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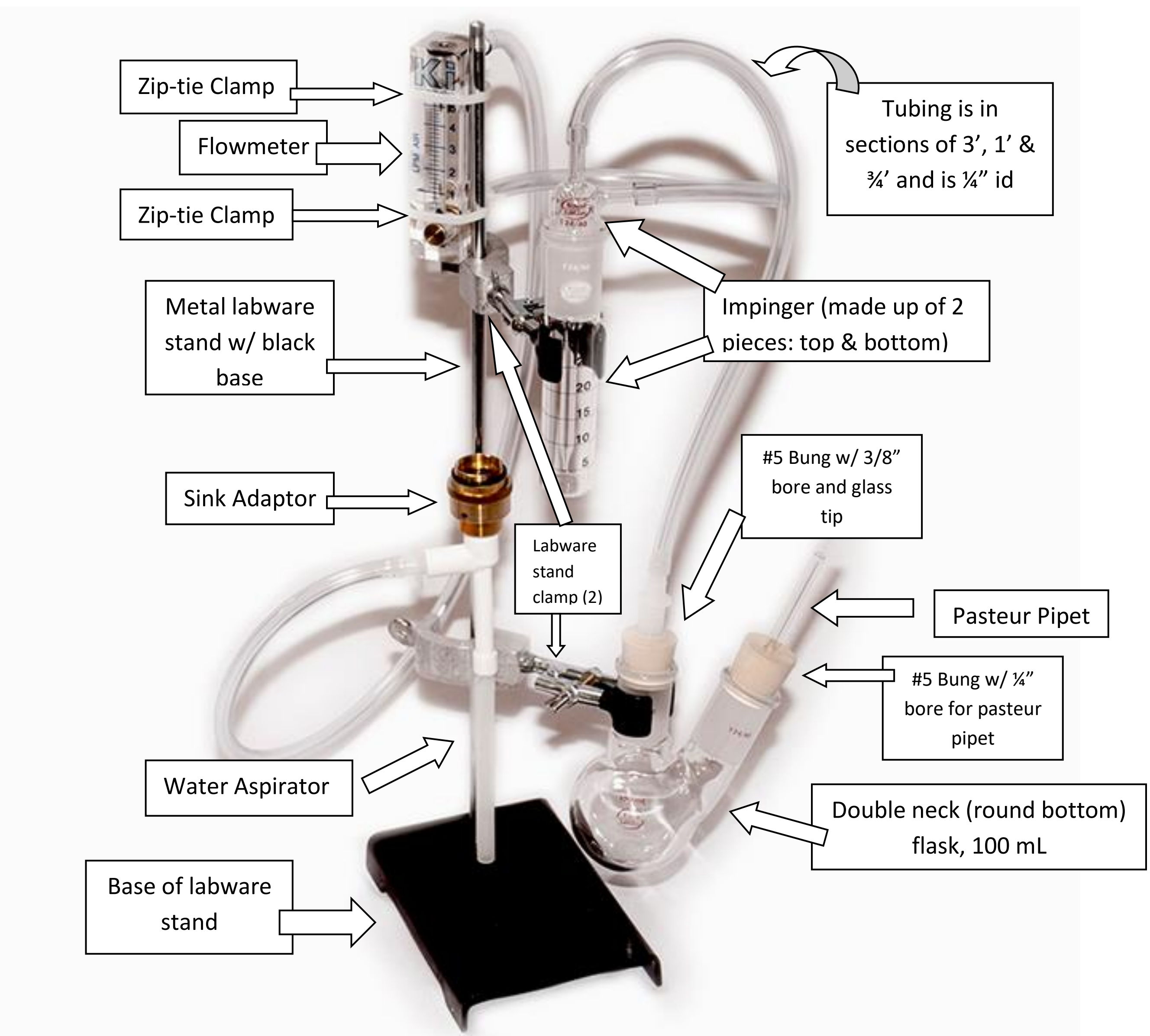
Aeration-Oxidation Apparatus

for measuring Free + Bound (=Total) Sulfur Dioxide



<u>Testing For Free (Unbound) Sulfur Dioxide In Wine</u>
<u>Using The Aeration Oxidation Method</u>

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Categories of Sulfur Dioxide *(hereafter called SO_2)*: SO_2 is added to wine as a preservative and protector.

- Free ***(unbound)* SO_2** – It is this free SO_2 that is the 'workhorse' at protecting wine.
- Bound SO_2 – This can be measured but it is not as critical that it be done. Some of the bound SO_2 is loosely bound and can release into free SO_2 when running some tests. Care must be taken to prevent this to avoid a false reading.
- Total SO_2 – This is merely the sum of the bound SO_2 and the free SO_2.

For many, Aeration Oxidation is the most accurate method to test SO_2 levels in wine. There is a slight overestimation of the SO_2 level in red wine because some of the SO_2 that is bound to color pigments will release and be erroneously read as being free SO_2. The older the red wine is the less this occurs. At any rate the overestimation is slight and not of significant consequence.

Labware Needed:

I. Aeration Oxidation apparatus for measuring free SO_2

A. The parts include: ***(if more than one is needed that number is shown in ( )***:
   - Metal stand ***(rod & base)***
   - 100 ml round bottom double neck flask
   - Clamp ***(to attach the 100 ml flask to the metal rod)***
   - Bored rubber stopper # 5 with a ¼-inch hole
   - Bored rubber stopper # 5 with a $^3/_8$-inch hole
   - Pasteur pipet ***(2 &1 is extra)***
   - Glass tip
   - Tubing to run from the top of the 100 ml flask to the upright on the impinger top
   - Complete impinger unit ***(this means it has a top & a bottom)***.
   - Clamp ***(to attach the impinger bottom to the metal rod)***
   - Tubing to run from the side attachment of the impinger top to the inlet ***(bottom)*** of the flowmeter
   - Flowmeter
   - Tubing to run from the outlet (top) of the flowmeter to the water aspirator ***(or a catch basin if an aqua pump is used)***
   - A plastic quick disconnect to insert somewhere inline on the tubing that runs from the aspirator to the impinger ***(this allows for disconnecting the A/O apparatus from the water aspirator)***.
   - Plastic tie clamps ***(2) (to secure the flowmeter to the metal rod on the stand)***
   - Water aspirator ***(an aqua pump can be substituted with 1200 being a good size)***
   - Sink adaptors ***(2) (to accommodate attaching the aspirator to the sink)***
   - **Note:** To test for bound SO_2, the addition of a condenser, a heating element and a longer metal rod would be required. These may be special ordered. An aqua pump cannot be used when testing for bound SO_2. The water aspirator must be used because water must be drawn through the condenser to cool the sample during testing.

Note: The recommended number of the labware listed below can possibly be reduced if there is a willingness to clean a single piece before using it to handle a different substance.

II. 10 ml volumetric pipet ***(2)***
III. 20 ml volumetric pipet
IV. Pipet bulb (used to draw fluid into a pipet) (adding a glass tip to the bulb increases ease of use
V. Small beakers ***(3)***
VI. 10 ml or 25 ml buret ***(2)*** with stopcock ***(a smaller volume buret simply may require refilling)***
VII. Buret stand with buret clamp
VIII. Some type of ice bath container in which to suspend the 100 ml double neck flask ***(Tupperware works well)***. During the test, keep the wine sample temperature below 20^0 C ***(68^0 F)***
IX. 100 or 200 ml volumetric flask ***(3)*** These are used to prepare and/or hold reagents
X. 250 or 500 ml plastic rinse bottle with rinse spout (for distilled water)
XI. Safety glasses ***(recommended when using chemical reagents)***
XII. A timing device ***(having a second hand is desirable & a stopwatch would be ideal)***
XIII. Grease pencil with which to label beakers, pipets, etc.

Reagents Needed: These can be purchased or made from stronger solutions by proper dilution using distilled water. Instructions for some dilution procedures are included with this document.

XIV. 0.3% hydrogen peroxide ***(hydrogen peroxide should be stored in a refrigerator as it is unstable at room temperature)***
XV. 25% phosphoric acid
XVI. SO_2 indicator solution ***(made from methyl red and methylene blue)***
XVII. 0.01 normal ***(0.01N)*** sodium hydroxide ***(NaOH); this can be diluted from 0.1N***
XVIII. Distilled water

Procedures: There are many steps but don't panic, they are quite easy.

Step One: Consider labeling the beakers, pipets, flasks, etc. with a grease pencil as to what reagents they will contain. Label a large beaker as a waste beaker in which to dump waste reagents. **It is recommended that safety glasses be worn when working with chemicals. Make sure all labware is clean & dry.**

Step Two: Set up two burettes on stands.
Part A: Pour a small amount of 0.01 ***(0.01N)*** normal sodium hydroxide ***(NaOH)*** into a burette. Empty into waste beaker. Then, fill the burette with the NaOH. (A small burette funnel can be very helpful in pouring liquid into the burette.) **It is not necessary to fill the burette completely to the zero level at the top but be sure to have enough for the needed task.** You should dispense liquid out of the burette into the waste beaker until all air bubbles are eliminated.
Part B: You can hold off on pouring hydrochloric ***(or other acceptable)*** acid in the second burette until it's needed. When ready, use the same procedure as in Part A.
These burets can now be set aside for use later. Using a burette to dispense a liquid is known as titration.
Step Three: If not already done, assemble the aeration oxidation apparatus ***(see above schematic)***.

Step Four: Use a plastic rinse bottle of distilled water to rinse out the 100 ml round bottom double neck flask. Discard the water into the waste beaker. Clamp the top neck of the flask to the metal rod and suspend it in the ice bath to begin cooling. Nothing will be added to the flask just yet. Insert the heavier but shorter glass tip into the # 5 rubber bung with the $^{3}/_{8}$ hole and insert into the top neck of the flask. Attach the proper tubing to the protruding tip and attach the other end of the tubing to the impinger top. **The ice bath will be most effective and easiest to use if it contains mostly cold water with some ice**. It is important that the flask be cold when the wine sample is added to reduce the chance of some of the loosely bound SO_2 becoming free SO_2.

<u>Step Five</u>: Make sure the collecting impinger is clean & dry. Prepare a small beaker and a 10 mL volumetric pipet to receive an adequate amount of 0.3% hydrogen peroxide. In a similar fashion as was done for the sodium hydroxide above, give a final cleansing rinse to the beaker and the pipet with hydrogen peroxide 0.3%. Discard this rinse into the waste beaker. Using a pipet pump, draw the hydrogen peroxide solution to fill a 10 mL volumetric pipet. Draw the liquid up past the fill line, then release enough until the bottom of the meniscus reaches the fill line. Release the 10 mL of hydrogen peroxide into the bottom part of the impinger unit.

<u>Step Six</u>: Add three drops of the SO_2 indicator solution into the impinger bottom. Swirl to mix it into the already present hydrogen peroxide. You should get an olive-turquoise green color in this mixture that is in the impinger. If you have that color at the start, there will be no need to adjust it. However, if you don't have that color, then you can adjust it by first adding a drop of the 0.01N sodium hydroxide into the burette you may have prepared earlier followed, if needed, by a drop of mild hydrochloric acid ***(or suitable substitute)*** from another burette. Toggle back and forth between the two until you get the required olive-turquoise green color. (***And substitute a different kind of mild acid such as mild phosphoric or mild sulfuric acid as well as a different normality of NaOH as the purpose is merely to adjust the color).***

<u>Step Seven</u>: Clamp the impinger bottom to the metal rod. Then the impinger top can be inserted into the impinger bottom. Setting up the impinger with its solution should be done first before the double neck flask is prepared. This will increase the chance of still capturing any SO_2 that may blow off prematurely when the wine sample is added to the 100 mL flask.

<u>Step Eight</u>: Prepare, by rinsing with 25% phosphoric acid, a beaker and 10 mL volumetric pipet using the same techniques as described previously. Then draw a 10 mL amount of 25% phosphoric to then be released into the double neck flask via the side neck. It is important to add the phosphoric acid to the flask before the wine sample is added to reduce the chance of any premature loss of any of the free SO_2 for which you are testing. The phosphoric acid will make the wine more acidic (lowering the pH) so it more easily will release the free sulfur dioxide (SO_2).

<u>Step Nine</u>: From the wine sample you hopefully collected in advance, you will now measure out the correct amount ***(20 mL)*** to be added to the double neck flask. The wine to be tested should have been stored in an inert ***(glass)*** container that is full to the brim. This will reduce the risk of prematurely losing any free SO_2. Again, rinse a beaker and 20 mL volumetric pipet with a small amount of the wine and discard into the waste beaker. Pour an adequate amount of wine **down the side** of the beaker. Then draw the 20 mL sample of wine and release it into the double neck flask via the side neck. Use the ice bath to keep the sample below 20^0 C (68^0 F). Stopper the side neck with the # 5 rubber stopper with the ¼ inch hole. Prior to this, slip the long, thin and very delicate Pasteur pipet into the stopper hole ***(lubricate the pipet with Antifoam or glycerine to ease insertion of the glass tube)***. Position the bottom tip of this pipet so it rests into the wine/phosphoric acid mixture inside the flask. This will allow the air to bubble through the sample.

<u>Step Ten</u>: It is now time to aerate the wine sample. This can be done one of two ways. Either hook up a vacuum pump to the Pasteur pipet protruding from the side neck of the double neck flask to push air through the system or attach the aspirator to a sink faucet and then connect the other end of the tubing to the flowmeter outlet ***(top)*** to pull air through the system. The aeration should be at a rate of 1000 to 1500 mL/minute ***(1.0 to 1.5 liters per minute)*** as measured by the flowmeter. Run the aeration for ten ***(10)***

minutes at which time the airflow is shut off. The collecting of the free SO_2 in the impinger causes a reaction with the hydrogen peroxide resulting in a conversion to sulfuric acid which can then be titrated as discussed in steps 11 & 12 below.

<u>**Step Eleven**</u>**:** Now it is time to analyze the mixture collected in the impinger. Pull the tubing off the top of the impinger and lift the top partially out of the bottom container. While keeping the tip of the top's glass tube inside the bottom, use the rinse bottle of distilled water to rinse off any of the purple collection still clinging to the outside circumference of the tube. Also squirt some water down inside the impinger top to push any collection back into the liquid in the bottom. **Distilled water will not have any affect on the collection material.** Now the impinger bottom can be dismounted from the apparatus. The contents will be a purple color. Using the burette containing 0.01N sodium hydroxide, titrate ***drop by drop*** the contents of the impinger. It is vital that you first <u>record an initial reading</u> of the level of sodium hydroxide contained in the burette. **Swirl the impinger contents at each addition of NaOH**. Titrate until the purple color changes back to its original olive or turquoise green color. It is imperative that the exact last drop that permanently changes the color from purple to the olive or turquoise green is observed and the titration stopped immediately. If titration takes you past the olive or turquoise green color, a false reading will occur and the whole test will require repeating. You know you went past the end point if the color changes to a lighter & brighter green. The further past you go the lighter will be the color. **A sign that the endpoint is very close is that the purple will start to turn dingy with a gray hue followed by a gray color. The next drop of sodium hydroxide may indeed cause the transition back to the original olive or turquoise green color so definitely go drop by drop.** After reaching the proper end point, read the level of sodium hydroxide left in the burette. Subtract the initial reading from the final reading to know how much sodium hydroxide it took to get back to the original color. **Record these numbers to avoid error.**

<u>**Step Twelve**</u>**:** Calculate the free SO_2 level using the following formula:

$$\text{Free } SO_2 = \frac{(V) * (N) * (32) * (1000)}{(v^1)}$$

Where: ***(V)*** =The amount of sodium hydroxide used to titrate back to the original color ***(this variable is the only truly unknown one and won't be known until you do the titration)***.

(N) = The normality level of the sodium hydroxide used ***(you know this really before you start and unless you change to a different normality it will cease to be an unknown variable).***

(v^1) = The size of the wine sample ***(if you followed these directions the wine sample was 20 mL so that also ceases to be an unknown variable)***.

Therefore: You can work out most of the formula ahead of time to be: **Free SO_2 = (V) * 16**

For Example: If it took 2.85 ml of the 0.01N sodium hydroxide to turn the purple color back to the proper green color, the free sulfur dioxide level would be 2.85 * 16 = 45.6 parts per million (ppm) sulfur dioxide.

<u>Procedures For Making Various Reagents</u>

Hydrogen Peroxide 0.3 %:

- Use one milliliter ***(mL)*** of 30% hydrogen peroxide and then add distilled water to make a solution that totals 100 mL. Use a 100 ml volumetric flask to make one batch or a 200 ml volumetric flask to make a double batch.

- An alternative is to use 10 ml of 3% hydrogen peroxide and bring to a 100 ml volume by adding distilled water. 3% hydrogen peroxide is readily available from your local drug store.
- Hydrogen peroxide decomposes quickly at room temperature so store the 30% or 3% solutions in the refrigerator until needed. It is best to make up the 0.3% solution as fresh as possible before use.

Phosphoric Acid 25%:

- Add 294 mL of 85% phosphoric acid to a one-liter flask. Add distilled water to bring to the one-liter volume. 25% solution already made up is available from PIWC.

Sulfur Dioxide Indicator:

- Into 100 ml of 50% ethyl alcohol, dissolve 0.1 gram of methyl red and 0.05 gram of methylene blue.
- Ready make solution is available in small quantities from PIWC.

Sodium Hydroxide (NaOH) 0.01 Normal: Also written as N/100 or 0.01 N or 1/100N.

- Put 10 mL of 1/10 Normal ***(0.1N)*** NaOH into a 100 mL volumetric flask and add distilled water to bring the flask to volume.
- NaOH can stray from its intended normality level especially with higher exposure to oxygen in the air. There is potassium acid phthalate *(or hydrochloric acid & methyl red)* available from PIWC that can be used to recalibrate the normality of NaOH. Or, fairly regularly repurchase a new batch of NaOH.

Following Are Two Formulas That May Be Of Value:

For Normality - Amount To Add = $\frac{\text{Normality Desired * Volume Desired}}{\text{Normality of the Concentrated Solution}}$

For Example – $\frac{0.01N * 100 \text{ ml}}{0.1N}$ = 10 ml of 0.1N NaOH added to a 100 ml flask, brought to volume with distilled water will make 0.01N NaOH.

For Percentage – Volume To Add = $\frac{\text{\% Desired * Total Volume Desired}}{\text{\% Of The Concentrated solution}}$

For Example – $\frac{0.3\% * 100 \text{ ml}}{30\%}$ = 1 ml 30% hydrogen peroxide added to a 100 ml flask, brought to volume with distilled water will make 0.3% hydrogen peroxide.