CHEMISTRY OF WHITE WINE

Adapted with permission from the University of Illinois; Revised 2013.

INTRODUCTION

The fermentation of fruit juice producing alcoholic beverages is one of the oldest ways in which man has used chemistry to serve himself. Today, the manufacture of wines is a multi-million dollar industry in which chemistry plays a central role.

The process of wine making begins with growing and harvesting the fruit (usually grapes). Next the juice, called must, is extracted from the grapes. The must is the reagent for the fermentation process. Fermentation is an oxidation carried out in an anaerobic (no oxygen) environment by microorganisms that grow and thrive by breaking down sugars. Yeasts are the most common microorganisms used to carry out the fermentation process, but there are many other species, primarily bacteria, which can also cause fermentation.

The end products of yeast fermentation are carbon dioxide and ethyl alcohol (ethanol):

$$\text{Yeast} + C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

where $C_6H_{12}O_6$ is the molecular formula for a sugar, fructose, and its many isomers, including glucose and galactose.

The wine making process has been refined over the centuries. There are many schools of thought on the proper length of time to ferment the wine, the temperature at which the fermentation should be carried out, the length of time the wine should age, the kind of container in which the wine should age, the position in which it should age, the final alcohol content, the final sugar content, etc.

In addition to general properties, certain wines have special characteristics. Champagne and other "sparkling" or bubbly wines undergo a second fermentation after being bottled. The CO_2 given off in this second fermentation is unable to escape and is preserved as "sparkle". Some wines, notably the ports, sherries, and Madeiras, have higher alcohol contents than the average table wine because brandy or some other distilled spirit with a higher alcohol percentage has been added. Some particularly sweet dessert wines are made from grapes that have been permitted to rot while still on the vine. The grapes are attacked by fungus, *Botrytis cinerea*, which drastically reduces the liquid content of the grape but leaves the amount of sugar unchanged.

Investigation of the Components of Wine by Chemical Methods

Commercial wine making today is a scientific process. All the steps of fermentation are rigidly controlled, and the final product is subject to numerous regulations as to alcohol content, sugar content, acidity, percent of each kind of grape which can be used in its production, amount of preservatives, etc.

In this experiment, you will analyze three components of wine: alcohol content, acidity, and sufur dioxide content.

PART A. Determination of Alcohol Content

Ethanol and certain other alcohols react with ammonium hexanitrato cerium(IV), $(NH_4)_2Ce(NO_3)_6$, to yield a corresponding carboxylic acid and a cerium(III) complex. The reaction forms a deep red intermediary complex as the alcohol is oxidized. This colored species obeys Beer's Law, meaning that the absorbance of light by the colored substance is directly proportional to the concentration of the substance in solution:

$$A = \varepsilon l c$$

where A = absorbance
ε = molar absorptivity
l = path length of cell
c = concentration

A Beer's Law plot of absorbance vs. concentration of the red complex, called a calibration curve, allows for the determination of the quantity of alcohol oxidized by ammonium hexanitrato cerium(IV). By measuring the absorbance of the complex formed, the percent alcohol in a sample can be determined from the calibration curve.

PART B. Acid Content Analysis

In order to determine the acidity of the wine, you will need to perform a titration. The acidity in wine is primarily due to tartaric acid, a dicarboxylic acid with the chemical formula:

HOOCCHOHCHOHCOO**H**

Tartaric acid, $H_2C_4H_4O_6$, is a weak diprotic acid with two acidic protons; these two acidic protons have been highlighted in the chemical formula above. Because tartaric acid has two acidic protons, neutralization of each mole of this acid will require two moles of NaOH base:

$$H_2C_4H_4O_6 + 2\ NaOH \rightarrow Na_2C_4H_4O_6 + 2\ H_2O$$

PART C. Sulfur Dioxide Analysis

The sulfur dioxide concentration will also be determined by titration; however the reaction is a little different from what you have seen before. Keep in mind that the technique is the same, a titration, and the quantitative information obtained in this case is similar.

Sulfur dioxide, SO_2, is used commercially as a preservative in wines to protect the wine from bacteriological growth and subsequent infection. Sulfur dioxide is generated by the addition of potassium metabisulfite to the slightly acidic must:

$$K_2S_2O_5 + H_2O \rightarrow 2\ KHSO_3$$

$$KHSO_3 + H_3O^+ \rightarrow SO_2 + 2\ H_2O + K^+$$

The preservative's concentration is strictly regulated because the added sulfites and the SO_2 produced from the reaction can be harmful to certain people. Legal limits vary from country to country, from 200-450 mg/L. Much of the added SO_2 combines with other components of wine leaving about 20-40 mg/L free in solution to protect the product from spoilage.

In the laboratory, <u>the concentration of free SO_2</u> can be determined by the following reaction:

$$SO_2(aq) + I_2(aq) + 2H_2O(1) \rightarrow 2\ I^-(aq) + SO_4^{2-}(aq) + 4\ H^+(aq)$$

An aqueous solution of iodine is reddish-brown, whereas the products of the reaction are colorless. As iodine is added, the color of the wine will remain unchanged until all the sulfur dioxide is consumed. As soon as iodine is present in excess, the following reaction occurs:

$$I_2 + I^- \rightarrow I_3^-$$

The triiodide ion is slightly yellow. However, if starch is added, a dark blue-black starch-triiodide complex forms and serves as an endpoint indicator:

$$I_3^- + \text{starch} \rightarrow \text{blue complex}$$

To measure <u>the total concentration of SO_2</u>, sodium hydroxide is added to the wine to break down bisulfite complexes. Then it is acidified and titrated using the same technique.

LABORATORY PROCEDURE

"White" wine samples are provided, and some reagents are set out in pump dispensers. Analyze the brand of wine assigned by your instructor.

PART A. Measuring the Alcohol (ethanol) Content of Wine

1. Double click the "Logger Pro" icon and allow the screen to open.

2. The Spectrometer needs to be powered for about 5 minutes before using so do this step before preparing your solutions. Do not use the Go!Link with the spectrometer. Plug the Spectrometer via provided USB cable to the computer USB port.

3. Prepare Dilutions of the Stock Ethanol Solution:

   a. Prepare and load a 25 mL buret to deliver the volumes of ethanol stock solution (10% by volume, % v/v) shown in the table below. Cap the buret to reduce evaporation of ethanol.

   b. Load a 50 mL buret with deionized (DI) H_2O.

   c. Clean and dry eight labeled large test tubes (TT). Deliver the volumes given below into the TT. Mix well.

TT	Stock Ethanol soln (mL)	DI H_20 (mL)	Final Ethanol concentration (%v/v)
A1	0.0	10.0	0
B1	1.0	9.0	1.0
C1	2.0	8.0	2.0
D1	3.0	7.0	3.0
E1	4.0	6.0	4.0
F1	5.0	5.0	5.0
G1	6.0	4.0	6.0
U1	1.0 mL wine	9.0	Unknown

4. Prepare Standard Ethanol Solutions

   a. Clean and dry a second set of eight large test tubes (TT) and label them A2, B2, ….

   b. Rinse the pipet with about 1.0 mL of the contents of TT A1 and discard. Carefully, pipet 1.0 mL of the contents of TT A1 into TT A2. Repeat this process for the rest of the solutions.

   c. Add 5.0 mL of color reagent from the pre-set pump dispenser to each TT in this second set. Mix well. Fold a sheet of paper over the TT to protect the contents. The contents of TT A2 will now be used as the blank in the calibration of the spectrometer as well as in the generation of the calibration curve. The contents of TT B2 will be the most dilute solution.

d. Let the color develop for 5 minutes then immediately begin making absorbance readings. Read over steps 5 and 6 during this 5 minute wait. The solutions in this second set of test tubes are the solutions that will be used in the remainder of this experiment.

5. Calibrate the Spectrometer ; Do not unplug the spectrometer during this experiment or you will have to start over.

    a. Return to the Logger Pro screen on the computer. Click: Experiment ; Calibrate ; Spectrometer:1.

    b. Allow the lamp to warm up for 90 seconds as displayed on the computer screen.

    c. Only touch the ridged faces of the cuvette, never touch the clear faces. Rinse and fill one cuvette (about ¾ full) with the blank (TT A2). Rinse and fill a second cuvette with the most dilute solution (TT B2). **Gently blot** (don't scratch the sides of the cuvette) any drips on the outside of the cuvette with a Kimwipe. These cuvettes will be used again in the next few steps so keep them handy and keep track of what the contents are in each.

    d. Place the cuvette containing the blank in the spectrometer so that one of the clear sides is aligned with the white arrow at the top of the cuvette slot. Click: "Finish Calibration" ; OK. Remove the cuvette from the spectrometer but keep it handy.

6. Determine the Wavelength of Maximum Absorbance

    a. Place the cuvette containing the most dilute solution in the spectrometer. Click: Experiment ; Data Collection ; Full Spectrum ; Done. Click the rainbow icon labeled "Absorbance=…" in the upper left hand corner of the window. Change the "Wavelength Range" to 380-750nm. Close this box by clicking the "x" in the upper right hand corner of the window.

    b. Click the small green triangle in the toolbar labeled "Collect". After the line graph appears on the screen, click the small red square in the toolbar labeled "Stop".

    c. To automatically store the maximum wavelength go to the toolbar and select: Experiment ; Store Latest Run.

7. Generate the Calibration Curve

    a. In the toolbar click: Experiment ; Data Collection ; choose Events with Entry in the Mode box. Highlight the word Event in the Column Name box and replace it with Concentration. Put percent in the Units box. Clear "Short Name". OK. You are ready to begin collecting data. Remove the cuvette from the spectrometer but keep it handy.

    b. Place the cuvette containing the blank back in the spectrometer. Click the begin data collection button (triangle) in the toolbar labeled "Collect". When the absorbance reading stabilizes, click the KEEP button located in the toolbar just to the right of the Red Stop Button. Type in the concentration of the solution that is in the cuvette (don't include units). OK.

    c. Working in order of most dilute (you already have a cuvette containing the most dilute solution) to most concentrated of the standard solutions (not the unknown), rinse and then fill a cuvette with the solution that will be analyzed. Place the cuvette in the

spectrometer. When the absorbance reading stabilizes, click the KEEP button (be careful that you **don't** accidentally click the stop data collection button (square)), and enter the concentration of the analyzed solution. Repeat until the absorbance of each solution has been determined.

Pour your samples back into the appropriate TT (second set) after you have measured each absorbance and discard them in the waste container only after you have acceptable data.

d. When the absorbance of all standard solutions (not the unknown) has been measured, click the stop data collection button (square) located in the toolbar.

e. To determine the equation of the line for your calibration curve click: Analyze ; Linear Fit. A box should appear with the equation and a correlation.

To receive full credit for this lab your calibration curve must be a good, straight-line graph, with a correlation coefficient of 99% or better (Corr: on the screen reads 0.9900 or greater). You should repeat the experiment until you get this proficiency.

Work carefully. If you need to repeat the experiment, you still must be done with the write up and post-lab questions before the end of the lab period.

8. Each lab partner's report must have a Logger Pro generated printout of the calibration curve attached to it. The printout must show the graph, the information needed to generate the equation (slope and intercept) for the line, and the correlation reading. To do this click: File ; Print. Uncheck the "Print Visible Spectrum on Wavelength Graphs" and change the "orientation" to landscape under properties. Be sure that the names of all lab partners are entered in the "Name" section and that the date box is checked.

9. Rinse and fill a cuvette with your assigned wine sample from Step 4 (TT U2). Place the cuvette in the spectrometer. Record the absorbance in your lab report once the reading has stabilized.

10. When you are done, return your bin to Lab Services.

PART B. Determining the Acid Content of Wine

1. Clean a 50-mL buret and rinse it twice with the standard (0.05 M) NaOH solution. Load the buret with the same NaOH solution and prepare to titrate.

2. Pipet 10.0 mL of the white wine into a conical flask. Add about 40 mL of DI water, and 3-4 drops of phenolphthalein. Titrate the sample. The endpoint is characterized by a persistent faint pink color.

3. Repeat the titration with a second sample of wine. Adjust the sample size if necessary. Repeat the titration a third time.

4. Measure the pH of your wine sample by adding a drop of wine to a piece of pH indicator paper. Record this value in your laboratory report.

PART C. Determining the available SO_2 concentration (C.1) and the total SO_2 concentration (C.2).

C.1 Determining the Concentration of **available (free) SO_2**

Pipet 20.0 mL (2 x 10.0 mL) of wine into a conical flask. Add 4 mL of 6 M H_2SO_4 and 3 mL starch indicator. Perform a quick titration with standard iodine solution to determine the approximate volume of I_2 needed to oxidize the SO_2. It may be necessary to titrate more or less wine to get a suitable titer of iodine solution.

The end of this titration is the appearance of a dark blue color that persists for 2 minutes. Oxidation by air will then occur and the color will fade and disappear. DO NOT ADD MORE IODINE. Titrate two more samples, or until you get consistent data. Adjust the sample size if necessary.

C.2 Determining the Concentration of **total (free and combined) SO_2**

Measure 10.0 mL of wine into a conical flask. Add 7.5 mL of 1 M NaOH. Swirl the flask and wait 15 minutes. Add 4 mL of 6 M H_2SO_4 and 3 mL starch indicator. Titrate to the same end point as in part C.1 and collect consistent data. Adjust the sample size if necessary.

Specific Gravity (use as density) of Aqueous Solution Ethanol at 15ºC

Specify Gravity	% Alcohol by Volume	Specify Gravity	% Alcohol by Volume	Specify Gravity	% Alcohol by Volume	Specify Gravity	% Alcohol by Volume
1.00000	0.00	0.99417	4.00	0.98897	8.00	0.98435	12.00
0.99984	0.10	0.99403	4.10	0.98885	8.10	0.98424	12.10
0.99968	0.20	0.99390	4.20	0.98873	8.20	0.98413	12.20
0.99953	0.30	0.99376	4.30	0.98861	8.30	0.98402	12.30
0.99937	0.40	0.99363	4.40	0.98849	8.40	0.98391	12.40
0.99923	0.50	0.99349	4.50	0.98837	8.50	0.98381	12.50
0.99907	0.60	0.99335	4.60	0.98825	8.60	0.98370	12.60
0.99892	0.70	0.99322	4.70	0.98813	8.70	0.98359	12.70
0.99877	0.80	0.99308	4.80	0.98801	8.80	0.98348	12.80
0.99861	0.90	0.99295	4.90	0.98789	8.90	0.98337	12.90
0.99849	1.00	0.99281	5.00	0.98777	9.00	0.98326	13.00
0.99834	1.10	0.99268	5.10	0.98765	9.10	0.98315	13.10
0.99819	1.20	0.99255	5.20	0.98754	9.20	0.98305	13.20
0.99805	1.30	0.99241	5.30	0.98742	9.30	0.98294	13.30
0.99790	1.40	0.99228	5.40	0.98730	9.40	0.98283	13.40
0.99775	1.50	0.99215	5.50	0.98719	9.50	0.98273	13.50
0.99760	1.60	0.99202	5.60	0.98707	9.60	0.98262	13.60
0.99745	1.70	0.99189	5.70	0.98695	9.70	0.98251	13.70
0.99731	1.80	0.99175	5.80	0.98683	9.80	0.98240	13.80
0.99716	1.90	0.99162	5.90	0.98672	9.90	0.98230	13.90
0.99701	2.00	0.99149	6.00	0.98660	10.00	0.98219	14.00
0.99687	2.10	0.99136	6.10	0.98649	10.10	0.98209	14.10
0.99672	2.20	0.99123	6.20	0.98637	10.20	0.98198	14.20
0.99658	2.30	0.99111	6.30	0.98626	10.30	0.98188	14.30
0.99643	2.40	0.99098	6.40	0.98614	10.40	0.98177	14.40
0.99629	2.50	0.99085	6.50	0.98603	10.50	0.98167	14.50
0.99615	2.60	0.99072	6.60	0.98592	10.60	0.98156	14.60
0.99600	2.70	0.99059	6.70	0.98580	10.70	0.98146	14.70
0.99586	2.80	0.99047	6.80	0.98569	10.80	0.98135	14.80
0.99571	2.90	0.99034	6.90	0.98557	10.90	0.98125	14.90
0.99557	3.00	0.99021	7.00	0.98546	11.00	0.98114	15.00
0.99543	3.10	0.99009	7.10	0.98535	11.10		
0.99529	3.20	0.98996	7.20	0.98524	11.20		
0.99515	3.30	0.98984	7.30	0.98513	11.30		
0.99501	3.40	0.98971	7.40	0.98502	11.40		
0.99487	3.50	0.98959	7.50	0.98491	11.50		
0.99473	3.60	0.98947	7.60	0.98479	11.60		
0.99459	3.70	0.98934	7.70	0.98468	11.70		
0.99445	3.80	0.98922	7.80	0.98457	11.80		
0.99431	3.90	0.98909	7.90	0.98446	11.90		