WIZARDS OF THE WINERY
CHEMISTRY OF WHITE WINE

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INTRODUCTION

The fermentation of fruit juice to yield alcoholic beverages is undoubtedly 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 then 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 the 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). Other types of fermentation yield lactic acid; alcohols such as methanol, and 2,3-butanediol; carboxylic acids such as acetic, propanoic, butanoic, and heptanoic; and less common substances, including acetoin and diacetyl. In any case, yeast or bacteria carry out fermentation to produce energy for living, reproduction, and growth. The other products, in addition to energy, for the fermentation reaction by yeast, are carbon dioxide and alcohol.

A simple representation of the chemical equation for fermentation is:

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

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 much 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, hence, 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. Thus the application of chemistry to wine making is not a trivial consideration.

In this experiment, you will analyze three components of wine. In this lab you are responsible for putting together your own report sheet.

PART A. 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 bee highlighted in the chemical formula above. Because tartaric acid is has two acidic protons, neutralization of each mole of this acid will require two moles of NaOH base. Here is the neutralization reaction

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

PART B. 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, the concentration of free SO_2 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 the total concentration of SO_2, sodium hydroxide is added to the wine to break down bisulfite complexes. Then it is acidified and titrated using the same technique.

PART C. 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:

$$A = \varepsilon lc$$

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

This direct proportionality will be used to determine the percent by weight of alcohol in the wine. A Beer's Law plot of absorbance vs. concentration of the red complex allows direct determination of the quantity of alcohol oxidized by ammonium hexanitrato cerium(IV). By measuring the absorbance of the complex formed by the wine, the percent alcohol can be read from a computer-generated calibration graph.

LABORATORY PROCEDURE

"White" wine samples are provided, and some reagents are set out in pump dispensers. Analyze a brand of wine assigned by your instructor. We will compare a few different commercial wines as a class project.

PART A. Determining the Acid Content of Wine

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

A.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.

A.3 Repeat the titration with a second sample of wine. Adjust the sample size if necessary. If the results from your two trials are consistent, then you may proceed with the next section; otherwise, repeat the titration a third time.

A.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 B. Determining the available SO_2 concentration (B.1) and the total SO_2 concentration (B.2).

B.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. Add less than 1 g of solid KI to the flask and let it dissolve. 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 which persists for 2 minutes. Oxidation by air will now occur and the color will fade and disappear. DO NOT ADD MORE IODINE. Titrate at least two more samples, or until you get consistent data. Adjust the sample size if necessary.

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

Measure 15.0 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 6 M H_2SO_4 and 3 mL starch indicator, and less than 1 g of KI. Titrate to the same end point as in part B.1 and collect consistent data. Adjust the sample size if necessary.

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

The first thing that you need to do is prepare a set of standard solutions, via dilution, that have **known** alcohol contents. You then can "compare" your assigned wine sample in order to determine the alcohol content.

C.1 Preparing Dilutions of the Stock Ethanol Solution (10 % aqueous v/v)

1. Prepare and load a 25 mL buret to deliver the volumes of ethanol stock solution shown in the Table below. Cap the buret to reduce evaporation of ethanol.

2. Load a 50 mL buret with DI H_2O.

3. Have eight clean, dry , labeled TT ready to receive the solutions. Deliver each volume into the TT. Mix well. Transfer each prepared dilution to a clean dry test tube.

4. Pipet 1.0 mL of any one variety of wine into tube U

Tube	Stock Ethanol soln (mL)	DI H_20 (mL)	Final Ethanol con (% v/v)
A	0.0	10.0	0
B	1.0	9.0	1.0
C	2.0	8.0	2.0
D	3.0	7.0	3.0
E	4.0	6.0	4.0
F	5.0	5.0	5.0
G	6.0	4.0	6.0
U	1.0 mL wine	9.0	Unknown

C.2 Preparing Standard Solutions of Ethanol

1. Carefully pipet 1.0 mL of each dilution that you prepared into another set of clean, dry test tubes. Start with the most dilute solution (A). Rinse the pipet with about one mL of solution (B) before taking 1.0 mL of B for further use. Discard the rinse every time.

2. Add 5.0 mL of color reagent from the pre-set pump dispenser to each tube in this second set. Mix well. Fold a sheet of paper over the tubes to protect the contents.

3. Let the color develop for 5 minutes. Read the absorbances immediately thereafter **using the PSL Photometer to take the absorbance readings and produce your standard curve.**

4. After completion of the standard curve, take a photometer reading of your assigned wine sample in order to determine the alcohol content.

5. Be sure to print out two copies (one for each lab partner) of your graph and data before quitting the program.

References

1. Adapted from a laboratory exercise used by the University of Illinois (General Chemistry Experiments: Chemistry 102. Stipes Pub. Co., Champaign, IL. 1985).
2. Seliger, B. *Chemistry in the Marketplace*. Harcourt Brace Jovanovich (Australia). 4th ed. 1989.
3. De Moura, John M., and S. D. Gammon, *J. Chem Ed :Software,* vol. 4, #2, 1997
4. Lange's *Handbook of Chemistry,* McGraw-Hill, 1967

DATA REPORT - WIZARDS OF THE WINERY

Hand in a report of your own making that is organized to show the data collected and key observations made during the experiment. The density of pure ethanol (alcohol) is 0.798 g/mL. Use the table on the next page to determine the density of your wine solution at various alcohol concentrations. When using the table, assume that density and specific gravity are the same.

Your report must include the following:

1. A **brief** description of experimental procedure, quantities (data), and results from Parts A, B, and C. A person reading your report should know what you did and how you did it. Do not present a detailed rehash of the procedure.

2. Complete work and answers for:

    a. tartaric acid concentration in units of M.
    b. free SO_2 concentration in units of **both** M and ppm.
    c. total SO_2 concentration in units of **both** M and ppm.
    d. percent alcohol (ethanol) by **both** volume (%v/v) and weight (%w/w). **When reporting this result, don't forget that you diluted your wine by a factor of 10 in order to take a measurement.**

3. Comparison of your results with typical data for bulk wines of the kind you tested (look at the label).

Specific Gravity (use as density) of Aqueous Solution Ethyl Alcohol at 15°C

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		