

Cellar Dweller

Cornell Extension Enology Lab

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Things we're dwelling on now...

Selling the Spec

By Chris Gerling

You there. Yes, you in the winery laboratory surrounded by the burettes and beakers. Look at all of that delicate, breakable glassware. Look at all of those dangerous chemicals. Look at that paper chromatography kit resembling some kind of middle school art project gone wrong. That kit can't even give you quantitative data. Isn't there an easier way? There is an easier way, and it's called a spec. But wait (I hear you asking), why should I spend my hard-earned money on a spectrophotometer when I already have all the fancy lab equipment surrounding me? I can titrate and calibrate and even aerate/ oxidate (actually, hold on to that one- I'll explain later). Why do I need a new instrument? I'm glad you asked.

Lots of Assays, One Method

The first great thing about the spec is flexibility. It will allow you to measure things you couldn't measure before (YAN) or measure things quantitatively where you couldn't previously (malic/ lactic conversion). The spec can also do all sorts of things besides the two biggies I just mentioned: acetic acid, glucose, fructose, ethanol, tartaric acid, ascorbic acid. If you want to go beyond the usual, you can analyze for tannins, acetaldehyde, citric acid, glycerol, sorbic acid and more. Sound too good to be true? There are catches, of course, and we'll cover those soon. The point is that there are a lot of assays you'll now be able to run should you feel the need, and if you can do one test, you can pretty much do them all.

That's right, if you invest in a spec and learn how to use it, you will be able to run any and all of these tests. The magic is in the test kits that have been developed for each analysis. You simply order the appropriate kit for the assay. The protocols are sometimes different, but not very different. If you can master any one of them, you can master any other. There are definitely some skills required, not the least of which is careful direction-following, but the skills are broadly adaptable across the assays. You will not need to re-align tubes and pumps, adjust burette stands and fittings, mix up dangerous titrants or work over open flames. Sounds great, right? Now you're wondering about those catches, and why everyone doesn't have a spectrophotometer next to the coffee maker in their kitchen. To answer those questions, I suppose I should go through the fine print.



"Before I got a spec, my lab bench was covered with chemicals."-John Fillquote

Details, Details

There are expenses associated with buying and operating a spec; besides just purchasing the instrument, you will need micro-pipettes and possibly even a small centrifuge. Most of the kits required for colorimetric analysis are enzymatic and have a shelf life of a few months to a year, so each kit may need to be replaced for each vintage. The costs will not be that different for 50 wines than for 5, so good planning can go a long way toward saving money. Beyond expense, there is a bit of a learning curve. Micro-pipetting is a skill unto itself, and learning to do it accurately takes practice. The spectrophotometer will also require a little trial and error before you get the hang of it. We're not talking about brain surgery here, but running a spec will be more complex than the ebulliometer. Finally, as I alluded to earlier, although the spec can technically do SO₂, this is one assay that is probably not nearly as good as the standard A/O.

So how does it work? The secret is in the color changes that the test kits can trigger. Most enzyme assays are colorimetric, which means simply that the compound measured is colored, and the degree of color is proportional to the concentration of the compound of interest. Enzymatic tests are indirect measurements- ie, they don't measure malic acid, but involve a reaction producing a proportional quantity of a light-active compound. In winery applications, the compound of interest is commonly coupled to the redox reaction of nicotinamide adenine dinucleotide (NAD⁺ to NADH), because NADH is yellow and easily measured at 340nm. All enzymes are biological compounds, however, and have specific storage requirements and expiration dates that can't be ignored. If the reagents are stored improperly, have expired, or otherwise lose activity, their response to the compound of interest WON'T be proportional, and results will be badly skewed.

Is a Spec Right For You?

In the end, like any real world product that is not advertised on infomercials late at night, the

spec has costs and benefits, disadvantages and advantages, pluses and minuses, etc. We like the fact that it is relatively simple and very flexible, doesn't require hydrochloric acid, formaldehyde or any other dangerous chemical (and the chemicals it does use are in very small amounts so there's very little chemical waste) and gives quantitative results. (Cut to me looking at a paper chromatograph: "is that malic acid? Pickle juice from lunch?") The cost to get set up is not minor (more in the next article), but the expense is mostly up front, and then becomes a matter of planning and usage. You probably already have inexpensive methods on-hand that can at least provide rough estimates of remaining sugar or MLF progress, however, and adding nitrogen prophylactically is not listed as one of the seven deadly sins. Would you be able to use this information to make better wine? That's for you to decide.

Like the workout equipment and kitchen time-savers that infomercials sell, the big risk is that a spec will be bought and then used as a \$2,000 paperweight. If you're going to make the monetary investment in the instrument, spend some time getting comfortable with it. Students practice on a spec using variable concentrations of water and green food coloring, so there are methods for learning without wasting expensive enzyme kits. Many people who buy a spec want to run the basics at first (ML, sugar, YAN), but think about moving up to looking at tannins and more advanced phenolic assays later. These tests require a broader range of wavelengths and a more expensive instrument, however. The accompanying article will go into more detail, but essentially you are now stepping up to a "UV-Vis" spec, which includes both the visible light spectrum and beyond (ultraviolet). The questions to ask yourself are whether you really plan to run these tests or if you just like the idea of being able to do so, and whether you'll be more upset about burning a few hundred dollars for wasted capacity or about the need but not the wherewithal to do more advanced work.

Coming Events

We will be offering a course covering all aspects of the spec on January 18-19. We will have a session to just learn pipetting and we will have practice sessions with a couple of different instruments. You are obviously not required to buy a spec before, during or after the workshop. We are not receiving commissions from any company based on the amount of specs sold in the east over the next few months. We've spent a fair amount of time reviewing wine analysis basics in the last year or so, and we wanted to talk about what might be a good next step for those who were curious. Information and registration contacts for the workshop can be found at the link below. More technical information can be found in the accompanying article. For anyone who would still like to learn about standard winery analytical techniques, we will be repeating that workshop in late March, and that information is in the second link.

http://cornell.localist.com/event/wine_analysis_part_ii

http://events.cornell.edu/event/wine_analysis_part_i



Now the lab bench looks sleek and semi-modern. Don't look in the refrigerator, however.

Shedding some light on wine chemistry- Spec use in the winery

Anna Katharine Mansfield

The most common question we've been getting recently is about in-house winery upgrades. Frankly, it's exciting to see that winemaker/chemists who have mastered the benchtop arts of titration, A/O, and paper chromatography are looking for the next challenge, or have decided that more parameters measured leads to a better understanding of their wine. So, beaming proudly, we offer up the following information on spec- on spectrophotometers, that is.

What it is: The spectrophotometer (or 'spec' to its friends), is an instrument that shines a certain wavelength of light through a liquid sample, then measures the intensity after it passes through. For winery applications, there are two kinds of specs worth considering- Vis specs, that measure visible light at wavelengths of 400-700nm, and UV/Vis instruments, that measure both visible and ultraviolet (wavelengths <400nm) light. The technical classification of these instruments is a little confusing, as most Vis specs actually measure as low as 320nm. Generally, the decision to buy a VIS spec or the more expensive UV/Vis is dictated by the

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& FL Grape Grower Convention
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analyses a winery wants to run. All colorimetric enzymatic assays (YAN, malic acid, reducing sugars, etc.) require a Vis spec that can read as low as 340nm, while phenolics/color measurements require a UV/Vis that can read at 280nm.

Beer's Law- in the winery?

While it sounds like a theorem predicting the an individual's attractiveness in relation to the concentration of alcohol in your system, Beer's law is actually considerably more useful in most lab settings. Simply put, Beer's law ensures that a proportional relationship exists between the concentration of a solution and its absorbance of a particular wavelength of light passing through it. Subsequently, if you record the absorbance of light passed through a blank sample (usually water) and of a sample, it's possible to calculate the amount of a given compound dissolved in the sample. The main thing to remember is that this relationship makes spec measurements robust, accurate, and relatively easy.

Using a calibration curve:

Beer's law is applied through the use of a calibration curve, which is merely a plot of the absorbance measured for solutions with increasing concentrations of the compound of interest. This plot is generally linear (see sample, above), allowing you to calculate the concentration of an unknown sample using that familiar staple:

where:

$$y = mx + b$$

- y** = sample absorbance
- m** = slope of the calibration curve
- x** = sample concentration
- b** = y intercept of the calibration curve

While this may require a brief review of algebra, once you remember (or Google) how to calculate a slope, the whole equation can quickly be set up in a spreadsheet for future use.

Undergrads in Cornell's wine chemistry class often balk at the time and hassle involved in building the calibration curve and doing all these calculations. They rightly point out that the enzyme kit includes a pre-packaged equation to prevent their wasting time on things like lab work when they're so behind on their smartphone texting. These equations will work, in theory; they're

devised using the known molecular extinction coefficient of the compound measured in a given assay. Unfortunately, neither the students nor most winery labs are running analysis in theory, and reality dictates that most are using inexpensive instruments with less-than-optimal accuracy. In other words, the equation will give you accurate data if you're using a very expensive instrument with no built-in variability, but on specs in the <\$10K range, building a calibration curve is the only way to correct the inherent inaccuracies in the system. The good news is that going through this effort WILL correct for the inaccuracies, yielding pretty accurate numbers for most analyses. See the charts below for more information about parameters that can and can't be corrected by running a calibration curve.

Other equipment: As with any instrumental analysis, the spec comes with a short list of accessories necessary for accurate measurements. These include:

Cuvettes: Samples are placed in plastic, glass, or quartz cuvettes for spec measurement. Cylindrical cuvettes (which look like test tubes) have more variable wall thickness, and thus may skew measurements more than square cuvettes, which are built to line up exactly with the beam of light. Less expensive instruments are often designed to use cylindrical cuvettes. Also, cuvettes are made of different materials for UV, Vis, and UV/Vis measurements, so make sure to buy the appropriate cuvettes for your instrument and the wavelength (UV or Vis) measured in a given analysis.

Micropipettes & disposable pipette tips: Most samples require dilution or precise additions of a series of reagents. To minimize errors, dilutions and additions **must** be made with automatic pipettes that can accurately dispense between 20µl and 1000µl. Pipettes cost \$150-300 each, and require additional expenditure for disposable plastic pipette tips. Small errors in measurement are multiplied when calculating final concentrations, so there's no real way around micropipette purchase.

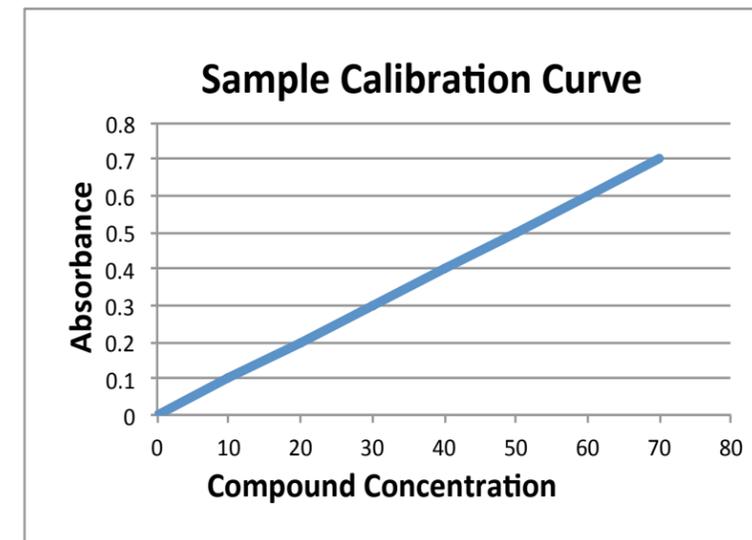
Enzymatic test kits: Unless you have the time and lab facilities to make and store your own reagents, you'll have to buy commercial enzyme test kits for most analyses. Megazyme (www.megazyme.com) and Unitech Scientific (www.unitechscientific.com) are two suppliers who have focused on kits marketed for winery use, though there are several other suppliers. Test kit cost varies by analyte measured, ranging from \$1-4 per test in theory. In practice, costs per test may be closer to \$5-8, depending on the number of samples and technician skill.

Choosing a spectrophotometer

Besides budget, one of the first things to consider when choosing an instrument is the type of analyses you'd like to run. As mentioned above, enzymatic analyses can generally be run on a Vis spec, but phenolics and color analyses require a UV/Vis instrument. The tables below outline the minimum requirements for a functional instrument- the first covering parameters essential to acceptable accuracy, and the second reviewing parameters which induce errors that can be corrected using a calibration curve. Extras that can run up the price include aesthetic design, autosampling capabilities, and computer interface options.

Essential specifications:

Parameter	Min/Max	Notes
Wavelength	at least as low as: 340nm for enzymatic assays 280nm for phenolic and color measurements	Wavelengths below 320 require a UV/VIS spec
Photometric range	0-2AU (Absorbance Units)	The linear range for an instrument is generally 50-70% of the entire range, depending on other parameters
Stray light	0.1% is good 0.5% is the maximum acceptable	Stray light shortens the effective linear photometric range- so an instrument with a 0-2AU published range and 0.1% stray light may actually have a range of 0-1AU in practice.



Important specifications:

Parameter	Min/Max	Notes
Spectral slit width <i>or</i> Spectral bandwidth	20nm at least 5-10nm is acceptable	The larger this number is, the more important it is to run a calibration curve and NOT rely on calculations provided with the enzyme test kits.
Wavelength accuracy	2% generally acceptable for winery applications	As above, a calibration curve will negate any errors caused by variation in wavelength accuracy

Affordable Spec recommendations (from Gavin Sacks, our wine chemist):

Jenway 6320D or 6300 – VIS spec with slightly better specifications and more modern design. Around \$1350.

Spectronic 20D+ by Thermo Scientific – the standard VIS spec we use in undergrad labs. Around \$1500.

GENESYS 20 by Thermo Scientific – an updated version of the Spectronic 20D+. Around \$2000.

GENESYS 10 UV/VIS by Thermo Scientific – a dependable UV/VIS spec with USB output. \$4500 - \$5300.

Unico S1100 –the cheapest VIS spec available with acceptable specifications for enzyme assays. Around \$700.

Cornell Extension Enology Websites

New Wine Lab Site:

<http://grapesandwine.cals.cornell.edu/cals/grape-sandwine/outreach/nys-analytical-lab.cfm>

Events:

<http://grapesandwine.cals.cornell.edu/cals/grape-sandwine/outreach/enology/workshops.cfm>

Newsletters:

<http://grapesandwine.cals.cornell.edu/cals/grape-sandwine/newsletters.cfm>

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