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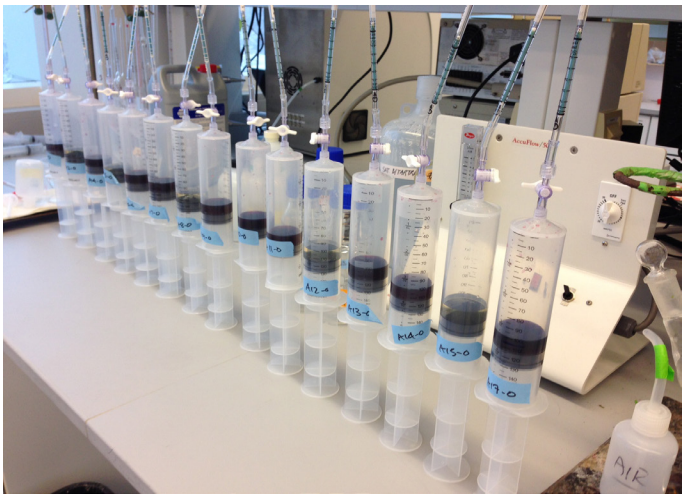
Research Focus

"Free" Doesn't Always Mean Free: Rethinking SO_2 Measurements in the Winery

Gavin L. Sacks[1] and Patricia A. Howe[1,2]

[1]Department of Food Science, CALS, Cornell University, Ithaca, New York 14853
[2]Constellation Brands, St. Helena, California 94574



A simple, inexpensive method for measuring SO_2 in wines uses headspace gas detection tubes developed for the mining industry to accurately measure molecular SO_2 in wines. Using this method, we found that standard approaches (Ripper titration, aeration-oxidation, FIA) overestimate the amount of free and bound SO_2 – particularly in red wines.

Photo by Patricia Howe.

Measurements of free and molecular SO_2 are routinely performed in wineries to ensure microbial and oxidative stability. However, standard approaches to SO_2 suffer from poor reproducibility across laboratories. More importantly, literature indicates that all standard SO_2 methods badly overestimate SO_2 in red wines. We developed an easier, inexpensive and more accurate way to measure free and molecular SO_2. The values provided by this technique may be better predictors of wine stability than standard analytical methods – and could simplify SO_2 measurements for small wineries.

Key Concepts

- Although many forms of SO_2 exist in wine, only the "molecular" form of SO_2 has strong antimicrobial activity (and the ability to singe nostrils). Proper measurement of molecular SO_2 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 SO_2.
- All standard approaches to free SO_2 badly overestimate the amount of free and molecular SO_2 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 SO_2 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 dioxide[1]? SO_2 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 SO_2 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 SO_2 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 SO_2 in winemaking. Sulfur dioxide (SO_2, 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 SO_2 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 SO_2 in winemaking arises from its unique preservative properties:

- As thoroughly discussed in a recent review, SO_2 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.
- SO_2 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).

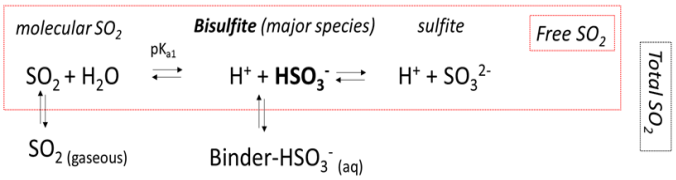


***Figure 1**: Distribution of different SO_2 species in wine.*

In wine, SO_2 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* SO_2 forms which account for the preservative effects of SO_2, and the *bound* forms of SO_2 that arise from the reaction of bisulfite and other wine components. Both bound and free SO_2 count towards *total* SO_2, which is regulated in most wine producing countries.

Free and "Molecular" SO_2. Most free SO_2 in wine exists as bisulfite (>90%), with a smaller fraction (typically, <5%) existing as so-called "molecular" SO_2. As described later, the proportion of free SO_2 that exists as molecular SO_2 is pH dependent. A key point from Table 1 is molecular SO_2 and free SO_2 (the sum of molecular SO_2 and bisulfite) must be considered independently - having sufficient free SO_2 to prevent unwanted oxidation does not ensure adequate molecular SO_2 to prevent microbial spoilage.

Because bound SO_2 has a minimal preservative effect, winemakers are usually more concerned with measuring free SO_2 rather than total SO_2. Despite the apparently large number of methods in use, standard strategies for free SO_2 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 SO_2, then separate and quantify SO_2. Common techniques are aeration-oxidation (A-O) and flow injection analysis (FIA).

***Table 1**. Overview of the different SO_2 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)

$$[\text{Molecular } SO_2] = \frac{[\text{Free } SO_2]}{1 + 10^{(\text{pH-p}K_{a1})}}$$

***Equation 1**: Relationship of molecular SO_2 to free SO_2. In water at 20 C, $pK_{a1} = 1.81$.*

Once free SO_2 is measured, molecular SO_2 is then calculated from the pH and free SO_2 using the Henderson – Hasselbalch (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 SO_2. 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 SO_2 is necessary at higher pH to prevent spoilage – at lower pH, a higher percentage of SO_2 will be in the molecular form than at a higher pH. For example, in water at room temperature, the fraction of free SO_2 existing as molecular SO_2 decreases from pH 3 (6%) to pH 3.3 (3.2%) to pH 3.6 (1.6%).

The pK_a of SO_2 changes. One complication with using the H-H equation is that the pKa of SO_2 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 SO_2 is present (**Figure 2**). Conversely, wines are often stored in relatively cool cellars – which decreases the pK_a and the fraction of molecular SO_2 present – but may be analyzed at warmer temperatures in the wine laboratory. This will result in an overestimation of molecular SO_2 as compared to what is present in the actual storage conditions.

Online molecular SO_2 calculator. A Cornell Food Science graduate student, Greg Dlubac, has recently developed an online calculator for determining molecular SO_2. While several calculators for SO_2 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 SO_2, and thus should provide more accurate estimates of SO_2.

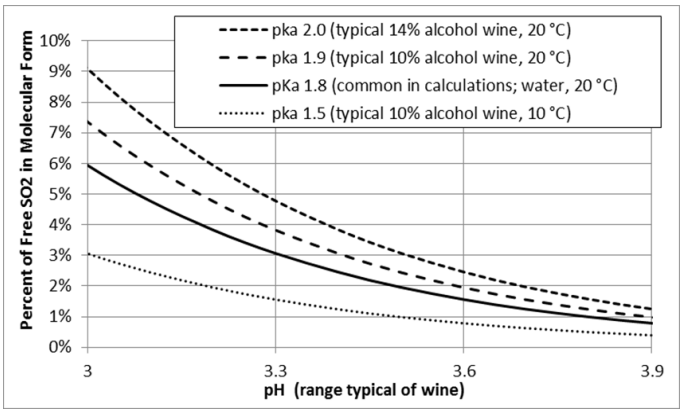


***Figure 2**: Impact of pK_a values on percentage of free SO_2 that exists as molecular SO_2 as a function of wine pH.*

Laboratory analysis vs. magic 8-ball – How well does the industry perform SO_2 measurements? Although free SO_2 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 SO_2. Performance for several other common analytes in wine, including ethanol and titratable acidity, were also considered.

The reproducibility of free SO_2 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 interlaboratory 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 injec-

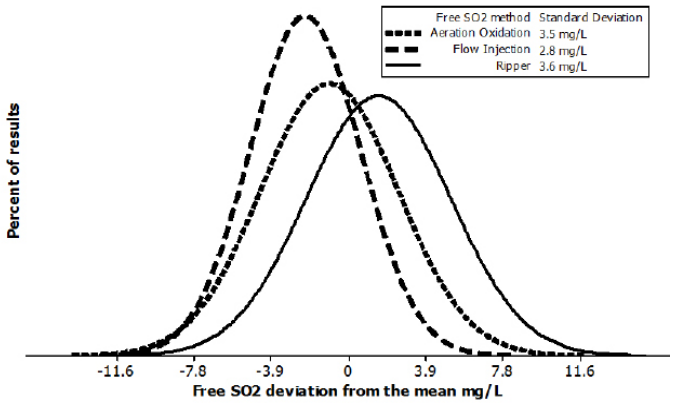


***Figure 3**: Histogram of free SO_2 performance for three common analytical approaches based on 13 years of CTS interlaboratory proficiency testing: Aeration-oxidation (1893 measurements), flow injection (161 measurements), and Ripper (1118 measurements). Data from (Howe, Ebeler et al. 2015).*

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 SO_2 would be even worse than for free SO_2, since it requires determination of both pH and free SO_2 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 SO_2 measurement. Finally, as mentioned above, most calculations of molecular SO_2 use significantly incorrect values for the acidity constant, pK_a.

A new approach: Simple, direct headspace measurements of SO_2. 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 SO_2 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 SO_2 measurement. HS-GDT uses headspace SO_2 as a proxy for molecular SO_2 (see **Figure 1**). In contrast, earlier described methods (Ripper, A-O, FIA) measure all free SO_2 forms, and require subsequent calculation of molecular SO_2. Furthermore, these standard methods require sample dilution and/or pH shifts, resulting in partial hydrolysis of "bound' SO_2 and overestimation of both free and molecular SO_2.

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 SO_2 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, r^2=0.97), but for red wines the standard techniques overestimate molecular SO_2 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.

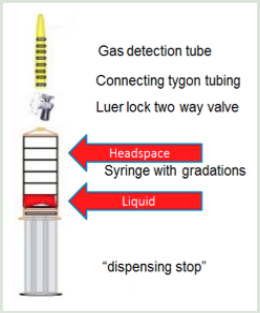
The new HS-GDT method: A better predictor of microbial stability. A reasonable response to our new method is "Does it matter? We've been using standard techniques for decades...maybe they overestimate molecular SO_2, but do a better job predicting spoilage because the bound anthocyanin-bisulfite complexes are still antimicrobial."

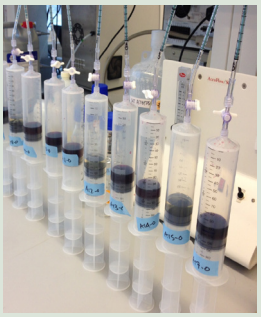
To test this hypothesis, we performed "challenge studies" by inoculating sweet wines with a commercial yeast, and determining molecular SO_2 through both the new HS-GDT method and standard methods (A-O, FIA) through-

The Headspace-Gas Detection (HS-GDT) Method for Measuring SO_2

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 SO_2 in wine.

Photos by Patricia Howe.

The Headspace-gas detection tube (HS-GDT) method uses these gas detection tubes to measure SO_2 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 SO_2 gas detection tube, which changes color in direct proportion to the concentration of SO_2 in the sample's headspace. Because the headspace SO_2 concentration is proportional to the molecular SO_2 concentration of the wine (a linear relationship described in chemistry textbooks as *Henry's Law)*, it is then possible to determine wine molecular SO_2 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

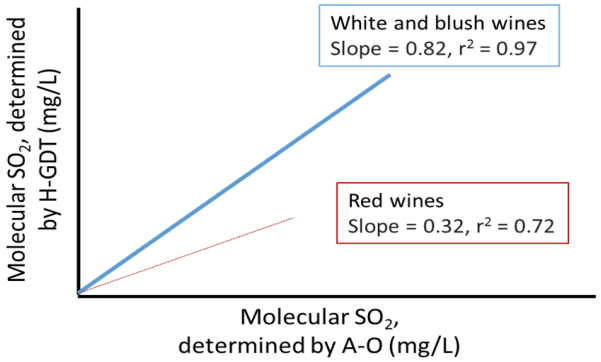


***Figure 4**: Comparison of new HS-GDT SO_2 method to traditional aeration-oxidation (A-O) method using commercial wines (14 white, 9 red, 4 blush). Red wines contain anthocyanin-bisulfite complexes that dissociate during standard SO_2 measurements, resulting in a considerable overestimation of the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

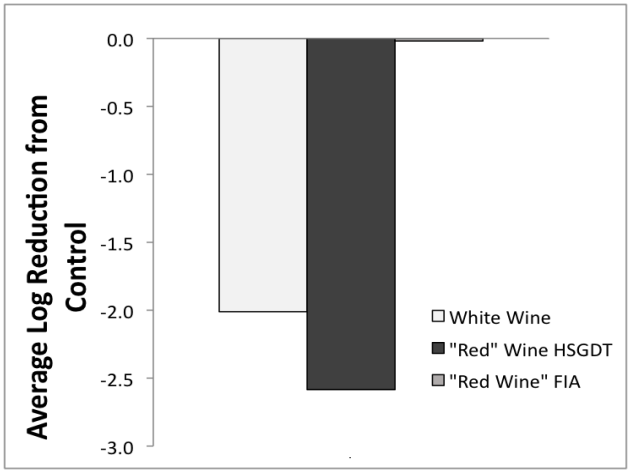


***Figure 5**: Comparison of the validity of HS-GDT vs. standard approach (FIA) for measuring molecular SO_2. Wines were adjusted to ~0.6 mg/L molecular SO_2 based on each technique prior to inoculations, and viability was evaluated by YM plating. The approaches yielded near identical values for white 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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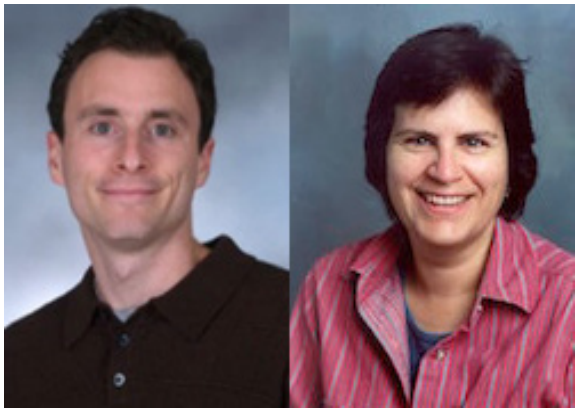

Gavin Sacks joined the Cornell faculty in 2006. He is associate professor of food science and director of the undergraduate Viticulture and Enology program at Cornell. Pat Howe joined the Cornell food science department in 2013 as lecturer, following 30 years in the wine business in California. She completed her PhD at Cornell in 2015, and is currently senior director for quality at Constellation Brands based in the San Francisco bay area.

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