**Wine Analysis Lab I: The Color of Red Wines and Beer’s Law**

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**Introduction**

Since the advent of settled, communal living, getting enough clean, uncontaminated water to drink has been a high priority for human beings. We, recipients of the 19th C revolution in public health and sanitation, tend to forget that for most of history the best way to stay hydrated was by drinking fermented beverages: wine, beer or mead. The drinks are pathogen-free, and their acidity and alcohol kill off most microorganisms in the local waters that used to dilute them. Better still, such libations provide a way to store necessary calories and nutrients from foodstuffs that would otherwise spoil. Finally, the mind-altering properties of ethanol exert a magical effect on human bodies and imaginations. Fermented beverages appear in mythology, ritual, medical practice, and economic structures of very early human societies.

Wine continues to be popular. In 2010, 330 million cases of wine were sold in the US at an estimated retail value of $30 billion, while in Washington State, wine sales exceeded $600 million. California leads the nation in the production of premium wines, while the wine industry in Washington is in second place.

**Purpose**

The purpose of this lab is to explore some of the complex mixture of chemicals that cause wine to look, smell, taste and feel delectable to a large percentage of humans. Phenolic compounds, the primary flavoring and coloring agents in red wines, are bactericidal, antioxidant, and beneficial to the cardiovascular system. The phenolics originate from grape skin or woody parts of vines, and contain a myriad of covalently bound species, as well as colloidal aggregates that are poorly understood. The profile of phenolic compounds changes considerably with fermentation and aging techniques, which is why winemaking is still a prodigious art.

Phenol is the hydroxylated benzene with this structure, depicted as a modified line-angle drawing:

![Phenol Structure Diagram](image)

Phenolics are any structure where the hydrogens on the five non-hydroxylated carbons of the benzene ring are occupied by a dizzying variety of organic structures. The four major groups of phenolics include phenolic acids, flavanoids, anthocyanins, and tannins.

Phenolic acids are the carboxylic acid derivatives of phenol:

![Phenolic Acid Structure Diagram](image)

Any of the unoccupied carbons can be substituted. These molecules contribute to the low pH of wine. While the acids themselves are mostly tasteless, some of them are precursors to volatile phenolics produced by microorganisms, and some coveted, flavorful derivatives of the acids are achieved by toasting the wood in the barrels that wine is aged in.

The flavanoids are yellow-hued derivatives of flavone, where R and R₁ are substituents.

![Flavanoid Structure Diagram](image)

A related compound is the major red pigment in wine, malvidin-3-monoglucoside, an anthocyanin.
The chromic properties of malvidin-3-monoglucoside are pH reactive and unstable. It goes from bright red at low pH (the flavylium cation shown above) to murky blue at high pH (the quinoidal base, which is uncharged). Furthermore, the sugar molecule at the lower right of the structure (arrow) can be replaced with sulfur dioxide, bleaching the structure. Complex anthocyanin polymers also contribute color in wine, but their structures are poorly understood. They are resistant to bleaching, so that any color left over after SO₂ treatment is due to these large polymers.

Tannins are mostly brownish in color; tannins impart the characteristic color of black tea. Tannins are defined by their ability to form covalent bonds with large molecules, such as polysaccharides and proteins. The structural diversity of tannins produced in wine is huge, as phenolic compounds polymerize together and gain and lose substitutions. Tannins are responsible for some of the wine flavor and mouth feel, as they will react with glycoproteins in saliva.

**Beer’s Law**

Beer’s law is a simple, useful equation. It provides a direct relationship between the concentration of a compound in a sample and the amount of light that sample absorbs:

\[ A = abc \]

where \( A \) = the absorbance, \( a \) = the molar absorptivity constant (a characteristic of a particular compound at a particular wavelength), \( b \) = the path length, and \( c \) = the molar concentration of the compound. The greater the absorbance, the greater the concentration of the colored compound(s).

Depending upon the chemical structures in the compound (or mixture of compounds), the absorbance of particular wavelengths will be more or less pronounced. A compound will absorb at a particular wavelength if that wavelength possesses the energy necessary to move an electron from the ground state to an excited state. The remaining wavelengths of visible light are transmitted, or pass through, a sample, and the sum of these wavelengths gives rise to the color you see. (See Ch. 24 in the textbook for an explanation of the artist’s color wheel if you want more on this subject.)

Since wine is such a complex mixture, we will not be using Beer’s law to calculate directly the concentrations of single compounds. Nevertheless, we can use absorbance as a rough guide to the relative amounts of pigment classes. In this lab, the absorbances at 420, 520, and 620 nm are used as a qualitative measure of the phenolic compounds that give the characteristic coloration to red wines. Some vintages are brighter or darker, some are more ruby or more garnet. If you were performing these assays in a winery setting, you would likely have historical guidelines for your particular product, and you would follow the absorbances through different stages of the winemaking process to monitor wine quality. The data we will collect correspond to the phenolics given below.

- \( A_{420} \) Yellow or brown pigments (flavanoids and tannins; some anthocyanins)
- \( A_{520} \) Red pigments (mostly anthocyanins)
- \( A_{620} \) Blue pigments (mostly anthocyanins)

A few convenient manipulations of the data give the density and hue of the wine. Wines with color density of 0 to 6 are classified as lightly colored, those with color densities of 6 to 10 are medium colored, and those with color densities of above ten are considered to be deep red wines. Generally speaking, the deeper the red color, the more likely the wine is to have compounds that are valuable for the taste and smell they impart to full bodied wines. The hue indicates the relative contributions of yellow/brown and red to the color of the wine; the smaller the hue value, the more reddish the wine.

\[ \frac{A_{420} + A_{520} + A_{620}}{A_{520}} \quad \text{Color density at the natural pH of the wine} \]

\[ \frac{A_{420}}{A_{520}} \quad \text{Color hue at the natural pH of the wine} \]

Chemical treatment of the wine reveals a bit about the state of the pigment molecules. Addition of HCl converts anthocyanin structures that are more blue or yellow at the natural pH of the wine into pigments that are red at lower pH.
The sulfur dioxide—produced by sodium metabisulfite—bleaches any monomeric (single-unit) anthocyanins. Any color left after this treatment is due to polymerized phenolics.

\[ A_{520}^{HCl} \]  
Total red pigments

\[ A_{520}^{SO_2} \]  
Polymeric anthocyanins

Finally, you will decolorize your wine sample in order to prepare for next week’s lab.

**Procedure**

**A. Preparation of Samples**

a. Using a graduated cylinder, draw 100 mL of your denatured wine sample into a flask. Mark the name of the sample on your report sheet (e.g. Zin, Young Red, Blush, etc.)

b. Mark a large test tube with “HCl”. Using a serological pipet, add 10 mL 1 M HCl to this test tube. Using a plastic transfer pipet, add 3 drops of your wine sample. Cap with some Para film, invert 3x, and leave for 90 minutes before measuring the absorbance. While this is sitting, you can complete the rest of the lab.

c. Mark a medium test tube with “Wine 1:10”. Using the buret in the front of the room, add 9.00 mL di water to the test tube. Using a clean 5 mL serological pipet, pipet 1.00 mL of wine into the test tube. Cover with Para film and invert 3x.

d. Mark a medium test tube with “SO\(_2\)”. With a serological pipet, add 5 mL wine to this test tube. With a plastic transfer pipet, add 3 drops of 25\% Na\(_2\)S\(_2\)O\(_5\) (sodium metabisulfite). Cover with Para film and invert 3x.

e. Mark a medium test tube with “SO\(_2\) 1:10”. Using the buret in the front of the room, add 9.00 mL di water to the test tube. Using your 5 mL serological pipet that you’ve used for wine, pipet 1 mL of the sample in the “SO\(_2\)” test tube into the “SO\(_2\) 1:10” tube. Cover with Para film and invert 3x.

f. Mark a medium test tube with “NaOH”. You will need to fill this test tube with a deep red wine sample, so if you have “Blush” as your unknown, get a sample from some other lab group of the Zin or Young Wine. Using your 5 mL serological pipet, pipet 2 mL of wine into this test tube and set aside.

**B. Check the Specs.**

a. Make certain the Spec-20s have been turned on for at least 30 minutes.

b. If there is a Spec-20 free, you may analyze your samples (Section C). If there is not a Spec-20 free, proceed to the Wine Decolorizing step (Section D), and the Chroinic Properties of Malvidin-3-monoglucoiside (Section G). Return to analyze your samples later.

**C. Measuring Absorbances of Wine Samples**

a. Obtain 4 of the special test tubes (also called “cuvettes”) for use in the spectrophotometer. They should be clean, but rinse them out with di water a couple times. DO NOT rinse with acetone to dry. Shake them as dry as you can.

b. Fill these test tubes 2/3 of the way full. When you take your readings, always line up the little line on the cuvette with the indentation in the instrument.

c. Fill the cuvettes with di water, wine 1:10, SO\(_2\) 1:10, and—if 90 minutes have transpired—HCl.

d. \( A_{420} \) readings

i. Using the top right knob, set wavelength to 420

ii. Use the Mode button to set the instrument to Transmittance

iii. Make certain there are no cuvettes in the instrument

iv. Using the lower left knob, set the instrument to read 0% transmittance

v. Insert the blank (di water cuvette). Use a Kimwipe to make sure no fingerprints are on the cuvette. Line up the line on the test tube with the indentation on the instrument.

vi. Using the lower right knob, set the instrument to read 100% transmittance

vii. Remove the blank.

viii. Push the Mode button to set the instrument to Absorbance
ix. Insert the “wine 1:10 sample” after wiping the outside with a Kimwipe.

x. Read the absorbance of the sample and record this on the Report page.

e. $A_{520}$ readings
   i. Using the top right knob, set wavelength to 520
   ii. Use the Mode button to set the instrument to Transmittance
   iii. Repeat steps iii through x, above, for the “wine 1:10” sample
   iv. Repeat steps ix and x for the “SO$_2$ 1:10” sample
   v. If 90 minutes have passed since you prepared your HCl sample, repeat steps ix and x for the “HCl” sample. (If not, you will skip this step for now, and you’ll do E, below, later.)

f. $A_{620}$ readings
   i. Using the top right knob, set wavelength to 620
   ii. Use the Mode button to set the instrument to Transmittance
   iii. Repeat steps e. iii through x, above, for the “wine 1:10” sample

D. pH Measurement and Wine Decolorizing
   a. Before you decolorize your wine, measure the pH of your wine sample. Use the pH meters and the LabQuests, making sure to rinse your pH probe with di water before and after analysis of your sample. Record the pH on the Report sheet.
   b. Weigh out ~10 g of decolorizing charcoal into a plastic weigh boat on the balance. Please use care—the decolorizing charcoal is very finely divided and floats all over. Clean the balance with a brush when you are finished.
   c. Add 10 g decolorizing charcoal to the wine in your flask. Stir with a stir rod. Let sit for 5 minutes, swirling occasionally.
   d. Rinse out a Buchner funnel flask several times with di water. Place a filter in the funnel and attach the tubing to the aspirator.
   e. With the water running on high, drip a little water onto the filter so that it adheres well. Swirl the flask of wine plus charcoal, and transfer into the filter funnel.
      i. The decolorized wine will collect in the sidearm flask, and it may look a bit cloudy—no worries.
   f. When you’ve filtered all of your wine, disassemble the Buchner funnel, and transfer the decolorized wine to a new flask. Cover with Para film and store in your lab drawer for next week.
   g. Clean the Buchner funnel and sidearm flask VERY WELL with soapy water and a test tube brush. Rinse all components well with di water.

E. $A_{520}$ reading for the HCl sample
   a. If you did perform C.e.v. above, skip to F. If you did not perform step C.e.v. above, you will need to place some of your HCl sample in a cuvette.
   b. You will likely need to recalibrate the Spec-20 if other groups have used it.
      i. Using the top right knob, set wavelength to 520
      ii. Use the Mode button to set the instrument to Transmittance
      iii. Make certain there are no cuvettes in the instrument
      iv. Using the lower left knob, set the instrument to read 0% transmittance
      v. Insert the blank (di water cuvette). Use a Kimwipe to make sure no fingerprints are on the cuvette. Line up the line on the test tube with the indentation on the instrument.
      vi. Using the lower right knob, set the instrument to read 100% transmittance
      vii. Remove the blank.
      viii. Push the Mode button to set the instrument to Absorbance
      ix. Insert the “HCl” sample after wiping the outside with a Kimwipe.
      x. Read the absorbance of the sample and record this on the Report page.
F. Clean all cuvettes
   a. Dump the contents down the drain
   b. Add ~2 mL of detergent and cap with your finger or thumb
   c. Rinse 2x with tap water
   d. Rinse 2x with di water
   e. Store in rack near the Spec-20

G. Chromic Properties of Malvidin-3-monoglucoside
   a. To your “NaOH” test tube, count as you add drops of 1 M HCl, with mixing, until the color turns bright red. Record the number of drops it took to turn the malvidin-3-monoglucoside to the flavylium cation.
   b. To the same test tube, add the same number of drops of 1 M NaOH that you added of HCl in step a. You will notice that as the acid is neutralized, the color turns back to normal wine color. Describe this color on your report sheet (unleash your inner wine blogger).
   c. Keep adding drops of 1 M NaOH, with mixing, until the color turns murky blue. Record the number of drops it took to turn the malvidin-3-monoglucoside to the quinoidal base.

H. Finish your calculations
   a. Convert all absorbances to what they would be if undiluted, as indicated in the Report sheet.
   b. Express the results as indicated on the Report sheet, using the dilution-corrected values for all absorbances (the ones in the right-hand column).

Materials for each group of 24 students

   Equipment:
   Spec 20s, turned on for 30 minutes
   2 burets filled with d.i. water
   5 mL serological pipets
   10 mL serological pipets
   green pipet fillers
   Buchner funnels and Whatman #1 filters
   pH meters, calibrated

   Chemicals:
   200 mL 1 M HCl, divided into 2 bottles
   1 M HCl in dropper bottles
   1 M NaOH in dropper bottles
   decolorizing carbon/activated charcoal
   Wine samples, denatured with added methanol

Bibliography


Acknowledgments
Special thanks to Tim Donohue, WWCC Enology Instructor and Winemaker
Wine Analysis I Prelab Questions

Name________________

1. Write the characteristic color and name of the compound next to its structure:

   a. 
   
   b. 
   
   c. 

2. On the structure that corresponds to Malvidin-3-monoglucoside, circle the part of the molecule that gets replaced by SO₂ in the bleached sample.

3. How does the characteristic color of malvidin-3-monoglucoside change with pH?

4. At each wavelength, the absorbance measures a particular group of colored pigments. Write the wavelength you use to measure each color:

   a. Yellow-brown
   
   b. Red
   
   c. Blue

5. In the next experiment, we will be titrating wine samples for the concentration of reducing sugars using a rather complicated regime of indicators. Why do you suppose we need to decolorize the wine sample?
Wine Analysis I Report Sheet

Name ___________________________  Lab Partner(s):

C163, S11

Name of sample ________________________

Natural pH of wine sample ________________________

<table>
<thead>
<tr>
<th>Wine 1:10</th>
<th>Absorbance x 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_{420}</td>
<td>________</td>
</tr>
<tr>
<td>A_{520}</td>
<td>________</td>
</tr>
<tr>
<td>A_{620}</td>
<td>________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SO\textsubscript{2} 1:10</th>
<th>Absorbance x 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_{520}</td>
<td>________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HCl</th>
<th>Absorbance x 100 (yes, 10^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_{520}</td>
<td>________</td>
</tr>
</tbody>
</table>

In the following calculations, use the dilution-adjusted absorbances

\[
\text{Color density at the natural pH of the wine} \quad A_{420} + A_{520} + A_{620} = \_\_\_\_\_\_\_\_\_
\]

\[
\text{Color hue at the natural pH of the wine} \quad \frac{A_{420}}{A_{520}} = \_\_\_\_\_\_\_\_\_\_
\]

\[
\text{Degree of red pigment coloration (\%)} \quad \frac{A_{520}^{\text{HCl}}}{A_{520}^{\text{SO}_2}} \times 100 = \_\_\_\_\_\_\_\_\_\_
\]

\[
\text{Total red pigments} \quad A_{520}^{\text{HCl}} = \_\_\_\_\_\_\_\_\_\_
\]

\[
\text{Percent of polymeric anthocyanins} \quad \frac{A_{520}^{\text{SO}_2}}{A_{520}} \times 100 = \_\_\_\_\_\_\_\_\_\_
\]

Malvidin-3-monoglucoside

# drops of HCl added to change to bright red \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Color after adding equal number of drops of NaOH \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# drops of extra NaOH added to change to murky blue \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_