

1/31/2018

Goals:

- Set up the flow-cell
- Determine HPLC procedure to identify the different compounds in our honey stock solutions
- Create honey mediated gold nanoparticles

Division of tasks among group members:

- Sanuja and Pauline set up the flow-cell
  - Casey and Raquel found existing literature that used HPLC to test honey. The procedure from this literature will be used on 2/6/18 to prepare solutions needed for HPLC. The HPLC will be run on 2/7/18.
  - All four group members prepared honey and gold samples.
- 

➤ HPLC Procedure and Notes

- This procedure was obtained from Linda Stubbs' *Sugars in Honey by HPLC and Titrimetry* report.
  - [http://cffet.net/project/sample\\_report.pdf](http://cffet.net/project/sample_report.pdf)
- Step 1: Prepare Mobile Phase
  - 75% Acetonitrile: 25% DI water solution in a 1L beaker
  - Filter through a vacuum filtration system using a 0.45 um PTFE membrane filter
- Step 2: Prepare Sucrose Stock Solution
  - About 8g/L
  - Use parts of this solution to make up the required sucrose solution in the standard solutions
- Step 3: Prepare Mixed Calibration Standards at the Following Concentration Ratios
  - 1:1:0.2 g/L Fructose: Glucose: Sucrose
  - 2:2:0.4 g/L Fructose: Glucose: Sucrose
  - 5:5:1 g/L Fructose: Glucose: Sucrose
  - Prepare by accurately weighing appropriate masses of fructose and glucose and adding accurately pipetted volumes of sucrose stock solution
- Step 4: Prepare Honey Solutions
  - Honey samples were prepared at 10g/L
- Step 5: For Recovery Check Analysis
  - Solutions of about 5g/L were prepared by accurately weighing about 2.5g of honey and adding an accurately weighted mass of glucose of about 0.5 g and making up to 500 mL
- Step 6: All samples were filtered by a vacuum filtration system through cellulose-acetate membrane filter

- Notes
    - Standards were injected into the HPLC at 0.6 mL/min flow rate and at 0.9 mL/min flow rate.
    - Once optimum flow conditions had been established, repeated injections of standards were performed to obtain reproducible elution times and peak heights.
    - Average peak heights were used to construct calibration graphs for fructose and glucose.
    - Using the same conditions samples and recovery check samples were injected and calibration graphs were used to determine the fructose and glucose content of the honey sample.
- 

➤ Honey-mediated Gold Nanoparticle Procedure

- This procedure was obtained from Daizy Philip's *Honey mediated green synthesis of gold nanoparticles* report.
    - <https://www.sciencedirect.com/science/article/pii/S1386142509001346>
  - Step 1: Prepare H<sub>2</sub>AuCl<sub>4</sub> stock solution
    - 50 mg H<sub>2</sub>AuCl<sub>4</sub> was dissolved in 120mL DI H<sub>2</sub>O
  - Step 2: Prepare Honey stock solution
    - 20g honey was diluted to 70mL
  - Step 3: Prepare g<sub>3</sub>
    - 20 mL from honey stock solution was mixed with 30 mL from the gold stock solution
  - Step 4: Prepare g<sub>4</sub>
    - 25 mL from honey stock solution was mixed with 30 mL from the gold stock solution
  - Step 5: Prepare g<sub>5</sub>
    - 30 mL from honey stock solution was mixed with 30 mL from the gold stock solution
  - Step 6: Leave all three samples at room temperature for 3 hours for gold nanoparticles to form
    - The samples were monitored for all three hours to determine if and when the gold colloids would form. We hypothesized that all three will form gold nanoparticles, but the g<sub>5</sub> sample will form the nanoparticles faster than the other two samples.
  - Notes:
    - The honey used throughout this experiment was obtained from the American University Beekeeping Society. It is unfiltered, raw, and treatment-free. The honey was from the July 2017 harvest.
-

➤ Results of the Honey and Gold Samples

- Figure 1 below shows no change in any of the samples. According to the literature, if gold nanoparticles formed, the samples would have changed to a light purple. Thus, it is unlikely that any samples formed gold nanoparticles.
- There are multiple reasons why the solution did not form nanoparticles. For example, we might have used the wrong  $\text{HAuCl}_4$  that did not have a trihydrate. Our first step to address this issue is to check the  $\text{HAuCl}_4$  we used. Our second step forward is to double check the literature procedure.

Figure 1:  $g_3$ ,  $g_4$ , and  $g_5$  Samples

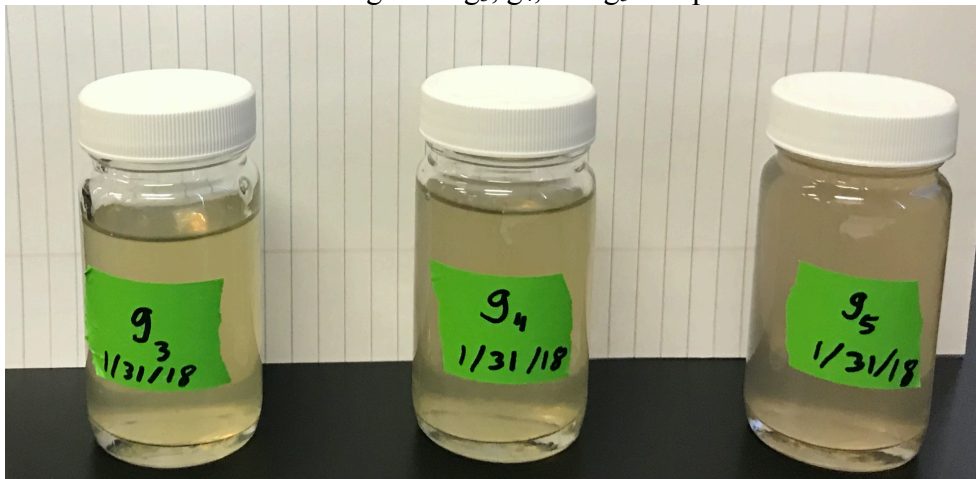


Figure 1 shows the  $g_3$ ,  $g_4$ , and  $g_5$  samples prepared on 1/31/2018. None of the samples changed colors.