Activity Sheet 1  
Testing for Vitamin C- Part One

Purpose: To test the level of vitamin C in a variety of fruit juices.

Procedure:

Part 1 – Testing Vitamin C Solution

1. Use the vitamin C solution for this experiment. The solution was made by adding 4 grams of vitamin C to 3 cups (750 mL) of water. This solution has more vitamin C in it than any of the fruit juices.

2. Pour approx. 10 mL of indophenol solution into a beaker. Set the beaker on a sheet of white paper so it will be easier to make an accurate color determination. This will be your “test beaker”.

3. Pour approx. 10 mL of water into a second beaker. Set this beaker next to the test beaker on the white paper. This will be your “control beaker”.

4. Use a medicine dropper to add one drop of the vitamin C solution to the test beaker and one drop of the vitamin C solution to the control beaker. After the addition of each drop, swirl the beaker thoroughly.

5. Continue to add drops of vitamin to each beaker, remembering to swirl after each drop. REMEMBER to keep track of the number of drops you are adding. Stop adding drops when the test beaker is the same color as the control beaker. Record the number of drops on your data recording sheet.

6. Dump out the contents of the beakers into a waste container and use a water bottle to rinse the beakers with water. Be sure to rinse well.
Part 2 – Testing Juices for Vitamin C

1. Pick one juice to do the experiment.

2. Pour approx. 10 mL of indophenol solution into a beaker. Set the beaker on a sheet of white paper so it will be easier to make an accurate color determination. This will be your “test beaker”.

3. Pour approx. 10 mL of water into a second beaker. Set this beaker next to the test beaker on the white paper. This will be your “control beaker”.

4. Use a medicine dropper to add one drop of your juice to the test beaker and one drop of juice to the control beaker. After the addition of each drop, swirl thoroughly.

5. Continue to add drops of juice to each beaker, remembering to swirl after each drop. REMEMBER to keep track of the number of drops you are adding, and be careful not to add too many. The goal of this test is to count how many drops of juice are required to cause the contents of the test beaker to become the same color as the contents of the control beaker. As a liquid with vitamin C is added, the indophenol will change from “blue” to “violet” to “pink” to “no color”. When the indophenol loses its color, the contents of the two beakers will become the same color. This is called the end point. The procedure of adding one liquid to another drop by drop until a specified end point is reached is called a titration.

6. When the end point has been reached, hold up the two beakers. Are they totally clear? The answer should be no. Since the juice is colored, there will always be a tint to the solution, even when the indophenol becomes colorless. This is why the control beaker is used. When the color of the contents of the two beakers is the same, we will know that all color due to the indophenol is gone.

7. Dump out the contents of the beakers into a waste container and use a water bottle to rinse the beakers. Be sure to rinse well.

8. Record your results on the data recording sheet. If it takes more than 50 drops to reach the end point, record “50+”.

9. Now repeat the experiment using a new type of juice.

10. Answer the questions on the following page.

Questions
1. What would happen if you did not rinse the beakers with water between juice tests?

2. What would happen to your results if you were not precise in the amount of water and indophenol solution you started with in the beakers?

3. How many drops did it take to titrate the indophenol with vitamin C solution?

4. What was the largest number of drops needed to titrate the indophenol with a juice? What was this juice?

5. Knowing that the vitamin C solution had more vitamin C in it than any of the juices, can you now rank the beverages from the most vitamin C (#1) to the least vitamin C (#4?)

6. How many drops do you predict something with very little vitamin C would take to titrate the indophenol?

7. Vitamin C reacts with the indophenol and causes its color to change. A liquid containing a lot of vitamin C needs just one drop to cause all the indophenol to react. Many drops of a liquid containing only a small amount of vitamin C must be added to cause all of the indophenol to react. Fill in the blanks below:

   The ___________ drops required to titrate indophenol, the ___________ the amount of vitamin C contained in the liquid.
Activity Sheet 2
Data Record Sheet

Steps:

1. Pour 10 mL indophenol into test beaker.

2. Pour 10 mL water into control beaker.

3. Add 1 drop of the test beverage to each beaker and swirl well.

4. Keep adding, drop by drop, until the contents in both beakers look the same. Remember to swirl after each drop and count the number of drops added.

5. After testing all beverages, rank the beverages in vitamin C content.

Indophenol Color Change: Blue ☐ Violet ☐ Pink ☐ No Color

<table>
<thead>
<tr>
<th>Test Beverage</th>
<th>Number of drops needed for Indophenol to lose all color</th>
<th>Ranking (1 = Most Vitamin C)</th>
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<tr>
<td>Vitamin C Solution</td>
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Activity Sheet 3
Chemical Equilibrium Calculations
The experiment you just completed used the following reaction to determine the amount of vitamin C in various fruit drinks:

\[
\text{HO-} \quad \text{O} \quad \text{OH} + \text{OCl} \quad \text{N} \quad \text{O} \quad \text{Na}^+ \quad \rightarrow \quad \text{HO-} \quad \text{O} \quad \text{OH} + \text{OCl} \quad \text{N} \quad \text{O} \quad \text{Na}^+. 
\]

The reaction between ascorbic acid and dichloro-indophenol is an example of an oxidation-reduction reaction. A more familiar example is the reaction of liquid laundry bleach, such as Clorox, which is a dilute solution of sodium hypochlorite, NaOCl. In solution, this compound dissociates to give Na\(^+\) and OCI\(^-\) ions. The hypochlorite ion oxidizes many colored compounds to give colorless or "bleached" products. Similarly, the dichloro-indophenol ion oxides ascorbic acid and produces a colorless compound, which is called the endpoint of the titration.

What does the word “reaction” mean? When a chemical reaction takes place spontaneously, the concentrations of the reactants and products change over time. After a certain time passes, most reactions come to chemical equilibrium—that is the ratio of reactants to products becomes constant. The equilibrium equation for the reaction of dichloro-indophenol and ascorbic acid is:

\[
\text{C}_6\text{H}_6\text{O}_6 + \text{OC}_6\text{H}_2\text{Cl}_2\text{NC}_6\text{H}_4\text{ONa}^+ \rightleftharpoons \text{C}_6\text{H}_6\text{O}_6 + \text{OHC}_6\text{H}_2\text{Cl}_2\text{NHC}_6\text{H}_4\text{ONa}^+. 
\]

Which states that for every one mole of ascorbic acid in your beaker, one mole of dichloro-indophenol reacts to form one mole of colorless products.
How many moles if dichloro-indophenol were in the beaker for each juice that you tested given that the concentration is $1.9 \times 10^{-4}$ M?

- The units used in chemistry to report concentration is molarity, which is defined as the number of moles of the substance of interest (in this case dichloro-indophenol) per unit volume in liters. If you know the volume and concentration of a substance, then you can calculate the moles.

\[ \text{liters of solution} \times \frac{1.9 \times 10^{-7} \text{ moles dichloro-indophenol}}{1.0 \text{ liter of solution}} = \text{moles dichloro-indophenol} \]

**How many moles of ascorbic acid were added to each reaction vessel?**

- Dichloro-indophenol is called an indicator because it changes color when all of the dichloro-indophenol in the reaction vessel has reacted with ascorbic acid. Look at the chemical equilibrium reaction to answer this question.

**How many liters of fruit drink were added to the dichloro-indophenol in each experiment?**

Refer to your data table to obtain the number of drops and then use the following information to calculate the liters of fruit drink added to each reaction vessel.

- 20 drops approximately equals 1 milliliter.

<table>
<thead>
<tr>
<th>Fruit Drink (Liters)</th>
<th>Number of Drops</th>
<th>Volume</th>
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**What is the concentration of ascorbic acid in each juice?**

Molarity of ascorbic acid = moles of ascorbic acid

**Activity Sheet 4**
Results

Summarize the results from your analysis in an easy to read format so that patterns and discrepancies can be easily recognized.

<table>
<thead>
<tr>
<th>Type of Juice</th>
<th>Number of Drops Added (#)</th>
<th>Concentration (molarity)</th>
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Activity Sheet 5
Explanation

What does the percent vitamin C given on the fruit drink container mean? For more information see: http://www.nal.usda.gov/fnic/dga/rda.pdf

Does the trend in juices you found agree with the percentages reported by the manufacturers (make note of the individual serving sizes)?

Why is the number of moles of ascorbic acid added to the reaction vessel the same for all juices even though you added a different number of drops to each?

If you wanted to market a new juice using the slogan that it contained more vitamin C than any other juice, how many grams of ascorbic acid would you need to add to a 250 mL container? (The molecular weight of ascorbic acid is 176.13 gram/mole.)