

FROM GRAPE GROWER TO CHEMIST

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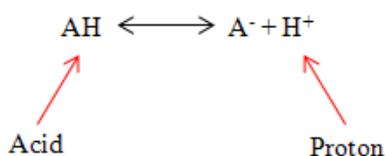
With the 2019 harvest underway, many grape growers have dusted off **their refractometers** and hopefully re-calibrated (with fresh buffer solution!) their pH meters. You have spent the last seven months growing grapes, and whether you like it or not, it's time to put on your chemist hat. Some folks don't want to know any more chemistry than is absolutely necessary, but since your beautiful and valuable grape crop depends on an accurate assessment of maturity, it's worthwhile to make sure that you are getting good data.

Measuring acidity is an easy way to track grape maturity, and it is perhaps the single most important parameter for making quality wine. Why is acidity so important? It is critical to the balance and flavor of wine, microbial and chemical stability, and the color of red wine. Therefore, acidity should be a key aspect of harvest decisions, particularly in a hot climate where high pH is an ongoing challenge. The information written below summarizes this essential parameter, and highlights how to avoid errors when measuring acidity.

What is Acidity?

We will start with defining just what an acid is and how pH and titratable acidity (TA), the two primary measures of wine acidity, differ. For you chemistry buffs, this article will leave a lot of meat on the bone, but surprisingly most people didn't actually enjoy chemistry class and don't want the extra details.

To begin, an acid can be defined as a proton (positive charge) donor and a base can be defined as a proton acceptor. When present in or added to a solution like wine, acids dissociate (split apart) releasing one or more protons in the form of a hydrogen ion (H^+). The acidity measurement, pH, is the concentration of H^+ (hence the H in pH) in solution. The more acid present in juice or wine, the more H^+ , or the more acidic it is. pH can be confusing because it is measured on a negative logarithmic scale which means the difference between one pH unit, say pH 3 to 4, represents a ten-fold difference in H^+ concentration. And, the lower the pH value, the greater the H^+ concentration or more acidic. Wine pH generally ranges from 3.0 to 4.2, a seemingly narrow range, but it's actually a greater than 10-fold difference in H^+ !



In the reaction depicted above, AH symbolizes any acid. When added to a solution, acids dissociate or split into a proton (H^+) and an anion (A^-).

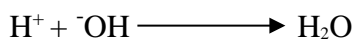
So why is a pH of 7 considered to be neutral? Pure water (H_2O) does not exist entirely in the form of H_2O molecules. Out of every 500 million molecules of water, 1 molecule is split into an H^+ and an OH^- . This gives a concentration of 0.0000001 mol/L or 1×10^{-7} mol of hydrogen ions and hydroxide (OH^-) ions. Thus, the pH of pure water is 7 (the molar concentration of H^+). For the chemistry adverse, it is probably sufficient to just know that a lower pH value means more acidic and more H^+ .

Titrateable Acidity

To explain titratable acidity (TA), it is necessary to discuss the organic acids in grapes and wine. Next to sugars, organic acids are the most abundant solids present in grape juice. There are three major acids in grapes and wine: tartaric, malic, and citric. In a hot climate like Texas, tartaric acid is usually present in the greatest concentration (around 2-6 grams per liter, g/L), followed by malic (2-7 g/L), then citric (0.2-1 g/L). These acids are produced in berries beginning at berry set, and then throughout ripening, acidity decreases primarily as a result of malic acid respiration. Malic acid is used by grape berries as an energy source and the longer the grapes hang during ripening and the warmer the temperature, the more respiration/more malic acid decreases. This is why cool climates generally produce grapes and wine with higher acidity than warmer climates.

Grape acids are considered to be weak acids which means in juice or wine, a predictable proportion of the acids do not completely dissociate or split apart releasing their H^+ . Why does that matter? pH only accounts for the free or dissociated H^+ but your tongue actually tastes both the free H^+ and the H^+ still attached or bound to the acid molecules. Therefore, to determine the

potential acid flavor (sourness) of grapes and wine, we measure both the free and bound H^+ with an acid/base titration. The titratable acidity (TA) of juice and wine is determined by adding a base (a proton acceptor) to a juice/wine sample until all of the acid has been neutralized. If we use a base of known concentration, we can calculate exactly how much acid was neutralized or titrated.

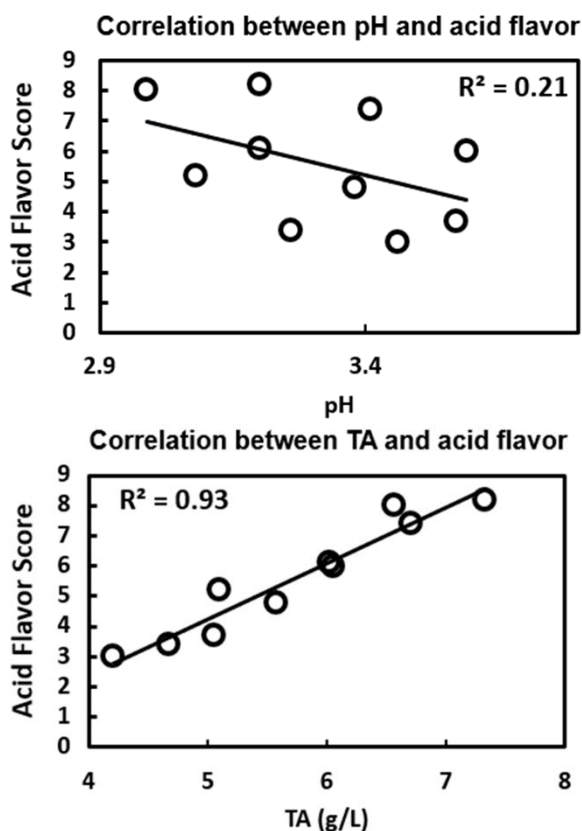


Neutralization reaction.

The base that we use to measure the TA in grapes and wine is sodium hydroxide (NaOH). When you add sodium hydroxide to a juice or wine sample, the sodium (Na) ion dissociates or separates from the hydroxide (OH^-). The negative charge of the hydroxide attracts the positive charge of the protons (H^+) in the sample and when the two combine, they form H_2O . This neutralizes the free acid, and causes the H^+ that are still bound to the acid (tartaric, malic, etc.) molecules to be released. As more sodium hydroxide is added and more H^+ is neutralized, the pH of the sample creeps up higher and higher. Once the pH reaches 8.2, all of the acid (that we care about) has been neutralized and we can determine the TA based the volume of sodium hydroxide that was used. Each molecule of sodium hydroxide, neutralizes one H^+ making the calculation simple.

The TA of wine generally ranges from 4 to 12 g/L tartaric acid equivalents. For the chemistry curious, TA is expressed as tartaric acid equivalents because the different acids in juice and wine have differing molecular weights and numbers of acid groups per molecule. We cannot distinguish between them when measuring TA. Rather, we are simply neutralizing their H^+ . We only know how much H^+ was neutralized, but it

is easier to conceptualize this by expressing it as a common wine acid, tartaric. Some countries express TA as sulfuric acid equivalents, but in it is much easier to understand it as tartaric acid. For example, if you wanted to raise the TA of a wine by 1 g/L, it would simply require that you add 1 gram of tartaric acid. If you wanted to raise the TA by 1 g/L in sulfuric acid equivalents, you would have to make a conversion to know how much tartaric acid is required.



These figures, reproduced from Plane et al.,1980, depicts how fourteen panelists ranked the acid taste of model wine solutions with differing pH values and TA's. TA was a much better predictor of acid flavor than pH.

Just the Facts:

- pH is the measure of free acid and is important for wine stability.
- TA is the measure of free and bound acid and is important for wine flavor.

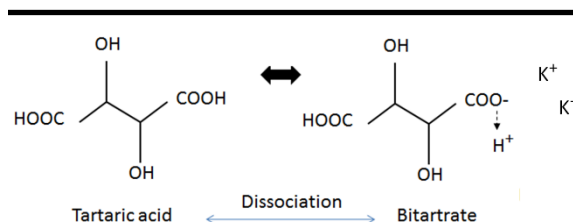
Potassium

At this point we have discussed the measure of free acid, pH, which relates to wine stability and the measure of free plus bound acid, TA, which relates to flavor, but there is one more aspect of acidity that should be mentioned, potassium. There is more potassium in grapes than any other mineral and it exists as a positively charged ion (K^+) similar to the H^+ . Potassium serves various roles in the plant cell and in order to maintain a favorable chemical balance, cells pump in and out different ions such as K^+ and H^+ . When potassium enters the cells of grape berries, H^+ may be correspondingly removed and used elsewhere resulting in a loss of acidity. This is particularly important when K^+ is in high concentrations. In a hot climate like Texas, we have to live with relatively high potassium as a result of the heat, but we do want to avoid situations that encourage excessive potassium. The word excessive is used here because potassium is an essential plant nutrient that serves a number of vital roles so we need it, but we would not want to over apply potassium fertilizer (this is my pitch for soil and tissue testing), for example. Other factors such as severely over- or under-irrigating, and excessively dense canopies that lead to leaf senescence also encourage higher potassium in fruit, but those details are beyond of the scope of this article.



pH continues to increase with hang time.

Another reason that we care about potassium is cream of tartar. Grapes are one of few fruits that produce high concentrations of tartaric acid. Tartaric acid is a diprotic acid, which means it has two acid groups (H^+) per molecule, and as we said earlier, it is a weak acid so some of the tartaric acid in juice and wine exists with both of its acid groups still attached or bound to the molecule (tartaric acid), some with one of its acid groups still attached (bitartrate) and some with none of its acid groups attached (tartrate).



When tartaric acid loses one of its protons it becomes bitartarte. Potassium can fill the now vacant site to form potassium bitartate or cream of tartar.

When an H^+ dissociates from a tartaric acid molecule, it leaves a negatively charged site that can attract other positively charged ions, namely K^+ (think soil cation exchange capacity where the negatively charged sites on soil colloids attract cations Ca^{++} , K^+ , Na^+). When K^+ binds to a bitartrate molecule at one of those sites, it forms the salt potassium bitartrate, AKA cream of tartar. This happens naturally when the two are in contact, particularly during winemaking, and like other salts, there is a limit to how much potassium bitartrate can dissolve in a solution of juice or wine. Once the limit is reached (saturation), additional salt will begin to precipitate or fall out of solution. During winemaking, the alcohol produced reduces the saturation point of cream of tartar so it often precipitates or falls out of solution. Likewise, the saturation point is affected by temperature. The colder the temperature the lower the saturation point (think of adding sugar

to ice tea versus hot tea). This interaction between tartaric and potassium can result in seemingly strange changes in pH throughout winemaking, but to avoid putting you to sleep, it has been excluded from this article. Perhaps a second, chemistry-nerd edition will be in order in the future to explain why the precipitation of cream of tartar can result in both increases and decreases in pH and why skin contact during winemaking can exacerbate this phenomenon.

Saturation Point	Temperature (°F)
0.492 g/L in water	68
0.251 g/L in 14% alcohol	68
0.081 g/L in 14% alcohol	25

The saturation point of cream of tartar as influenced by alcohol and temperature.

Just the Facts:

- Potassium is an essential nutrient that plants can't live without, but excessive levels of potassium can contribute to high juice/wine pH.
- Potassium forms a salt with tartaric acid called cream of tartar.
- Cream of tartar precipitates out of wine when it exceeds the point of saturation.

pH Meter Use & Maintenance

Now that you've heard more about acidity than you ever cared to, let's discuss how to measure it, or at least how to avoid major sources of error. As grape grower, it can be easy to overlook lab equipment maintenance and proper use, but it's quite easy to make a mistake when measuring

acidity so care should be taken. First and foremost is starting with the proper equipment. While I admit that I am not a pH meter expert, I have had the opportunity to use quite a few different types of pH meters and I can tell you that some of the bargain hand-held meters are flat out unreliable. I have seen certain brands not work properly straight out of the box, and others drift badly. That said, if you are shopping for a pH meter it is recommended that you do your homework and not just instinctively go for the cheapest. A good pH meter can last for years if it is properly maintained (i.e., not stored under the back seat of your truck).

pH meters should be calibrated according to the manufacturers recommendation prior to each use. Incorrect calibration or not calibrating can result in significant measurement errors. Since calibration methods vary, you'll have to read the instruction manual on this one. You should calibrate your pH meter each time it is used and if you are running a large number of samples, it should be recalibrated after approximately 4 hours.



Opened pH buffer solutions should be replaced after 3-6 months so it's a good idea to write the date of opening on the bottle.

Some pH electrodes contain a thermocouple for automatic temperature correction, while others have separate temperature probes. This is

important because sample temperature affects the measurement. If your pH meter does not correct for temperature it should be done manually. It is important to always store the sensing bulb of the pH electrode in an appropriate storage solution and not dry.

There are a few different types of pH meter solutions that you will need for your meter. The exact solutions may differ by model so once again, you'll have to refer to the manual, but in general there are buffer solutions for calibration, electrode filling solution, and storage solution. pH meters come with two or three buffer solutions that are used to calibrate the meter. These solutions, unopened, are usually good for a couple of years, but once they have been opened they should be replaced after 3 to 6 months. So, it's easiest just to buy new solution each season. Most importantly, do not store your pH probe dry and do not let the solution inside the probe dry out. You wouldn't run your tractor without regular maintenance would you? A pH meter doesn't cost all that much in comparison to your tractor, but you make harvest decisions based on the data it generates, and your crop may be even more valuable than your tractor.

Measuring Titratable Acidity

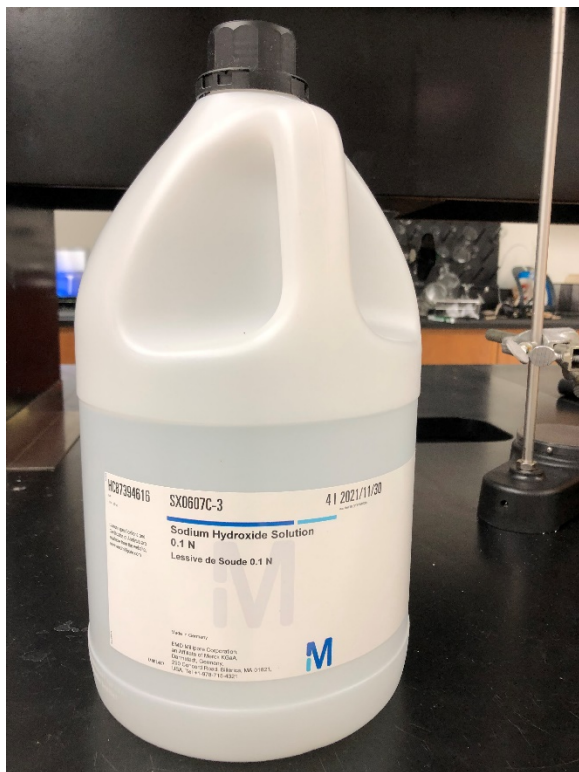
The most common source of error when measuring TA is the sodium hydroxide titrant. Sodium hydroxide is a base and once it comes in contact with air, it begins to lose strength. This is not an instantaneous reaction, but if you have half a bottle of sodium hydroxide on your shelf from last season it has lost significant strength. How? Carbon dioxide in the air dissolves in the titrant solution forming carbonic acid, which is then

neutralized by the hydroxide just like grape acids are when you titrate. The more air the titrant has been exposed to, the weaker it will be. That doesn't necessarily mean that you can't use it any longer, it just means that you need to account for the error. Otherwise, it will appear that your TA is higher than it actually is because it will take more of the weaker titrant.

How do you determine the strength of a sodium hydroxide after it has been opened? There are two easy ways: take an acid of known concentration such as hydrochloric acid (HCl) and titrate it, or create a tartaric acid standard. For example, the sodium hydroxide used for juice and wine titration is 0.1 N. If you take 10 mL of 0.1 N HCl it should require exactly 10 mL of 0.1 NaOH to neutralize or titrate it. If it requires more than that to reach an endpoint pH of 8.2, the titrant has lost strength and you need to use a correction factor in your TA calculation. For instance, if it took 11 mL of NaOH to titrate 10 mL of HCl, the correction factor would be $10/11 = 0.909$. After titrating an actual sample you would multiply the result times the correction factor (0.909) to account for the loss of strength. Otherwise, your TA reading will be artificially inflated by almost 10%.

You can often find 0.1 N HCl from suppliers that sell wine lab reagents, but if not you can make up a tartaric acid standard instead. To make an 8 g/L TA standard, fill a beaker or flask with 50 mL of distilled water, add 0.8 grams of tartaric acid, then bring to volume to 100 mL with the distilled water. You now have an 8 g/L tartaric acid solution that you can titrate to determine the strength of your sodium hydroxide. If can't accurately measure out quantities that low, then you should make your standard in a larger volume. Once complete, you can use your standard to determine the correction factor for your titrant.

The water that you use to increase the volume of



Once opened, sodium hydroxide loses strength and should be tested at each use against a standard.

your juice or wine samples should be distilled water and not tap water. The pH of distilled water is often below 7 and can add a very small amount of error to your measurement, but in my opinion, it's not worth worrying about. A single drop of sodium hydroxide is typically sufficient to raise the pH of 5 mL of distilled water to ≥ 8.2 , and this tiny volume is not measurable with most burettes. Tap water, on the other hand, can range from basic to acidic and can interfere with your measurement so you want to avoid using it.

Another possible source of error is indicator. Titration is complete when the pH of the sample reaches 8.2. This can be measured with a pH probe (recommended, especially for red juice/wine) or phenolphthalein indicator. Phenolphthalein indicator is added to the sample prior to titration where it remains colorless until a pH of 8.2 is reached. At that point, the indicator

turns pink/red. This can be easy to see in white wine/juice, but is difficult in red wine/juice.

Folks that titrate a large number of samples may want to invest in an automatic titrator. The same principles described above apply, but the machine incrementally adds the titrant and stops when once the endpoint pH has been reached. If you can't justify the cost of an automatic titrator, I suggest that you never use one so you don't know what you are missing out on. But, manual titration is a good character building exercise and I highly recommend it for all interns.



Automatic titrator with sample changer.

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General Procedures for Measuring Acidity

pH

Materials

pH meter with electrode, 10 to 50 ml beaker, pH buffer solutions, pasteur pipette, distilled water.

Method

1. Turn on the meter, slide the rubber sleeve off of the vent hold in the electrode (if need be), check the level of internal filling KCl solution (to ¼ inch below the hole), add if needed.

Calibration of the meter: You must first calibrate the meter with two or three buffer solutions before proceeding with the sample measurement. This must be done every time the meter is turned on. Once calibrated, do not turn the meter off until analysis is complete for the day. Recalibration may be required after approximately 4 hours if a high volume of samples is measured.

Measurement

1. Place approximately 10 - 50 ml of sample in beaker.
2. Remove electrode from buffer and rinse with distilled water. Place electrode into the beaker with sample.
3. Allow pH meter to stabilize and record pH.

Note: When not in use, store electrode immersed in storage solution. DO NOT store electrode dry.

Titrateable Acidity (TA) by pH Titration

Materials

pH meter, 50-100 ml erlenmeyer or similar flask, distilled water, 0.10 N sodium hydroxide (NaOH), 5ml volumetric pipette or graduated cylinder, 10 to 50 ml burette, optional -magnetic stirrer and magnetic stir bar (1")

If no pH meter is available or on white wines, phenolphthalein indicator may be used to determine the endpoint.

Method

1. Pipette 5mL of wine (degassed)/juice into the Erlenmeyer flask. Degass by heating or inverting sample.
2. Add 5 to 10 (volume doesn't matter) ml of pH adjusted (8.2) distilled water
 - a. pH of water can be adjusted by adding a drop or two of 0.10 N NaOH until water has a pH of 8.2 (a few drops of phenolphthalein indicator may be added to the water in place of pH probe)
3. Fill burette with standardized 0.10 N NaOH and record initial burette reading
4. Slowly add (titrate) NaOH until a pH of 8.2 is reached and maintained for 10 seconds.
5. Record the final burette reading and determine the number of mls required to titrate the sample.

Calculations and Units of Reporting for Titrateable Acidity

TA as g/L of tartaric acid = (ml NaOH) * (N NaOH) * (15)