**Determination of Glucose by Titration with Fehling’s Reagent**

**Introduction**

Fehling’s test for reducing sugars has been used since the 1800’s to determine the amount of glucose and other reducing sugars (lactose in milk, for example). It has had many applications including use in agriculture (glucose determination in corn for use in corn syrup) and in medicine (glucose determination in urine for diabetes tests). The test works by taking advantage of the ability of aldehyde-containing sugars to reduce blue Cu\(^{2+}\) ions to Cu\(^+\) ions. A disadvantage of this reaction is that it must take place under basic conditions where Cu\(^{2+}\) tends to form an insoluble precipitate with hydroxide ions. In order to prevent this precipitation, tartrate ions are added to the solution in order to form a soluble complex with the Cu\(^{2+}\) (shown on the right), isolating it from the hydroxide.

The oxidation-reduction reaction between Cu\(^{2+}\) and the aldehyde portion of glucose is shown below.

\[
RCHO \text{(aq)} + 2 \text{Cu}^{2+} \text{(aq)} + 5 \text{OH}^- \text{(aq)} \rightarrow \text{RCOO}^- \text{(aq)} + \text{Cu}_2\text{O} \text{(s)} + 3 \text{H}_2\text{O} \text{(l)}
\]

\[\text{blue} \quad \text{rust red}\]

The reaction is self-indicating as the disappearance of the deep blue copper(II)-tartrate complex and appearance of Cu\(^+\) (in the form of a red Cu\(_2\)O precipitate) signals the equivalence point of the reaction. As the amount of Cu\(^{2+}\) in solution decreases, however, it becomes difficult to see the actual equivalence point accurately. This leads to only a poorly reproducible, qualitative determination of the amount of sugar present in the titrant. To compensate for this, after most of the blue from the Cu\(^{2+}\) complex is gone, the indicator methylene blue is added to the solution. Methylene blue is a commonly used indicator for oxidation-reduction reactions. It is a deep blue color in its oxidized form but colorless when exposed to reducing agents. After the glucose titrant has completely reduced all of the Cu\(^{2+}\) to Cu\(^+\), the methylene blue will be reduced by the glucose, completely removing the blue color from the solution. This total disappearance of color indicates the end point of the titration.

**Chemicals needed**

Fehling’s stock solution A
Made by dissolving ~70 g CuSO\(_4\)·5H\(_2\)O in DDI water and diluting to 1.000 L

Fehling’s stock solution B
Made by dissolving ~350 g K\(_2\)NaC\(_4\)H\(_4\)O\(_6\)·4H\(_2\)O (potassium sodium tartrate tetrahydrate) and 100.0 g NaOH in DDI water and diluting to 1.000 L. After sitting overnight, any sediment is filtered out.

Methylene Blue indicator, 1 % aqueous solution
Dextrose, dried 2 hours at 100 ºC
Unknown dextrose sample

**Dextrose standard solution**

1. Weigh accurately ~2.5 g of dried dextrose (i.e. glucose).
2. Dissolve in DDI water and dilute to 500.0 mL in a volumetric flask.
3. Mix well.
Fehling’s Standardization Procedure

1. Accurately transfer 10.00 mL Fehling’s solution A and 10.00 mL Fehling’s solution B into a 250.0 mL Erlenmeyer flask. Use a separate volumetric pipet for each solution. Add approximately 30 mL of DDI water. Mix well. (Figure 1)

2. Fill buret with dextrose standard. Make sure to drain to remove any bubbles.

3. Place flask on a hot plate turned to a medium heat. Heat to at least 70 °C but below boiling (use a thermometer). Throughout the titration, continue to monitor the temperature to make sure to keep the temperature of the solution consistently above 70°C. Remember that the addition of the dextrose titrant will cause the solution to cool.

4. Add dextrose standard until blue color nearly disappears. The mixture will remain a murky blue-gray with a reddish precipitate but won’t completely clear up. (Figure 2)

5. After the color mostly disappears (or after ~10 mL of added dextrose, whichever comes first), add 1 or 2 drops (no more) of methylene blue indicator.

6. Continue adding dextrose standard until the blue color disappears entirely and only a colorless solution with a red precipitate is present. (Figures 3 and 4) It will help to hold a piece of white paper behind the flask.

Figure 1: Mixture of Fehling's Solution A and B

Figure 2: After ~10 mL added dextrose, before methylene blue is added

Figure 3: Just before the last few drops

Figure 4: At the end point
7. Using the concentration of dextrose in your titrant and the volume of the dextrose solution used for the titration, determine the mass of dextrose that reacts per mL of Fehling’s reagent. (“Fehling’s reagent” refers to the mix of 10 mL each of stock solutions A and B.)
8. Repeat the titration at least three times.

Clean-up. Extra dextrose solution may be disposed of in the sink. The red precipitate (Cu₂O) should be collected by gravity filtration on filter paper and placed in the appropriate container in the hood. The remaining liquid may be disposed of in the sink. Any excess Fehling’s solution A should be placed in the copper waste container in the hood. Excess Fehling’s solution B may be disposed of in the sink.

Unknown Determination
Obtain an unknown dextrose sample. It may be either a pre-measured solution or a commercially available glucose tablet.

For a pre-measured solution
1. Quantitatively transfer the solution to a 500.0 mL volumetric flask, dissolve in DDI water and dilute to 500.0 mL. Mix well.
2. Fill buret with this dextrose unknown. Repeat titration as performed to standardize the Fehling’s solution.
3. Repeat the titration using your unknown dextrose solution at least three times.
4. Use the mass of glucose that reacts per mL of Fehling’s reagent (determined in standardization procedure step 7) to find the average concentration of dextrose in your prepared unknown (the 500.0 mL diluted solution).

For a glucose tablet
1. Examine the packaging for the tablet. Remove one tablet from the package and find its mass. Using the mass of a tablet and the amount of glucose per tablet listed on the package, calculate the predicted % w/w of glucose in your tablet.
2. Using the predicted % w/w of glucose determined above, find the mass of a tablet that would be needed to provide 2.5 g of glucose.
3. Using a mortar and pestle, grind one or two glucose tablets (as needed to provide at least the tablet mass determined in step 2).
4. Accurately weigh the amount of your glucose tablet that would be required to provide 2.5 g of glucose (as determined in step 2).
5. Dissolve the glucose tablet in DDI water in approximately 100 mL DDI water in a beaker over medium heat for ~10 minutes. Note that the tablet may not fully dissolve due to binders and other insoluble materials.
6. After removing the beaker from the heat, gravity filter the sample into a 500.0 mL volumetric flask to remove any undissolved solids. Dilute to the mark with DDI water. Mix well.
7. Fill buret with this dextrose unknown. Repeat titration as performed to standardize the Fehling’s solution.
8. Repeat the titration using your unknown dextrose solution at least three times.
9. Use the mass of glucose that reacts per mL of Fehling’s reagent (determined in standardization procedure step 7) to find the average % w/w dextrose in your glucose tablet.
**Data Analysis**

Determine the 95% confidence interval for your final results. Determine the uncertainty in your final measurement by propagating the uncertainties of the glassware and balance used in this procedure. Remember to include the uncertainty in the mass of glucose per mL of Fehling’s reagent in the final calculation of uncertainty for your glucose unknown. Compare the expected uncertainty to your actual uncertainty. If your unknown was a commercially available glucose tablet, quantitatively determine the accuracy of your results. Do your results indicate a systematic error at the 95% confidence level?

In your discussion, make sure to talk about any factors in this procedure that contribute to a loss of accuracy and precision. Using on-line or printed resources (not Wikipedia) find at least one alternative method of glucose determination. Describe the differences and similarities between the method you have found and the Fehling’s determination. When might one be more appropriate than the other?