



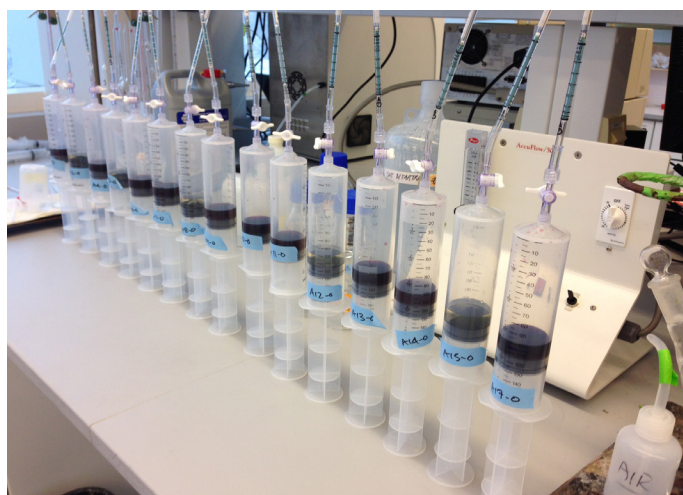
RESEARCH FOCUS

"Free" Doesn't Always Mean Free: Rethinking SO₂ Measurements in the Winery

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A simple, inexpensive method for measuring SO₂ in wines uses headspace gas detection tubes developed for the mining industry to accurately measure molecular SO₂ in wines. Using this method, we found that standard approaches (Ripper titration, aeration-oxidation, FIA) overestimate the amount of free and bound SO₂ – particularly in red wines.

Photo by Patricia Howe.

Measurements of free and molecular SO₂ are routinely performed in wineries to ensure microbial and oxidative stability. However, standard approaches to SO₂ suffer from poor reproducibility across laboratories. More importantly, literature indicates that all standard SO₂ methods badly overestimate SO₂ in red wines. We developed an easier, inexpensive and more accurate way to measure free and molecular SO₂. The values provided by this technique may be better predictors of wine stability than standard analytical methods – and could simplify SO₂ measurements for small wineries.

KEY CONCEPTS

- Although many forms of SO₂ exist in wine, only the “molecular” form of SO₂ has strong antimicrobial activity (and the ability to sting nostrils). Proper measurement of molecular SO₂ is critical for wine quality.
- A recent review of proficiency testing data shows that most wine laboratories can improve performance of standard approaches for determining molecular SO₂.
- All standard approaches to free SO₂ badly overestimate the amount of free and molecular SO₂ in all wines (particularly red wines) due to the presence of weak binders like anthocyanins.
- We have developed a simple, inexpensive headspace – gas detection tube (HS-GDT) method that accurately measures molecular SO₂ in red wines.
- This new HS-GDT method is a better predictor of yeast growth in sweet wines than conventional methods.
- We hope to extend the headspace gas detection tube method to become a practical, inexpensive alternative to standard titration or aeration-oxidation methods in small and large wineries.

Introduction. Is there any wine chemistry topic that inspires as many glazed eyes as sulfur dioxide¹? SO₂ is a widely used tool for preventing wine spoilage – not to mention for keeping dried apricots a perky orange color. In many wineries, however, thoughts about SO₂ extend no further than making a measurement, using a reference table, and possibly using a calculator to figure out the right addition. If these methods are acceptable, what’s the point of understanding the underlying chemistry? Furthermore, most of the currently used knowledge regarding SO₂ measurements and recommended concentrations is decades old. So what’s left to learn?

Dr. Patricia Howe, a former lecturer in enology at Cornell, investigated this question as part of her Ph.D. with Dr. Gavin Sacks (Food Science).

The role of SO₂ in winemaking. Sulfur dioxide (SO₂, often referred to colloquially as “sulfites”) is produced in trace amounts by yeast and other microorganisms as a normal part of their sulfur metabolism. However, most SO₂ in commercial wines is added by the winemaker, either as a gas (vapor or dissolved in liquid) or as a salt such as potassium metabisulfite (KMBS). The near-ubiquitous usage of SO₂ in winemaking arises from its unique preservative properties:

- As thoroughly discussed in a recent review, SO₂ has broad-spectrum antimicrobial properties (Divol, du Toit et al. 2012), and can inhibit or kill most of the spoilage yeast and bacteria that could affect wine, for example acetic acid bacteria.
- SO₂ is an excellent antioxidant, and is uniquely suited to scavenge many of the undesired compounds formed during wine oxidation that would otherwise result in off-aroma formation or color degradation (Ugliano 2013).

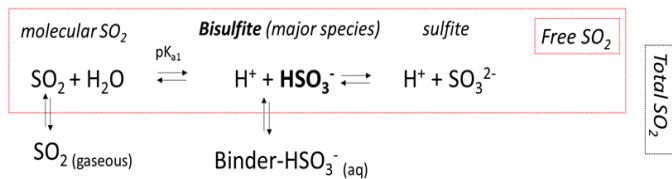


Figure 1: Distribution of different SO₂ species in wine.

In wine, SO₂ will exist as one of several different forms (Table 1). The equilibria among these forms are shown in Figure 1.

A key distinction is between *free* SO₂ forms which account for the preservative effects of SO₂, and the *bound* forms of SO₂ that arise from the reaction of bisulfite and other wine components. Both bound and free SO₂ count towards *total* SO₂, which is regulated in most wine producing countries.

Free and “Molecular” SO₂. Most free SO₂ in wine exists as bisulfite (>90%), with a smaller fraction (typically, <5%) existing as so-called “molecular” SO₂. As described later, the proportion of free SO₂ that exists as molecular SO₂ is pH dependent. A key point from Table 1 is molecular SO₂ and free SO₂ (the sum of molecular SO₂ and bisulfite) must be considered independently - having sufficient free SO₂ to prevent unwanted oxidation does not ensure adequate molecular SO₂ to prevent microbial spoilage.

Because bound SO₂ has a minimal preservative effect, winemakers are usually more concerned with measuring free SO₂ rather than total SO₂. Despite the apparently large number of methods in use, standard strategies for free SO₂ measurement fall into one of two categories:

1. *Direct addition of oxidants*, e.g. iodine titration following acidification (“Ripper method”).
2. *“Separation first” methods*, which use an acidification step to convert free to molecular SO₂, then separate and quantify SO₂. Common techniques are aeration-oxidation (A-O) and flow injection analysis (FIA).

Table 1. Overview of the different SO₂ species in wines.

What you measure	Defined as	Why you care	Typical target or constraint
Molecular SO ₂		Antimicrobial	Microbial stability: 0.5-0.8 mg/L (dry) or 1 mg/L (sweet) Sensory threshold: > 2 mg/L is irritating
Free SO ₂	Molecular SO ₂ + Bisulfite (HSO ₃ ⁻)	Antioxidant	Oxidative stability: 20-40 mg/L
Bound SO ₂	Includes both strongly and weakly bound forms	Contributes to total SO ₂ ; Can have minor antimicrobial activity. Weakly bound may eventually contribute to free SO ₂ pool.	
Total SO ₂	Free SO ₂ + Bound SO ₂	Regulatory, health issues	< 350 mg/L, TTB regulation (varies depending on country)

$$[\text{Molecular SO}_2] = \frac{[\text{Free SO}_2]}{1 + 10^{(\text{pH} - \text{pK}_{a1})}}$$

Equation 1: Relationship of molecular SO₂ to free SO₂. In water at 20 C, pK_{a1} = 1.81.

Once free SO₂ is measured, molecular SO₂ is then calculated from the pH and free SO₂ using the Henderson – Haselbalch (H-H) equation (Equation 1).

As a reminder of general chemistry: a pK_a value is the measure of the strength of an acid, in this case SO₂. A lower pK_a value means that the acid is stronger, and that the bisulfite form (i.e. the deprotonated form) is favored. Equation 1 also helps explain why more SO₂ is necessary at higher pH to prevent spoilage – at lower pH, a higher percentage of SO₂ will be in the molecular form than at a higher pH. For example, in water at room temperature, the fraction of free SO₂ existing as molecular SO₂ decreases from pH 3 (6%) to pH 3.3 (3.2%) to pH 3.6 (1.6%).

The pK_a of SO₂ changes. One complication with using the H-H equation is that the pKa of SO₂ is not constant: its value changes with several factors, most importantly ethanol, temperature, and concentrations of other dissolved ions (“ionic strength”). However, many texts or reference tables assume that the pK_a for water (1.81) is valid for all circumstances, even though a room temperature value of 1.9-2.0 would be more appropriate for a typical wine with 10-14% v/v ethanol and an ionic strength of 50-75 mM (Usseglio Tomasset and Bosia 1984).

This can result in a 30-50% underestimation (depending on pH) of how much molecular SO₂ is present (Figure 2). Conversely, wines are often stored in relatively cool cellars – which decreases the pK_a and the fraction of molecular SO₂ present – but may be analyzed at warmer temperatures in the wine laboratory. This will result in an overestimation of molecular SO₂ as compared to what is present in the actual storage conditions.

Online molecular SO₂ calculator. A Cornell Food Science graduate student, Greg Dlubac, has recently developed an

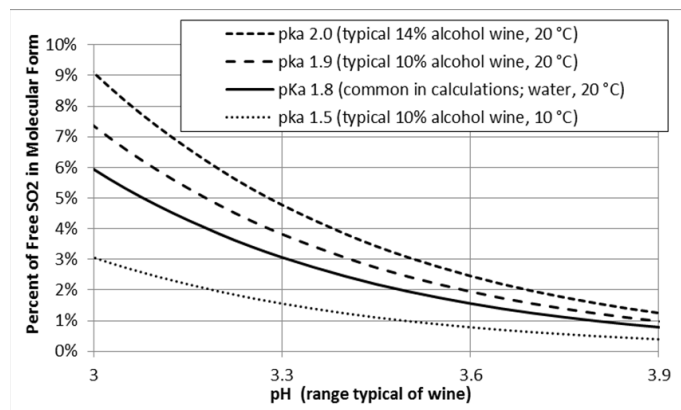


Figure 2: Impact of pK_a values on percentage of free SO₂ that exists as molecular SO₂ as a function of wine pH.

[online calculator](#) for determining molecular SO₂. While several calculators for SO₂ exist, Greg’s calculator is the first online tool to allow for inputting ethanol, ionic strength, and temperature to get an accurate pK_a value for SO₂, and thus should provide more accurate estimates of SO₂.

Laboratory analysis vs. magic 8-ball – How well does the industry perform SO₂ measurements? Although free SO₂ is measured on a routine basis (monthly or more often), recent data on analytical performance across individual labs (“reproducibility”) or among methods is lacking. This information would be timely because of the increasing adoption of automated techniques (e.g. FIA) in wineries.

In a recent review, Patricia Howe and Gavin Sacks (Cornell) collaborated with Sue Ebeler (UC Davis) to evaluate results from proficiency testing performed by Collaborative Testing Services (CTS) (Howe, Ebeler et al. 2015). The CTS data set included 78 different wines analyzed over a period of 13 years by dozens of laboratories: in total, we considered data from over 4000 measurements of free SO₂. Performance for several other common analytes in wine, including ethanol and titratable acidity, were also considered.

The reproducibility of free SO₂ measurements across methods (Figure 3) showed that the mean value for Ripper (iodine titration) was slightly higher than A-O (+2.5 mg/L) as expected – Ripper is known to measure a small portion of other reducing compounds in wine. The inter-laboratory reproducibility for Ripper was ±3.6 mg/L (one standard deviation), similar to performance reported over three decades ago (Vahl and Converse 1980)!

Surprisingly, although aeration-oxidation (A-O) is often thought to be superior to the Ripper method (iodine titration), the interlaboratory reproducibility for A-O was no better than Ripper (1 standard deviation = ±3.5 mg/L). This may be because A-O has many more steps and equipment, and thus more opportunities for error. Flow injection

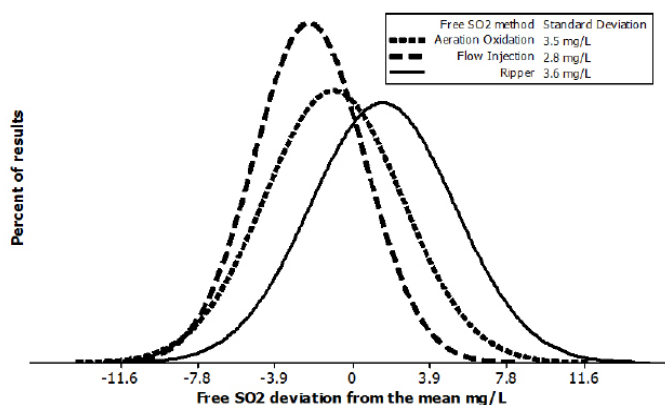


Figure 3: Histogram of free SO₂ performance for three common analytical approaches based on 13 years of CTS interlaboratory proficiency testing: Aeration-oxidation (1893 measurements), flow injection (161 measurements), and Ripper (1118 measurements). Data from (Howe, Ebeler et al. 2015).

tion analysis (FIA), which is effectively an automated version of A-O, yielded considerably better precision than both Ripper and A-O.

The interlaboratory performance for molecular SO₂ would be even worse than for free SO₂, since it requires determination of both pH and free SO₂ measurements. Reproducibility of pH measurements across laboratories was ±0.04 units, which would translate into an average additional 10% error on top of any error introduced by free SO₂ measurement. Finally, as mentioned above, most calculations of molecular SO₂ use significantly incorrect values for the acidity constant, pK_a.

A new approach: Simple, direct headspace measurements of SO₂. As part of her PhD work in the Sacks laboratory, Patricia Howe developed a fast, simple, and inexpensive method called the *headspace gas detection tube* method (HS-GDT) for directly measuring the molecular SO₂ in the headspace of a wine sample without acidifying, diluting, or otherwise changing the wine prior to analysis as is done with standard methods (See sidebar and Coelho, Howe et al. 2015).

We compared HS-GDT to classic approaches to SO₂ measurement. HS-GDT uses headspace SO₂ as a proxy for molecular SO₂ (see **Figure 1**). In contrast, earlier described methods (Ripper, A-O, FIA) measure all free SO₂ forms, and require subsequent calculation of molecular SO₂. Furthermore, these standard methods require sample dilution and/or pH shifts, resulting in partial hydrolysis of "bound" SO₂ and overestimation of both free and molecular SO₂.

This problem is particularly severe in red wines, due to dissolution of complexes of anthocyanin pigments and bisulfites – a problem that has been recognized since the 1970s (Rankine and Pocock 1970). Various approaches have been described in the literature for measuring molecular or free SO₂ without disrupting equilibria, e.g. (Davis, Barnett et al. 1983), but none have been routinely adapted by wineries due to technical challenges.

Our new headspace gas detection tube (HS-GDT) method and classic approaches show reasonably good agreement for white and rose wines (**Figure 4**) (slope near 1, r²=0.97), but for red wines the standard techniques overestimate molecular SO₂ by 3-fold on average (slope = 0.32). The inflated values observed with standard techniques like A-O are a consequence of the dissolution of anthocyanin-bisulfite complexes during analysis.

The new HS-GDT method: A better predictor of microbial stability. A reasonable response to our new method is "Does it matter? We've been using standard techniques for decades...maybe they overestimate molecular SO₂, but do a better job predicting spoilage because the bound anthocyanin-bisulfite complexes are still antimicrobial."

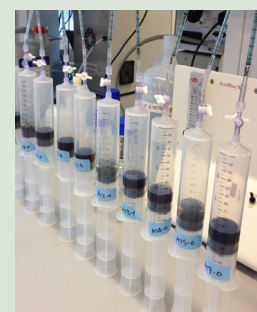
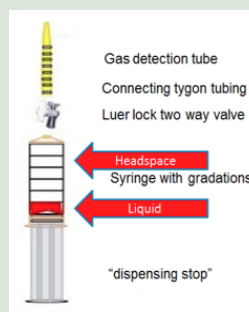
To test this hypothesis, we performed "challenge studies" by inoculating sweet wines with a commercial yeast, and determining molecular SO₂ through both the new HS-GDT method and standard methods (A-O, FIA) through-

The Headspace-Gas Detection (HS-GDT) Method for Measuring SO₂

Gas detection tubes are available for a larger number of target gases and concentration ranges. The tubes are calibrated to produce an easy-to-read color change proportional to the concentration of gases in the headspace. They are inexpensive and reliable, and are widely used in mines or other places where toxic gases in the air that can present occupational hazards to workers.



A wide variety of inexpensive gas detection tubes, used in the mining industry, are commercially available.



Headspace Gas Detection Tube (HS-GDT) apparatus for measurement of molecular and free SO₂ in wine.

Photos by Patricia Howe.

The Headspace-gas detection tube (HS-GDT) method uses these gas detection tubes to measure SO₂ gas in the headspace above a wine sample.

A measured amount of wine is placed into a syringe, and the wine and its headspace are allowed to reach equilibrium. The syringe plunger is then depressed to expel a measured amount of headspace (50-200 mL) into a SO₂ gas detection tube, which changes color in direct proportion to the concentration of SO₂ in the sample's headspace. Because the headspace SO₂ concentration is proportional to the molecular SO₂ concentration of the wine (a linear relationship described in chemistry textbooks as *Henry's Law*), it is then possible to determine wine molecular SO₂ without relying on acidification, dilution, or other changes to the wine prior to analysis.

out the experiment time course (Howe, Worobo et al. 2015). The wines were sterile filtered samples of white and red wines, with the latter generated by addition of an anthocyanin extract to a white wine. Prior to the introduction of yeast, the wines were adjusted to different SO_2 levels.

Based on plating data (Figure 5), we observed no correlation between yeast viability and the “standard” SO_2 techniques (A-O, FIA) for red wines. However, viability was well correlated with HS-GDT measurements of SO_2 . Thus, anthocyanin-bisulfite complexes contribute to standard SO_2 measurements, and standard SO_2 measurements

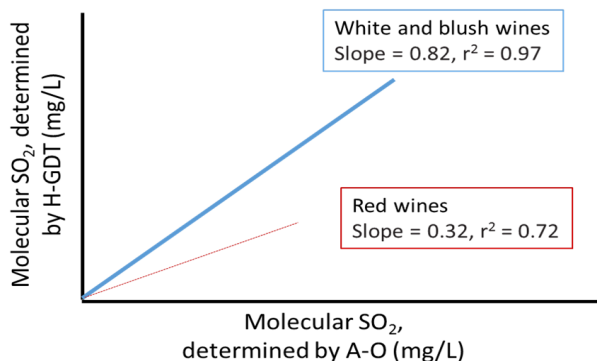


Figure 4: Comparison of new HS-GDT SO_2 method to traditional aeration-oxidation (A-O) method using commercial wines (14 white, 9 red, 4 blush). Red wines contain anthocyanin-bisulfite complexes that dissociate during standard SO_2 measurements, resulting in a considerable overestimation of the true molecular and free SO_2 using the traditional A-O method.

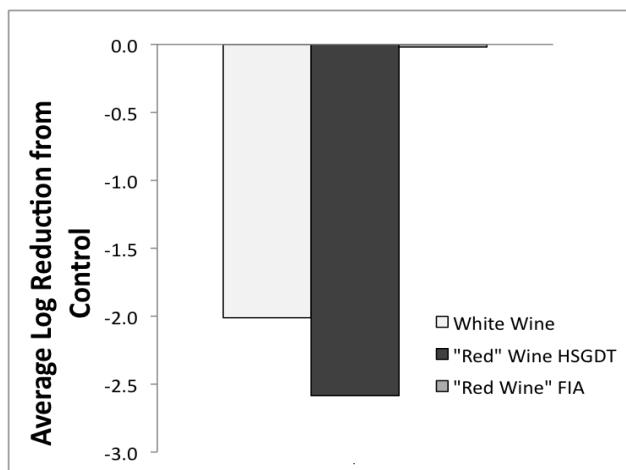


Figure 5: Comparison of the validity of HS-GDT vs. standard approach (FIA) for measuring molecular SO_2 . Wines were adjusted to ~ 0.6 mg/L molecular SO_2 based on each technique prior to inoculations, and viability was evaluated by YM plating. The approaches yielded near identical values for white wine, but necessitated very different SO_2 additions. The standard FIA approach overestimated the amount of active molecular SO_2 in red wine due to anthocyanin-bisulfite interferences. The new HS-GDT method does not perturb these complexes and yields valid results.

are of questionable value for predicting yeast spoilage of sweet wines.

What’s next for HS-GDT? Our recent work confirms previous studies that show standard approaches to SO_2 measurement can badly overestimate the true amount of free and molecular SO_2 , particularly in red wines. “Now what? If we have three-fold or less molecular SO_2 than we think we do, does that mean we should be adding three times or more SO_2 ?”

The short answer is “no”: Very high SO_2 risks exceeding legal limits, results in bleaching of red wine color, and may disrupt the normal aging of wine. Furthermore, many red wines appear to be stable even if they contain less than normal targets for molecular SO_2 concentrations –perhaps because of other factors such as low bioload, low available nutrients, and impact of temperature (or perhaps our target values are also overestimated?).

Instead, the new HS-GDT approach may be useful for identifying high-risk wines – that is, those wines with very low concentrations of molecular SO_2 due to the presence of anthocyanins and other weak SO_2 binders. Such wines could be checked more frequently for the presence of spoilage in the winery, or subjected to more stringent controls prior to bottling (e.g. dimethyl dicarbonate (DMDC), tighter filtration).

Finally, our investigation confirmed the importance of using the correct pKa value when determining the molecular SO_2 levels from free SO_2 (or vice versa). In particular, not accounting for ethanol or temperature can lead to considerable under-or over-estimation.

New questions: Our study on the validity of our new HS-GDT technique was limited to refermentation of sweet wine, but there are still several other questions.

- Do anthocyanin-bisulfite complexes have activity against spoilage bacteria?
- Do these complexes have activity against spoilage yeast, like *Brettanomyces*? If not, can they help explain why Brett occurs in barrel aged reds, but is virtually unheard of in barrel aged whites?
- How do anthocyanin-bisulfite complexes behave during wine oxidation? Does it make sense to count them as part of free SO_2 , since they rapidly dissociate and replenish any SO_2 consumed by oxidative reactions?

Practical use in the winery. Finally, the simplicity of the HS-GDT technique may make it particularly well suited for rapid screening of molecular SO_2 in a winery. The equipment needs are minimal, and no reagents are required, which means that the technique could be used outside of a laboratory. We are currently evaluating the accuracy and precision of HS-GDT measurements taken directly on a tank or barrel headspace.

Imagine a world without needing to take a tank or barrel sample over to the wine laboratory for SO_2 analyses. Sounds pretty nice, huh? Stay tuned.

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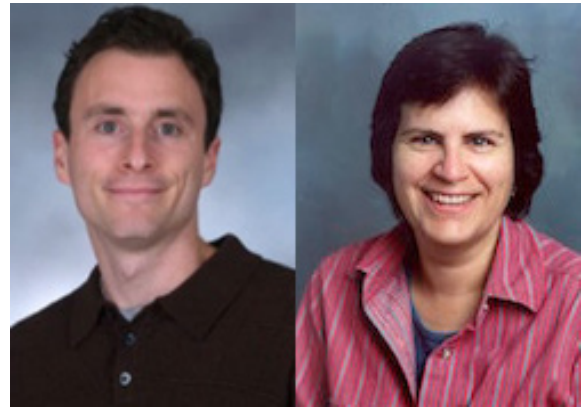
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