SO_2 Management in Winemaking

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Roles of SO_2 in Wine

- Antimicrobial
  - knock down yeast (esp. wild) and bacteria
  - Wild yeast and bacteria potential source of off flavors
- Anti-oxidant
  - Oxidation of wine leads to brown color, decrease in varietal aromas, development of nutty, sherry aromas
  - In juice, SO_2 inhibits enzymatic oxidation (PPO)
  - In wine, SO_2 reacts with chemical oxidation intermediates & oxidized compounds to reduce apparent effect
- The chemistry behind how SO_2 performs these activities fairly complicated

Three Areas to Cover Today

- Basic Chemistry of SO_2
- Managing SO_2 during Winemaking
- The A/O Method for Testing Free SO_2

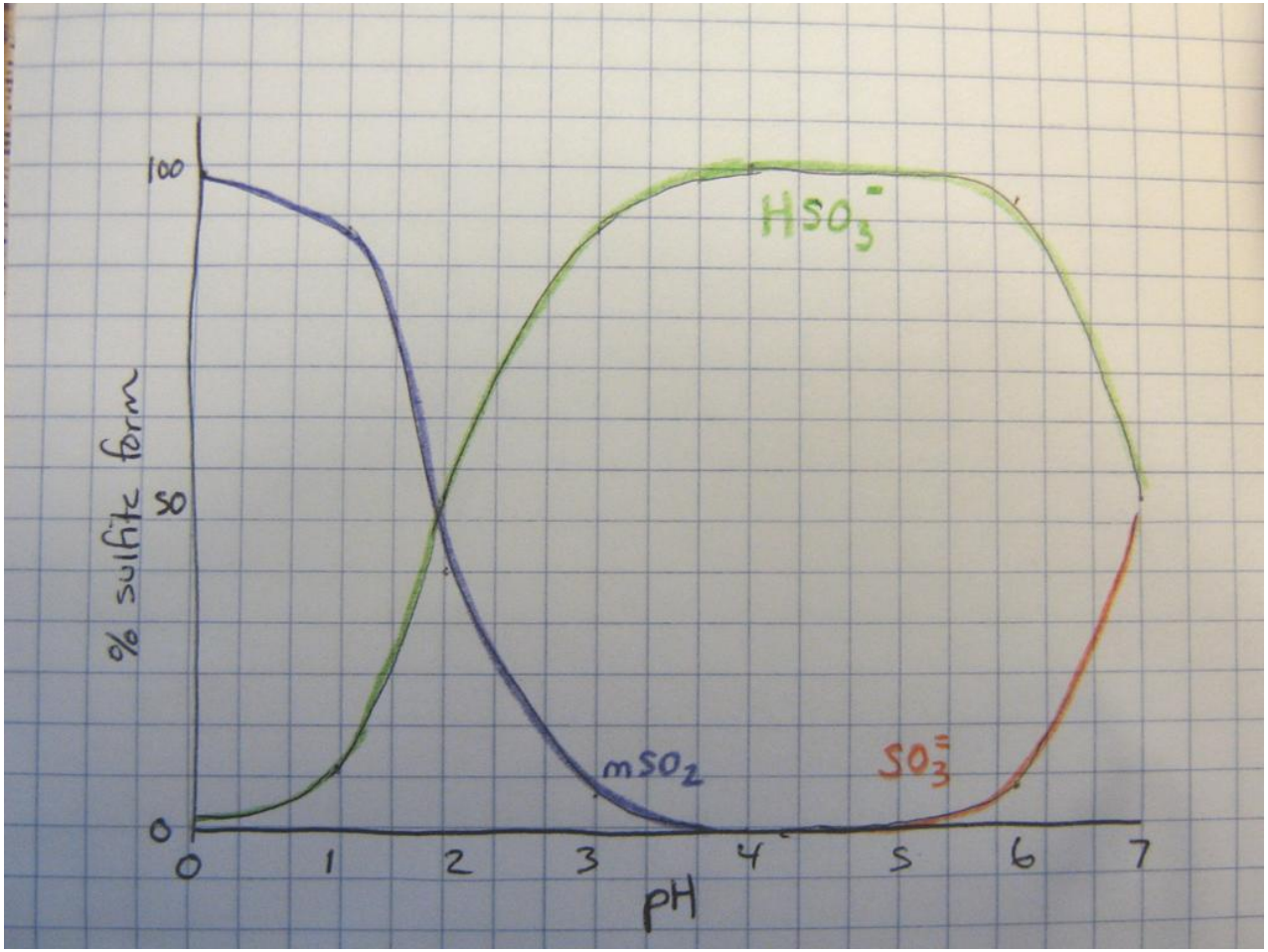
Basic Chemistry of SO_2

- SO_2 in wine exists as free SO_2 equilibrium, some free SO_2 can bind certain wine compounds to become bound SO_2
- All the SO_2 in the wine is referred to as the total SO_2
- Total SO_2 = free SO_2 + bound SO_2

Free SO_2 in Wine

- Exists in three forms: molecular SO_2, bisulfite ion (HSO_3^-), sulfite ion ($SO_3^=$)
- The forms are in equilibrium, amounts of each dependent on pH
- At wine pHs, mostly in bisulfite form
- Eyes on the Prize: molecular SO_2 is the form that is antimicrobial

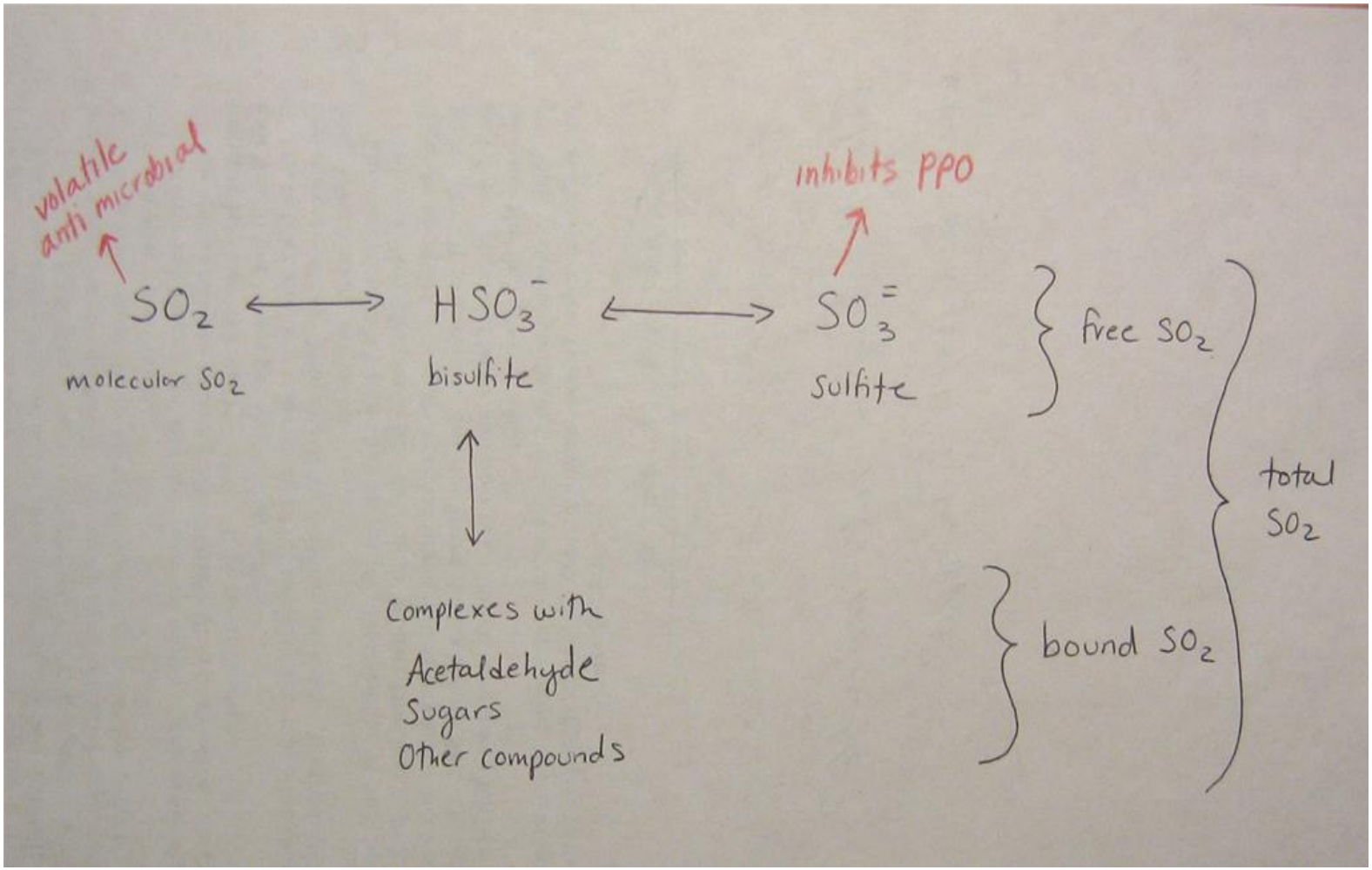
The Ever Present Free SO_2 Graph



Bound SO_2 in Wine

- The bisulfite form binds to wine components such as sugar, acetaldehyde, and phenolic compounds.
- Amount SO_2 bound varies (25 to 60%)
- When bisulfite ion reacts with these compounds, no longer takes part in free SO_2 equilibrium

Forms of SO_2 in Wine



Molecular SO_2 is Antimicrobial

- A level of 0.8 ppm molecular SO_2 generally viewed as sufficient for microbial stability
  - Amount of SO_2 added that is in free form is dependent on how much is bound
  - Amount of free SO_2 that is in molecular SO_2 form is dependent on pH
    - At pH 3.0, molecular SO_2 is 6% of free SO_2 and therefore need 13 ppm free SO_2 to have 0.8 ppm molecular SO_2
    - At pH 3.8, molecular SO_2 is 1% of free SO_2 and therefore need need 79 ppm free SO_2 to have 0.8 ppm molecular SO_2
- If goal is to ensure microbial stability, need to know pH & amount of free SO_2

Vinquiry – Free SO2 Chart

DISTRIBUTION OF FREE SO_2

From pH 3.0 – 4.0
(pKa = 1.81)

pH	% Molecular SO_2 (m)	% Bisulfite (HSO_3^-)	% Sulfite ($SO_3^=$)	Minimum ppm of Free SO_2	
				0.8 molecular	0.5 molecular
3.00	6.1	93.9	0.012	13	8
3.05	5.3			15	9
3.10	4.9	95.1	0.015	16	10
3.15	4.3			19	12
3.20	3.9	96.1	0.019	21	13
3.25	3.4			23	15
3.30	3.1	96.8	0.024	26	16
3.35	2.7			29	18
3.40	2.5	97.5	0.030	32	20
3.45	2.2			37	23
3.50	2.0	98.0	0.038	40	25
3.55	1.8			46	29
3.60	1.6	98.4	0.048	50	31
3.65	1.4			57	36
3.70	1.3	98.7	0.061	63	39
3.75	1.1			72	45
3.80	1.0	98.9	0.077	79	49
3.85	0.9			91	57
3.90	0.8	99.1	0.097	99	62
3.95	0.7			114	71
4.00	0.7	99.2	0.122	125	78

SO_2 as Antioxidant

- In juice, SO_2 reduces enzymatic oxidation by inhibiting the activity of polyphenol oxidase (PPO)
- In wine, chemical oxidation of compounds
- In wine, sulfite ion is the antioxidant form but at wine pH ineffective as antioxidant

Oxidation Reactions in Wine

- Phenolic compounds -> Oxidized compounds
  - H_20_2 is an oxidation intermediate, enables oxidation of ethanol to acetaldehyde
  - SO_2 scavanges H_20_2, preventing further oxidation of phenolics and ethanol
  - SO_2 can recycle quinone oxidation intermediate back to phenolic compound
  - SO_2 can bleach brown compounds
- Ethanol -> Acetaldehyde
  - SO_2 binds strongly to acetaldehyde, removes nutty, sherry like aroma

SO_2 as Anti-Oxidation Agent in Wine

- SO_2 masks effect of oxidation by binding acetaldehyde and bleaching brown pigments
- SO_2 curtails oxidation reactions by scavenging oxidation intermediates
- SO_2 recycles oxidation intermediates

Amount of SO_2 is not Stable

- Will lose free SO_2:
  - in headspace (molecular SO_2 is volatile)
  - as binds oxidation reactants/products
  - as binds solids in wine
- Test free SO_2 to ensure protection

Review Basic Chemistry of SO_2

- SO_2 exists as free and bound forms in wine
- Free SO_2 consists of molecular SO_2, bisulfite, and sulfite ion; amounts of each dependent on pH of wine
- The molecular SO_2 form inhibits yeast and bacteria, 0.8 ppm molecular SO_2 good for stability
- To ensure microbial stability, need to know wine pH and how much SO_2 is present as free SO_2
- SO_2 can inhibit enzymatic oxidation in juice
- SO_2 not a true anti-oxidant in wine, but does help to curtail oxidation reactions and covers up acetaldehyde smell quite nicely

Three Areas to Cover Today

- Basic Chemistry of SO_2
- Managing SO_2 during Winemaking
- The A/O Method for Testing Free SO_2

Using SO_2

- SO_2 is typically added to wine in the form of Potassium Metabisulfite.
- By weight, only 57% of Potassium Metabisulfite is released as SO_2
  - 1 gram of PMBS contains 0.57 grams SO_2
- SO_2 concentration measured in parts per million (ppm) which is equivalent to milligrams per liter (mg/L)
  - 1 gram of PMBS in 5 gallons gives 30 ppm SO_2

Adding SO_2 to Juice/Must

- SO_2 gives a little jump-start to wine yeast (around 30 ppm SO2)
- SO_2 lowers activity of PPO in juice and must
  - Add SO_2 to prevent browning, oxidation of fruity & floral aroms (oh, let's see, 30 ppm SO_2 for good inhibition)
  - Be stingy with the SO_2 to promote browning, oxidation (0 ppm SO_2)
    - Brown pigments will be removed by lees
    - Resulting wine more color stable, resistant to oxidation
- Add more SO_2 to moldy must/juice (>50 ppm SO_2)

SO_2 During Fermentation

- Yeast produce a small amount SO_2, 10 ppm, during fermentation
- Wine yeast neutralize SO_2 during fermentation with acetaldehyde
- SO_2 not bound to acetaldehyde is bound to solids
- At the end of alcoholic fermentation there is essentially no free SO_2

Adding SO_2 After Alcoholic Fermentation

- Add 0.8 ppm molecular SO_2 to prevent yeast and malolactic bacteria growth (also sufficient for anti-oxidation purposes).
  - 1 gram of PMBS dissolved in 5 gallons of wine would give 30 ppm SO_2
  - At a wine pH of 3.35, 30 ppm free SO_2 would provide 0.8 ppm molecular SO_2
  - But that assumes no SO_2 in bound form, 25% to 60% is typically bound
  - Need to determine amount of free SO_2 to ensure protection

SO_2 and Malolactic Fermentation

- Malolactic bacteria highly sensitive to free SO_2
- Delay additions of SO_2 until after MLF complete
- ML Bacteria also sensitive to bound SO_2, use a light hand when adding SO_2 to juice/must (>25 ppm total SO_2 may inhibit)
- Keep ML bacteria happy (temp, pH, alcohol) to ensure timely completion while wine not protected by SO_2

Adding SO_2 before Bottling

- Ensure have enough free SO_2 for 0.8 ppm molecular SO_2
- Add a little bit extra - to account for free SO_2 loss during bottling
  - I typically target a free SO_2 that is 10 to 15 ppm higher than the level of free SO_2 needed for 0.8 ppm molecular SO_2
  - Target more or less depending on trauma of bottling method (O_2 pick-up)

Let's give SO_2 a Helping Hand

- The combination of limiting O_2 exposure, using clean equipment, and maintaining free SO_2 leads to healthy wines
  - O_2 needed in oxidation of wine components
  - SO_2 doesn't prevent this oxidation, helps cover and curtail
  - Clean equipment and full containers helps limit yeast and bacteria contamination/growth

Review Managing SO_2 Winemaking

- Add SO_2 to must/juice to help wine yeast establish & prevent enzymatic oxidation
- After alcoholic fermentation, maintain 0.8 ppm molecular SO_2 to for microbial stability (also sufficient for anti-oxidation purposes)
- Delay SO_2 additions to wines undergoing MLF
- Ensure > 0.8 ppm molecular SO_2 at bottling for microbial stability

Three Areas to Cover Today

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Why We Test SO_2

- Survey says: audience home winemakers either don't test or use Titrets to test for free SO_2.
- Testing free SO_2 enables winemaker to know if wine is protected with 0.8 ppm molecular SO_2.
- Testing for SO_2 enables the winemaker to troubleshoot wines that are not behaving properly.
- Testing enables winemaker to figure out how SO_2 is being consumed in *their* wines.

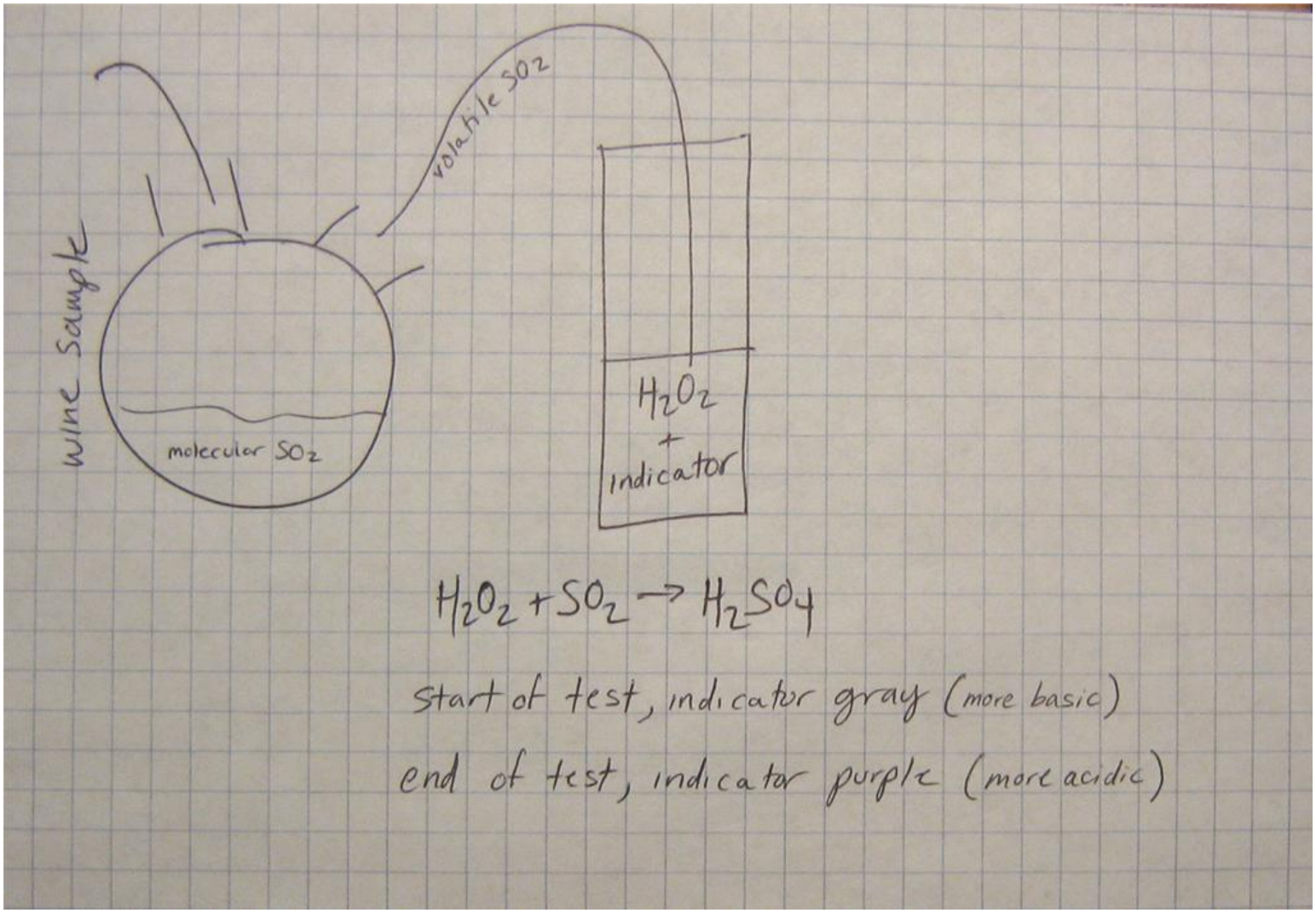
A/O method is A-OK

- Titrets based on Ripper method
  - Easy to use and quick (just over $1 per test)
  - Can only be used with whites
- Aeration/Oxidation method
  - Most accurate method
  - Can be used on reds
  - $$$ investment in equipment
  - Time investment to set-up & learn method

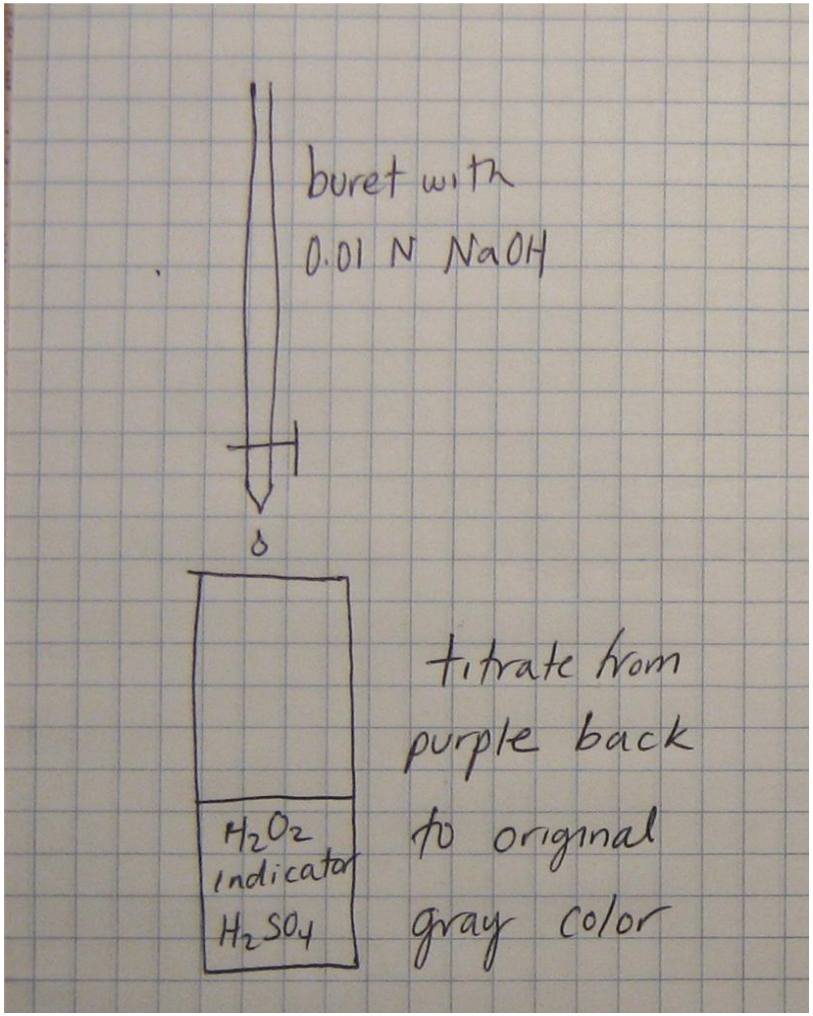
A/O Method Theory

- Acidify wine sample to drive all free SO_2 into the volatile molecular SO_2 form.
- Allow the molecular SO_2 to react with hydrogen peroxide (H_2O_2) to form sulfuric acid (H_2SO_4)
- Titrate sulfuric acid (H_2SO_4) with sodium hydroxide base (NaOH) to calculate starting amount free SO_2.

A/O Method Free SO_2 – Step 1



A/O Method Free SO_2 – Step 2



- Amount of 0.01 N NaOH used in titration directly proportional to amount of free SO_2 in original wine sample

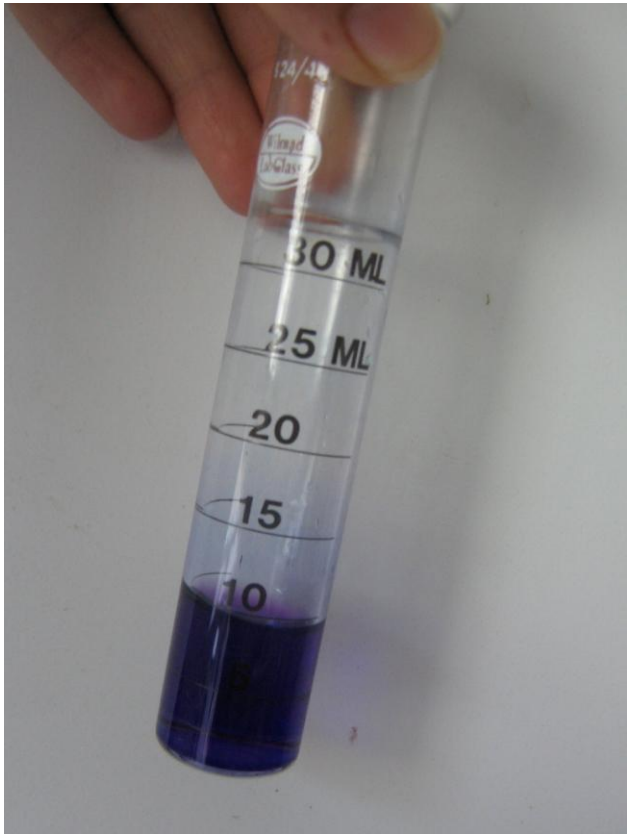
A/O Method in Practice

- 20 mL wine sample acidified with 10 mL 25% phosphoric acid to drive all the free SO_2 to the molecular SO_2 form (volatile form)
- Volatile SO_2 leaves round bottom flask via vacuum suction (aspirator) or air pump into an impinger containing 3% hydrogen peroxide and a few drops of SO_2 indicator
- Takes about 15 mins to complete, SO_2 indicator is gray at the start (more basic) and turns purple as hydrogen peroxide reacts with SO_2 to form sulfuric acid (more acidic)
- The sulfuric acid formed in the impinger is titrated with 0.01 N NaOH, as sulfuric acid is titrated to endpoint the indicator in the solution turns from purple back to initial gray color
- Amount of 0.01 N NaOH used in titration is proportional to free SO_2 present in original sample

Picture of A/O Method in Practice

H_20_2 and SO_2 indicator at start

After reaction with volatile SO_2



A/O Method – 0.01 N NaOH

- The 0.01 N NaOH prone to degrade overtime
- Using less than full strength 0.01 N NaOH affects accuracy of test
  - Use freshly opened 0.01 N NaOH
    - Expensive (16 oz is $16 at MoreWine.com)
  - Dilute 0.1 N NaOH to 0.01 N NaOH; check titre
    - Requires more equipment, time to test titre
    - Eventually inexpensive (16 oz 0.1 N NaOH is $5 at Presque Isle)

Make & Check Titre of 0.01 N NaOH

- Determining titre strength of 0.01 N NaOH with potassium acid phthalate ensures accurate results
- Will need: buret, buret stand, flasks, pipets, 0.1 N NaOH, graduated cylinder, dH_20, potassium acid phthalate, phenolphthalein indicator
- Use much of the same equipment set-up for titratable acidity

A/O Method – Aspirator vs Pump

- Need to volatilize SO_2 to enable reaction with H_2O_2
- Aspirator requires a sink faucet with water flow to create vacuum
  - Can control flow rate easily by opening up the faucet
- Pump requires electricity
  - Can control flow rate via pump itself or inline valve
  - Nice not to waste water, worry about flooding

A/O Method – Flowmeter vs Wing It

- The recommended 1 liter/min flowrate is to ensure one is not blasting the gaseous SO_2 right through your hydrogen peroxide sample and out the other end
- Flowmeters are expensive ($75 to $120)
- Might just want to wing it and track bubbling rate by eye

A/O Method – Bubble Rate

Set up A/O at Home - EASY

- Morewine.Com has a starter kit ready from the box with user friendly instructions
  - Uses air pump so no water source needed
  - With flowmeter ($490) and without ($295)
  - Includes reagents for 2 to 3 tests
  - Uses a syringe to titrate w/0.01 N NaOH (requires fresh NaOH)
  - Upgrade to buret titration system to check titre of 0.01 N NaOH (enables one to make own 0.01 N NaOH solution)

Set Up A/O at Home – Basic

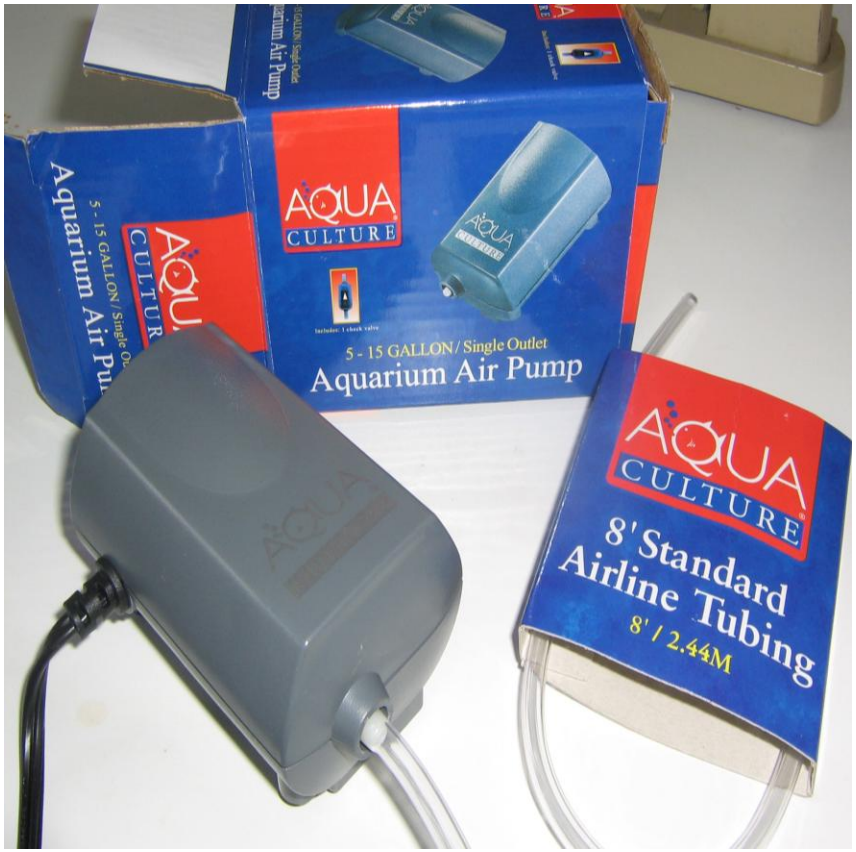
- Presque Isle has a starter kit with aspirator and flowmeter ($326)
  - Low price for including a flowmeter
  - Does not include reagents
  - Does not include titration set-up, pipets

Set Up A/O at Home – DIY

- Buy components separately to accommodate what is already owned
  - Round Bottom Flask ($45 MoreWine)
  - Impinger ($57 MoreWine)
  - Stand and Clamps (Cynmar)
  - Flowmeter ($75 to $120)
  - Aspirator or Pump (<$20)
  - Buret, Buret Stand, Buret clamp, flasks, cylinders, pipets ($60 Cynmar)
  - Reagents (varies)

DIY Further $$ Savings

- Buy an air pump & tubing from Walmart ($8)
- One for small fish tanks, 5 to 15 gallon, provides close to 1 L/min flowrate



Further $$ Savings

- Purchase 3% Hydrogen Peroxide at drug store ($1) instead of from wine suppliers ($16).

Further $$ Savings

- Cynmar is a great supplier for labware:
  - Support stands (<$10)
  - Clamps (~$5)
  - 25 mL Buret ($17.50)
  - Buret stand with clamp ($25)
  - 20 mL volumetic pipet ($2.90)
  - 100 mL Graduated cylinder ($9.80)

Review Using A/O Method

- Most accurate method, it is the real deal
- Best for home winemakers wanting to ensure consistency & interested in wine chemistry
- Determine titre strength of 0.01 N NaOH

Three Areas We Covered Today

- Basic Chemistry of SO_2
  - Free, Bound, molecular SO_2, pH, oh my!
- Managing SO_2 during Winemaking
  - After alcoholic fermentation maintain 0.8 ppm molecular SO_2 for microbial stability
- The A/O Method for Testing free SO_2
  - A/O and a pH meter is all one needs

The End

- Come visit us at Keuka Lake Vineyards down in Hammondsport
- ?’s -> stacinugent@klvineyards.com