **BOX 1** has a listing of companies and their websites.

Past articles on spectrophotometers have focused primarily on the automated systems that can measure up to hundreds of samples per hour. Equipment of this sort is highly desirable for larger-sized wineries because of both cost per sample for slower systems and the need for a large number of samples to be tested. This article will focus on systems more in line for small- to medium-sized wineries. Even within that group there is a variety of systems that can fit different winery methods of operation.

BOX 1

Companies Producing Spectrophotometers

Admeo Analytical Systems		**Hanna Instruments**	
California	*www.admeo.us*	Rhode Island	*www.hannainst.com*
Astoria Pacific		**Megazyme**	
Oregon	*www.astoria-pacific.com*	Ireland	*www.megazyme.com*
Blue Sun Sientific		**Thermo Fisher Scientific**	
Maryland	*www.bluesunscientific.com*	Massachusetts	*www.thermofisher.com*
CDR Wine Lab		**Unitech Scientific**	
Italy	*www.cdrfoodlab.com*	California	*www.unitechscientific.com*
Foss (FTIR)			
Denmark	*www.fossanalytics.com*		

BOX 2 contains information about four companies that produce instruments capable of analyzing a multitude of important wine compounds. Instead of wave splitters, they use specific wavelengths of light that pass through a test tube or cuvette using interference filters. Like a wave splitter, by measuring the degree to which the intensity of that wavelength is absorbed, either more or less by the substance in the test tube or cuvette in each of the steps in the reactions, a determination can be calculated on how much of the target material is in the sample. The difference between the two methods (aside from cost) is that a wave splitter can select any wavelength within the limits of the splitter's mechanics whereas interference filters select a narrow band width of wavelengths for each filter.

This advancement in spectrophotometer function lowered the cost of the instruments' production. The reason for the lower cost is that these filters can provide a relatively narrow bandwidth of filter (10nm) from a general light source to allow only the selected bandwidth to penetrate. In addition, it is now easier to add a secondary filter to the same test procedure that can analyze a complementary wavelength. The additional wavelength can remove some noise from the spectrum, thereby increasing the measurement's accuracy. In the more expensive system, the mechanics of wave splitting can provide very narrow bandwidths to reduce analytical variance. For wines, super-precision is not necessarily better. Repeatability is demonstrating a narrow enough range of analytical variability to be useful.

The skill set required to run these relatively simple instruments is based on the principle of "If you can bake a cake, one can run an enzymatic wine analyzer." In addition to the instrument, a laboratory must have some refrigeration available for storing test kits, and it is better if there is a freezer for some test kits in order to maintain a longer shelf life. In general, the shorter the test kit's shelf life, the fewer tests that should be purchased at one time so that tests are not lost because of their expiration date.

BOX 2

Analyses by Instrument Manufacturer

Analyses	Admeo	CDR WineLab	Megazyme	Unitech Scientific
Alcohol by Volume		●	●	●
Acetic Acid (volatile acidity)	●	●	●	
Acetaldehyde (Ethanal)	●	●	●	●
Anthocyanins Extraction on Grapes	●	●		●
Ammonia	●		●	●
Ammonia, L-Arginine Urea				
Ascorbic Acid	●		●	●
Calcium	●	●		●
Carbon Dioxide	●			
Catechins	●	●		
Citric Acid	●		●	●
Color (tonality and intensity)	●	●		
Copper	●	●		●
Fermentable Sugars	●	●	●	
Fructose	●		●	
Galacturonic Acid		●		
Gluconic Acid	●	●	●	
D-Glucose	●		●	●
Glucose-Fructose	●	●	●	
Glycerol	●	●	●	
HCl Index		●		
Histamine	●			
Iron	●			●
L-Lactic Acid	●	●	●	●
L-Malic Acid	●	●	●	●
Pectin			●	
pH	●	●		
Polymerised Anthocyanins		●		●
Polyphenol/Tannin	●	●		●
Primary Amino Nitrogen	●		●	●
Potassium, Enzymatic	●			●
Sucrose	●		●	●
Succinic Acid			●	
Sulfur Dioxide - Free	●	●	●	●
Sulfur Dioxide - Total	●	●		●
Tannins		●		
Tartaric Acid	●		●	●
Total Acidity	●	●		
Total Anthocyanins	●	●		
Total Polyphenols Index	●	●		
Total Polyphenols (Folin Ciocalteu)	●	●		●
Total Sugars	●			
Yeast Assimilable Nitrogen (YAN)	●	●		

Admeo

This company is one that I have not covered previously but appeared in research results. At this point in time, only their higher capacity instruments have had significant exposure to the wine industry. However, Admeo is currently introducing a product called the DNA Smart Analysis, which is a smaller instrument appropriate for small- to medium-sized wineries. I will review this instrument in an upcoming article that will describe the analytical protocols for a new series of analyses of anthocyanins, tannins, polyphenols and color balance. I have included the analyses that can be performed by the DNA Smart Analysis in BOX 2.

CDR Wine Lab

The CDR Wine Lab was introduced to the wine laboratory equipment market several years ago. CDR Wine Lab has two models: CDR Wine Lab (used for this article) and CDR Wine Lab Jr. The CDR Wine Lab (about $7,000) lists 25 separate analyses applicable to the wine industry whereas the Jr. ($4,000) performs 16 tests.

The instrument is built on a spectrometer-based technology that uses specific wavelength LED emitters. This technique has allowed the equipment to expand the measurable Absorbance Units (A) in the linear range to 6 A. There are four separate analytical bays that use six different wavelengths to perform the tests. The instrument has 16 heated slots (the Jr. model has three) where the sample cuvettes used for analysis reside in preparation for analysis.

The test is run using a touch-screen LCD. The menu lists the analyses that the instrument will run. There are several ways to store and retrieve data. First, the instrument itself can store thousands of analytical results. Second, there is a USB Type 2 port for technical service to the instrument and for connection to a PC, as well as a direct Ethernet LAN port and two USB 2.0 ports for database transfer of the performed tests, software updates and

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configuration. The instrument can conduct more than one test at a time and has an 80 mm graphic printer. The Jr. model does not have these features.

The CDR Wine Lab has a straightforward testing principle. Most test kits have one or more reagent bottles in a pack of 10 or 10 packs of 10 tests. A few tests have no additional reagent bottles; the user simply adds the sample to the cuvette for analysis. The shelf life of most tests is about 18 months from the date of manufacture (however, travel time to a winery may reduce the shelf life to about 12 months). Since the kits are mostly sold in 10-test packs, wineries should be able to use a pack within the usable time frame. Most test packs require storage at refrigerator temperature.

When performing a test, the LCD on-screen prompts direct every step. Once each sample is analyzed, a printout pops up with the necessary information. If direct-to-computer cables are installed, data can be saved to a computer file.

Most tests use one to three injections of 10 to 100 µl. This process is easy to learn (you can practice with water to develop your technique). While this is probably the most difficult part of learning the new lab procedures, it is necessary as the goal is to get consistent results.

The cost of the tests ranges from about $3.50 to $6.00, plus shipping. The common tests are usually in stock and, when not, the delay is often not long. Advance arrangements should be made for the test kits that are not used as frequently.

Proprietary software for the computer to display the results can be downloaded during setup.

Megazyme MegaQuant™ Wave and Unitech Scientific

The MegaQuant™ Wave, introduced by Megazyme, and Unitech Scientific, were one of the first spectrophotometers to offer a simplified way to perform enzymatic testing. The platforms of the two instruments are similar in that they use a tungsten lamp light source. Both come equipped with six interference filters. The Megazyme instrument provides wavelengths of 340, 405, 505, 545, 580 and 630 nm as standard, and Unitech Scientific provides wavelengths of 340, 420, 520, 580, 620 and 750 nm. Both instruments have a linear range of 0 to 3 Absorbance units that easily accommodate the range of most enzymatic tests.

Both systems accommodate 12 sample tubes in the incubation block. These blocks only accept round 12 mm diameter tubes. However, the actual measuring cell does accept both square 10 mm cuvettes, as well as the 1 mm X 10 mm cuvettes, in addition to the round tubes. The accuracy of a reading is 0.005A or about 1 percent of the reading.

On startup, the onboard touch-screen presents a list of items, including Utilities, Settings, Tests and Manage Test tabs; the user enters specific information to customize the printer output. Most of the time the screen input centers on the list of tests, and you select the one to run. The MegaQuant Wave has a repertoire of 24 separate test kits for the wine industry. The average test kit has three to five reagents that are added to the test tube or cuvette with micro-pipettes. The instrument provides step-by-step instructions for processes, such as necessary dilutions, sample insertions, and when to insert or remove a tube. On completion of the test, the instrument calculates and prints out the results or sends those results to external software.

The two systems are different in their instrument programming and their enzyme test kits. For example, Megazyme test kits come in different configurations of reagents. The stability of their reagents is generally two years. This is important as the number of tests in any one kit ranges from 50 to 100 tests. The cost per test is $3 to $5, plus shipping. However, if the user runs the half volume test, the lab can get twice the number of tests out of one test kit, which reduces all reagent costs by half.

Both Unitech Scientific and MegaQuant Wave test kits often require both refrigerated and freezer storage to get the two-year or more shelf life out of the kit. Freezer storage may not be required if all the tests are used within six to 12 months.

I have not used the Unitech Scientific equipment in my laboratory and consequently cannot make a personal assessment of its enzymes. However, I do know that the MegaQuant Wave protocol for analysis and the reagents used gives the instrument a greater sensitivity at low concentrations.

Using the Equipment: Analyses and Results

With the exception of Admeo, the equipment described above was used to conduct the appropriate analyses with the same wine, and each section of results that follows had triplicate analysis of the same sample.

When reviewing these results, it is important to understand the difference between precision and accuracy. Precision is the measure of the degree of variance in the results. A very low degree in variance has a high precision. However, high precision does not necessarily provide high accuracy. Great precision off the target is worse than larger variance on or near the target. The user needs to know what the target (most accurate) is to decide if a triplicate measure of +/- of 1 is better than +/- 3. For example, if an outside lab, such as ETS Labs, returns a result of 100 mg/L of malic acid in a wine, and one company's equipment consistently reported a value of 110 +/- 1 but another

company's equipment consistently returned a value of 100 +/- 3, the latter would be better than the former result.

Another consideration is tolerance. A highly accurate measurement with great precision is likely the best solution, but the cost of that equipment may be too high. The user must then determine their economic tolerance of what is good enough to obtain an affordable result.

The third factor is the time needed to get the result. New technology has streamlined analytical procedures, reducing the time to get an answer. When compared with traditional equipment, new methods are faster, more repeatable and many times more accurate. In addition, maintenance and calibration are much easier. Consequently, a lab can often run tests in a timelier manner and therefore help you make better wine.

When evaluating a company to supply lab equipment, there are several factors to consider. Probably the most important is the tests' shelf life. The greater the number of analyses in a single test kit, the longer into the future the expiration date should be in order to not lose any tests due to expiration. For example, 10 tests left in a 100-test reagent set will increase the cost per analysis by 10 percent. In addition, shipping adds a significant amount to a test's cost, especially if just a few tests are purchased. Investigation into the various ways of safely shipping test kits to minimize the shipping cost per test can save on the total cost of tests.

Options for the Small- and Medium-Sized Wine Laboratory

There is no one way to select the equipment for a winery lab. The choice depends partly on your starting point: Is this a new winery? Are you upgrading a legacy laboratory? Are you adding a satellite lab at a remote location or crush pad? These situations and more will influence decisions about winery laboratory equipment. The new technology available provides opportunities for a winery serious about their products and reputation at only slightly more cost than wet chemistry.

To maximize the usefulness of their investment, wineries should consider the CDR Wine Lab unit. This one instrument allows a winery to do over 20 tests in-house. There are compromises that a system like this presents but having this instrument and using it in the way it is intended will help a winery make better wine. The equipment to complete a lab would include a turbidity meter for heat stability, a conductivity meter (such as the Edge from Hanna) for cold stability and their refractive index meter for Brix determinations.

For greater precision, the MegaQuant Wave should be considered as part of the laboratory's evolution. I do not have any experience with Unitech Scientific's instrument, but all things being equal, this instrument should

FIGURE 5

Megazyme MegaQuant Wave and Unitech Scientific EnoLyzer Absorbance Mode Creation

Select from Main Display "Manage Tests" and then "Create Test"

Test Definition

Name		Exit
Mode	Absorbance	
Tube/Flowcell		^
Kinetic Rate		
Primary Filter		
Differential Filter		v
Print	Save	>>

Steps

For each step use up/down arrows to select from list choices

1. Enter test name in field
2. Select Absorbance mode
3. Select Tube
4. Select No for Kinetic Rate
5. Select the Primary Filter
6. If desired select a Differential Filter - this is not manditory

When finished, select Save.

Test is stored as a numbered test in the list of tests.

FIGURE 5. All users of either the Megazyme or Unitech Scientific instruments should have the absorbance mode tests for the wavelengths of the tests that are performed with their reagents. Then, when an error occurs in making an entry during automated test procedures, one can switch to the manual mode so as not to lose the values from multiple test runs. The potential for data loss happens when one removes a tube before a reading is made by the instrument or a reaction needs to continue to get a stable reading. It is also useful for obtaining simple absorbance readings for wine color.

provide the same analytical precision. In this particular case it will be the actual test kit that will provide the greatest potential for variability since currently, Unitech does offer a somewhat wider array of test kits. These two systems require a marginally greater attention to technique than the CDR Wine Lab, and it is important to add certain ancillary equipment to provide the analyses that cannot be done with the MegaQuant Wave, such as the refractive index meter, the Edge pH, EC and DO meter and the turbidity meter.

One significant factor about both the MegaQuant Wave and Unitech Scientific's instruments is that they can both be utilized as a spectrophotometer. An illustration showing how this is done for the MegaQuant Wave is shown in the figure above. **WBM**