UV DETERMINATION OF CAFFEINE CONTENT

Introduction:

Caffeine is a common organic molecule found in many beverages such as coffee, tea and cola. It is a stimulant to the central nervous system. That is why many students drink coffee or soda to help them feel alert.

Like many conjugated organic molecules, caffeine absorbs radiation with a wavelength around 260 nm. A conjugated system is one containing 2 double bonds separated by a single bond. This conjugated pattern may be repeated several times in the molecule. If a series of caffeine standards are analyzed in this region of absorption and a Beer's law is plot prepared, then the amount of caffeine in another substance can be determined. One should be aware that the assumption is being made that the unknown contain no other substances which absorb at this wavelength.

Purpose:

The purpose of this experiment is to determine the amount of caffeine in selected sodas.

Equipment/Material:

Spectorphotometer
quartz cuvets caffeine
standards degassed
soda samples
methylene chloride
droppers
ring stands
iron rings separatory funnels 50
mL graduated cylinders
Erlenmeyer flasks, with stoppers
Automatic pipets
50 ml, volumetric flasks (opt.)
waste container

Safety:

- Apron and goggles must be worn in lab.

Procedure:

1. Prepare 50.00 mL of the assigned caffeine standard (50, 100, 150, 200, or 250 ppm) by a quantitative dilution of the 1000 ppm stock solution.
2. Place the caffeine standard in a separatory funnel. Add 25 ml, of methylene chloride.
3. Extract the caffeine by inverting the funnel at least 3 times. Vent the separatory funnel after each inversion.
4. Remove the methylene chloride layer, which is the bottom layer, and save in a clean, stoppered Erlenmeyer flask.
5. Add another 25 ml, of methylene chloride to the separatory funnel.
6. Extract twice more by repeating steps 3-5. Combine the methylene chloride layers.
7. Add 50 ml of degassed soda to a clean separatory funnel separatory funnel. (The sep funnel may be rinsed with methylene chloride to be certain that no caffeine from the previous sample remains.)
8. Extract the soda 3 times with 25 mL portions of methylene chloride as above. Save the methylene chloride layers in another clean, stoppered Erlenmeyer flask.
9. With the sample compartment of the spec empty, turn on the power switch. Let the instrument initialize and print out a self test
10. Press the SCAN key. Scroll to BASELINE MENU and press E to enter. Scroll to Store Baseline and press E. Enter the following data: Starting wavelength 190nm, ending wavelength 350 nm, scan speed 200 nm/min.
11. Fill a clean cuvet with methylene chloride. Place it in the cell holder, close the cover, and press RUN to store the baseline data. When the screen displays the SCAN Menu and the printer prints Baseline Stored, samples can be run.
12. Rinse the cuvet twice with the first standard to be run; dispose of the methylene chloride in the waste container. Use the automatic pipet to fill the cuvet with 2.000 ml, of the standard. Insert the cuvet into the cell holder and press RUN to scan the sample. When the scan is completed, press RUN again to print the scan.
13. Repeat step 12 with the other standards and unknown solutions. Remember to label the print-outs with the sample name.
14. Plot a Beer's law curve of absorbance (y) vs concentration (x) for the caffeine standards.

Name:_____
Name:_____
Period:_____
Date:_____

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Data Table:

Caffeine Standards

Concentration (ppm)	Absorbance
0.00	
50.00	
100.00	
150.00	
200.00	
250.00	

Soda

Brand	Absorbance	Concentration ppm	Caffeine mg/L

Calculations:

1. Using the graph, determine the concentration of caffeine in each soda in ppm.

2. Calculate the mg of caffeine in a 12 oz serving (253 mL) of soda.

Questions:

1. Why **was it necessary** to extract the standards?

2. Why was it necessary to degas the soda?

3. Why was it necessary to extract the soda.

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TEACHER NOTES

Lab Time: 60-80 minutes

Preparations:

Time: 30 minutes

Prepare 1 L of 1000 ppm caffeine standard solution by dissolving 1.000g of caffeine in enough water to prepare 1 L of solution.

Assign standards and sodas to students. Suggested standards include 50, 100, 150, 200, 250 ppm caffeine.

Degas sodas by allowing the open containers to set at room temperature for at least 12 hours. If time does not permit the use of that method, place the sodas in beakers on stirring plates for a couple of hours.

Answers to Questions:

1. Why was it necessary to extract the standards?

   It was important to treat the standards the same as the sodas so that if any caffeine were lost during the extraction process it would also be lost from the standards.

2. Why was it necessary to degas the soda?

   If the soda were not degassed, the carbon dioxide bubbles would affect the UV light path through the solution.

3. Why was it necessary to extract the soda.

   The sugar and dyes in the soda would interfere in the UV analysis by absorbing in the same range as caffeine.

Considerations:

Time can be reduced by having standards prepared and having each group only analyze one soda. Both caffinated and decaffeinated sodas could be used. Other substances containing caffeine such as Vivrin and no-doz could be used. The range of the standards and/or solution concentration would have to be adjusted.

Science in Motion

Materials List

Lab: UV Determination of Caffeine Content

Number of Lab Groups Prepared:

Equipment per lab group	**Delivered**	**Returned**
Spectrophotometer		
Quartz Cuvets		
Ring Stands		
Separatory Funnels		
50 mL Graduated Cylinders		
Erlenmeyer Flasks with Stoppers		
Automatic Pi pets		
50 mL Volumetric Flasks		
Waste Container		

Consumables	Delivered
Caffeine Standards	
Degassed Soda Samples	
Meth lene Chloride	
Droppers	
Iron Rings	