Wine Analysis Lab II: Redox Titration for Reducing Sugars

Ruth Russo

Introduction

Wines are classified by how sweet or dry they taste. Many white wines, Riesling for example, have high sugar content and are sweet. Full-bodied reds, such as Merlot, are quite dry and have almost no sugar content. A winemaker may analyze the sugar content of the wine during the fermentation process, in which the yeast convert the sugars in the grape juice to alcohol. This assay for so-called “reducing sugars” is an important tool in enology, for it allows the winemaker to deliberately stop the fermentation at any point in order to produce a particular style of vintage with the desired degree of sweetness.

Reducing sugars

The purpose of this lab is to measure the concentration of the reducing sugars in a wine sample. The most common sugars in grape juice, glucose and fructose, are comprised of 6 carbon chains, the first or second carbon of which contains a carbonyl carbon, i.e. a carbon double-bonded to oxygen. The rest of the carbons are substituted with −OH groups. In aqueous solution, the open-chain form of the molecule is in equilibrium with a cyclic form of the molecule. The open-chain form of the sugar will react with an oxidizing agent, so that the carbonyl carbon adds another oxygen.

![Diagram of glucose in ring form, open-chain form, and oxidized form]

Glucose in ring form  Open-chain form  Oxidized form

Any carbohydrate that is able to react with an oxidizing agent is classified as a reducing sugar; in other words, it reduces the oxidizing agent and is itself oxidized in the process. Only simple sugars are reducing agents. Highly polymerized carbohydrates, such as starch, or highly substituted carbohydrates, such as in the malvidin-3-glucoside from last week, will not reduce the oxidizing agent.

The redox titration

In our lab, a series of colored redox reactions will be used to determine monosaccharide concentration. Copper sulfate pentahydrate and potassium sodium tartrate in strong base is known as Fehling’s solution, and has been used for over 150 years as a test for monosaccharides in organic chemistry. In enology, the Rebelein method refers to the use of an iodine/starch/thiosulfate indicator in conjunction with Fehling’s solution.

In the first step, the sample is mixed with CuSO₄•5H₂O and KNaC₄H₄O₆ in NaOH. The copper sulfate pentahydrate and the basic potassium sodium tartrate form a deep blue complex ion:

![Complex ion diagram]

If the sample contains reducing sugars, however, the copper (II) in the complex is reduced to copper (I) quantitatively.

Open chain sugar + Cu²⁺ → Oxidized sugar + Cu⁺
In the next step, iodide in a basic solution is added to the sample; the iodide reduces the copper (II) ion that is still left:

$$2Cu^{2+} + 2I^- \rightarrow 2Cu^+ + I_2$$

In this redox step, the products are copper (I) and molecular iodine. Notice that the production of molecular iodine is proportional to the copper (II) that is left over after any reducing sugar has acted upon the copper.

The solution is then neutralized with sulfuric acid, and a starch indicator is added to the sample. Starch binds to molecular iodine and gives the sample a blue-grey appearance. When the sample is titrated with a standardized sodium thiosulfate (Na$_2$S$_2$O$_3$) solution, the thiosulfate ions reduce the molecular iodine back to iodide:

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

At the endpoint of the titration, all the molecular iodine has been used up, and the starch turns—within a couple drops—from grey-blue to creamy white. Note that the volume of the thiosulfate used will be greater for a sample with no reducing sugars (such as a d.i. water blank), than it will be for a sample with some reducing sugar in it.

**Procedure**

**A. Set up and general comments**

a. Please work in groups of three or four

b. Set up a buret at your station with the standard thiosulfate solution. This is labeled Z6.

c. Clean (but do NOT dry) 2 or 3 125 mL Erlenmeyer flasks. **Do NOT use acetone today.**

d. Find a white piece of paper to place under your titration.

e. Obtain three 5 mL serological pipets and a green pipet filler.

f. Obtain a hot plate and turn it on medium.

g. Obtain tongs that can fit around the neck of your 125 mL flasks.

h. Each of the reagents Z1 through Z5 is in a buret in the front of the room. You may have a bit of waiting around for others to get done, but this is preferable to mixing up reagents and/or glass pipets.

i. Each sample needs to be made up fresh—they have to be done one at a time. When Z1 and Z2 get added together, they will start a side reaction if they sit too long. As you are titrating one sample, someone in the group can start preparing the next sample.

**B. Sample procedure**

a. Add 10.0 mL Z1 to a 125 mL Erlenmeyer. Add 5 mL Z2. Add 3-5 boiling chips.

b. Pipet 2.0 mL of the sample (d.i. water, decolorized wine, or known glucose) into the flask.

c. Place on the hot plate. Watch carefully. When boiling first begins, time this for 30 seconds. After 30 seconds, take the flask off the hot plate with your tongs and run the end of the flask under cool water until you can touch the base of the flask with the hand.

d. When the flask has reached room temperature, add 10 mL each of Z3, Z4, and Z5 in that order. Swirl to mix.

e. On the report sheet, record the initial volume of thiosulfate in the buret. Titrate to the creamy-white endpoint. Record the final volume.

f. The contents of the flask can go down the drain. Wash and rinse the flask.

**C. Trials**

a. Blanks: do three titrations using d.i. water as your sample

b. Wine: do three titrations using decolorized wine as your sample

i. If your sample is “Blush” wine, you will need to dilute this prior to analysis, since it is so sweet.

ii. Using your graduated cylinder, add 5 mL of decolorized wine to 45 mL water. Cover with Parafilm; invert 3x.
c. Known glucose: do one titration using 10 g/L glucose as your sample as a positive control.

D. Calculations
   a. The concentration of reducing sugars (g/L) is equal to (average mL blank)-(average mL wine sample)
   b. If you diluted your wine sample, you need to multiply this value by ten.
   c. Also calculate the reducing sugars for the known glucose sample and the percent error.
   d. Record these calculations on the Report Sheet.
   e. Answer the two questions.

Materials

Equipment
- Hot plates
- Burets
- 18 5 mL serological pipets
- green pipet fillers

Chemicals
- Reagents Z1 through Z6 prepared according to the Rebelein method
- Decolorized wine samples from last week

Bibliography

Acknowledgments
- Special thanks to Tim Donohue, WWCC Enology Instructor and Winemaker
- Special thanks to Jeanine Kay-Shoemake, WWCC Microbiology Instructor
1. Draw the open chain form of glucose, and circle the structural piece necessary for it to be a “reducing sugar”:

2. Write the balanced equation for copper(II) reacting with iodide, and label each reactant as a reducing agent or an oxidizing agent.

3. Write the balanced equation for iodine reacting with thiosulfate, and label each reactant as a reducing agent or an oxidizing agent.

4. What is the role of starch in the visualization of an endpoint?
Wine Analysis Lab II: Redox Titration for Reducing Sugars

Wine sample__________________________

<table>
<thead>
<tr>
<th>Titrations</th>
<th>Initial Volume (mL)</th>
<th>Final Volume (mL)</th>
<th>Difference (mL)</th>
</tr>
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<tbody>
<tr>
<td>Blanks</td>
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<td>Average</td>
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<td>Wine</td>
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<tr>
<td>Average</td>
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10 g/L glucose

<table>
<thead>
<tr>
<th>Calculation</th>
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</thead>
<tbody>
<tr>
<td>Wine sample</td>
<td>$RS(\frac{g}{mL}) = Blank_{avg} - Wine_{avg}$ multiply by ten if necessary</td>
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<tr>
<td>Positive control</td>
<td>$RS(\frac{g}{mL}) = Blank_{avg} - Glucose$</td>
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<tr>
<td>%error</td>
<td>$\left</td>
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</tbody>
</table>

Questions

1. Is your wine dry or sweet?

2. Did your positive control work well (i.e. are you within ~10% error)?