

**Student Guide: Pucker Up**

**Purpose:** To measure the vitamin C (ascorbic acid) concentration in various vitamin C supplements and drinks by determining the amount of indophenol indicator needed to react with reference solutions of known vitamin C concentration.

Similar to the idea presented in the article "[Tainted supplements flood the market](#)," you will qualitatively analyze the amount of a chemical in a supplement, however **the vitamin C sources used in the experiment will not contain the harmful chemicals mentioned in the article**. Chemicals used in a laboratory setting should always be handled with caution. Eating, directly touching or smelling chemicals is never permitted.

**Approximate class time:** One 45-minute class period.

**Chemicals:**

0.5 mg/ml indophenol solution

1.0 mg/ml vitamin C solution

Various drinks/juices to test

Various vitamin C supplement solutions to test

**Data table:**

Read through the following procedure, and take note of all the quantitative and qualitative data that needs to be recorded. For each part of the lab, create a data table that you can use while completing the activity.

**Procedure:****Part 1: Make and test vitamin C reference standards for comparison**

1. Make five tubes of different vitamin C concentrations. Label the tubes on the side so you can keep track of them:

**Tube A:** 1.0 mg/ml vitamin C = 16 drops of 1.0 mg/ml vitamin C

**Tube B:** 0.5 mg/ml vitamin C = 8 drops of 1.0 mg/ml vitamin C + 8 drops of water

**Tube C:** 0.25 mg/ml vitamin C = 4 drops of 1.0 mg/ml vitamin C + 12 drops of water

**Tube D:** 0.125 mg/ml vitamin C = 2 drops of 1.0 mg/ml vitamin C + 14 drops of water

**Tube E:** 0.0625 mg/ml vitamin C = 1 drop of 1.0 mg/ml vitamin C + 15 drops of water

2. The pH test strips change colors to indicate how acidic ( $\text{pH} < 7$ ) or alkaline ( $\text{pH} > 7$ ) a sample is. Cut the test strips in half lengthwise, dip a strip into the vitamin C solution for two seconds and then let it dry. Compare the strip's color to the color code on the packaging to determine the sample's pH. Record all results in your data table.

If necessary, you can make further dilutions or intermediate dilutions to test.

3. Make five new tubes with 16 drops of 0.5 mg/ml indophenol per tube.

4. As you can see from your indophenol solutions, indophenol dissolved in water makes a blue solution. Adding vitamin C reduces the color to the point that the solution becomes colorless. You can evaluate how much vitamin C is in a sample by counting how many drops of the sample must be added to indophenol to make it colorless. Complete the following steps below, and record in your data table the number of drops of vitamin C solution needed to turn each indophenol solution clear. Make sure to gently shake the test tube in between the addition of every drop to ensure the solutions properly mix.

**Tube 1:** Add drops from Tube A to Tube 1 until Tube 1 becomes colorless.  
Record how many drops of 1.0 mg/ml vitamin C make the indophenol become colorless.

**Tube 2:** Add drops from Tube B to Tube 2 until Tube 2 becomes colorless.  
Record how many drops of 0.5 mg/ml vitamin C make the indophenol become colorless.

**Tube 3:** Add drops from Tube C to Tube 3 until Tube 3 becomes colorless.  
Record how many drops of 0.25 mg/ml vitamin C make the indophenol become colorless.

**Tube 4:** Add drops from Tube D to Tube 4 until Tube 4 becomes colorless.  
Record how many drops of 0.125 mg/ml vitamin C make the indophenol become colorless.

**Tube 5:** Add drops from Tube E to Tube 5 until Tube 5 becomes colorless.  
Record how many drops of 0.0625 mg/ml vitamin C make the indophenol become colorless.

5. Graph the reference vitamin C concentrations versus the number of drops of vitamin C solution required to turn the indophenol solution colorless. Check with your teacher to see what program you should use to graph your data, or if you should graph your data by hand on graph paper. Once these values are graphed, determine a line of best fit.

7. Rinse and dry all test tubes, so you can reuse them for the next part.

## **Part 2: Test vitamin C in five drink or juice samples**

1. Add 16 drops of a drink or juice sample to a test tube. Do this for five different drinks or juices. Label each tube with the name of the drink. Record each sample name in your data table.

2. Use colorimetric pH strips to measure the pH of each sample. Record the pH values in your data table.

3. Make five new tubes with 16 drops of 0.5 mg/ml indophenol per tube.

4. Using a different pipette for each drink or juice sample, add each drink or juice sample one drop at a time to the indophenol tubes until the solutions become colorless. Make sure to gently shake the test tube in between the addition of every drop to ensure the solutions properly mix. In your data table, record how many drops it took for each drink or juice sample to turn the indophenol solution clear.
5. Using the line of best fit from your graph of reference data, determine the concentration of vitamin C in each unknown solution. Record your results in your data table.
6. Rinse and dry all test tubes, so you can reuse them for the next part.

### **Part 3: Test vitamin C supplement pills solutions**

1. Add 16 drops of a vitamin C supplement pill solution to a test tube. Do this for five different supplement pill solutions. Label each tube with the name or letter of the supplement pill. Record each sample name or letter in your data table.
2. Use colorimetric pH strips to measure the pH of each sample. Record the pH values in your data table.
3. Make five new tubes with 16 drops of 0.5 mg/ml indophenol per tube.
4. Using a different pipette for each supplement solution, add each supplement solution one drop at a time to the indophenol tubes until each becomes colorless. Make sure to gently shake the test tube in between the addition of every drop to ensure the solutions properly mix. In your data table, record the how many drops it took for each supplement solution to turn the indophenol solution clear.
5. Using the line of best fit from your graph of reference data, determine the concentration of vitamin C in each unknown supplement solution. Record your results in your data table.
6. Clean your equipment and your lab area.

### **Part 4: Analysis questions**

1. **Which drink or juice had the most vitamin C?**
2. **Which drink or juice had the least vitamin C?**
3. **Which supplement pill had the most vitamin C?**
4. **Which supplement pill had the least vitamin C?**

**5. How did pH correlate with vitamin C concentration? What does this tell you about vitamin C?**

**6. Which result surprised you the most?**